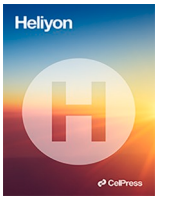
Heliyon 10 (2024) e24614

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Establishment of high performance liquid chromatographic fingerprint and determination of 4 kinds of phenolic acid bioactive substances of fruitless *Lycium barbarum* leaves from Ningxia at different harvesting periods

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A R T I C L E I N F O

Keywords:
Ningxia fruitless *Lycium barbarum* leaves
Fingerprints
HPLC
Phenolic acid bioactive substances

A B S T R A C T

"Fruitless *Lycium barbarum* leaf (FLBL) are the leaves of a new variety of *Lycium barbarum* in Ningxia, which exhibit higher content of various nutrients, trace elements, and bioactive substances compared to *Lycium barbarum* fruits and leaves. However, the health and medicinal value as well as the by-products derived from FLBL have not received sufficient attention, and the contents of main components vary at different harvesting periods. Therefore, for the first time this study aimed to establish high-performance liquid chromatography (HPLC) fingerprints and determine the contents of four phenolic acid bioactive substances during different harvesting periods in order to provide an experimental basis for cultivation, collection, and research on FLBL. The results revealed 17 common peaks among 10 batches samples with a similarity ranging from 0.71 to 0.976. The linear relationships R2 for catechin, epicatechin-catechin, chlorogenic acid, and rutin were determined as 0.9999 each; meanwhile, the average recovery rate ranged from 93.92 % to 120.11 %, with an RSD between 0.91 % and 2.82 %. The precision, repeatability stability (24 h), and recovery rate met the requirements outlined in "Chinese Pharmacopoeia". Catechin, epicatechin, and rutin exhibited higher levels from June to August, while chlorogenic acid showed increased levels from July to September. The findings serve as a foundation for quality control measures such as identifying optimal harvest periods or facilitating development and production processes related to Ningxia FLBL."

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https://doi.org/10.1016/j.heliyon.2024.e24614
Received 26 August 2023; Received in revised form 10 January 2024; Accepted 11 January 2024
Available online 18 January 2024
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1. Introduction

Lycium barbarum, a member of the Solanaceae family, has been utilized as a traditional Chinese herbal medicine for over 2000 years [1]. *Lycium barbarum* leaves are abundant in protein, amino acids, vitamins and trace elements, flavonoids, terpenoids, alkaloids and other bioactive compounds that possess significant nutritional and health-promoting properties. Ancient Chinese medical texts provide extensive documentation on the efficacy of *Lycium barbarum* leaves. The "Compendium of Materia Medica" describes various applications such as decorations made from flowers, fruits, roots, stems and leaves; as well as single-ingredient juices and creams with their respective functions. It is believed that *Lycium barbarum* leaves can tonify kidney essence, clear heat to improve vision quality and delay aging processes. Consequently, it has gained global popularity as a tea infusion ingredient or dietary supplement [2–5]. The *Lycium barbarum* produced in Zhongning County of Ningxia, China has been included in the Chinese Pharmacopoeia (2020). Numerous studies have reported that the leaves of *Lycium barbarum* exhibit antioxidant, anti-inflammatory, immune-regulatory, antineoplastic, and hypoglycemic effects [6–10].

Fruitless *Lycium barbarum* leaves (FLBL) refer to the leaves obtained from a novel variety of fruitless *Lycium barbarum* trees in Ningxia. These trees were derived through clonal reproduction of wild *Lycium barbarum* species and Ningqi No. 1 species. According to the report tested by the Dairy Testing Center of the Ministry of Agriculture of China, FLBL exhibited significantly higher levels of nutrients, bioactive substances, and trace elements compared to LBLs. These included elevated concentrations of protein, flavonoids, polyphenols, catechins, vitamin E as well as essential minerals such as calcium (Ca^{2+}), magnesium, and zinc [11]. In different regions, variations in soil composition, climate conditions, and harvesting time lead to differences in the composition and bioactive content of the aforementioned substances, resulting in varying nutritional value and effects. However, current focus within the pharmaceutical, agricultural, and food industries primarily revolves around analyzing the main components, pharmacological mechanisms, regional disparities in these components' presence, cultivation practices, quality control standards as well as product research and development related to *Lycium barbarum* and *Lycium barbarum* leaves [11–16].

Our previous study revealed that Ningxia FLBL exhibits antioxidative effects and improves sleep disorders and functional constipation. However, there is a lack of research in several crucial aspects including cultivation techniques, analysis of main components, nutritional value assessment, pharmacological mechanisms, product development, and quality control standards. This knowledge gap significantly hampers the progress in planting, harvesting, further processing, research advancements, and practical applications of Ningxia FLBL. Therefore, this study aimed to establish HPLC fingerprints of FLBL and determine the contents of four phenolic acid bioactive substances (catechin, epicatechin,chlorogenic acid and rutin) at different harvesting periods. These findings will serve as a valuable reference for optimizing cultivation practices, harvesting strategies, and ensuring quality control while also contributing to the understanding of the pharmacological mechanisms underlying Ningxia FLBL.

2. Experimental materials and instruments

2.1. Main instruments

A high-performance liquid chromatograph (1260, Agilent, USA) equipped with a YMC-Triart C18 chromatographic column was used. Electronic scales (XPE26, Mettler Toledo, Switzerland) and an ultrasonic cleaning instrument (CQ-200B-DST, Shanghai Yuejin Medical Optical Instrument Factory) were employed.

2.2. Experimental materials

Reference samples, including chlorogenic acid (batch number: AF8112791), epicatechin (batch number: ABP16102905), catechin (batch number: ABP0117202), and rutin (batch number: 080–9002), were procured from Chengdu Elfa Technology Biological Co., LTD and China National Institute for the Control of Pharmaceutical and Biological Products. Primary reagents such as methanol and acetonitrile (chromatographic grade) were obtained from Fisher Chemical Company; methanol, absolute ethanol, trifluoroacetic acid were purchased from Tianjin Damao Chemical Reagent Factory; diammonium hydrogen phosphate was sourced from Shanghai Guanguo Chemical Technology Co., LTD.

Fig. 1. displays a photograph of fruitless *Lycium barbarum* leaves from Ningxia (provided by Ningxia Qiya Food Technology Co., LTD).

Tested samples: Ningxia FLBL, provided by Ningxia Qiya Food Technology Co., LTD, were collected from the planting base of Zhongning County, Ningxia, China at ten points with an interval of 10 m between adjacent collection points. Samples were collected twice per month from May to September and dried before use in this study (Fig. 1). Sample information is presented in "Table 1."

2.3. Experimental methods

2.3.1. Preparation of tested solution

FLBL samples were pulverized into powder, accurately weighed (0.5 g) after passing through a 60-mesh sieve, and then transferred to a 50-mL volumetric flask containing 50 mL of 90 % methanol. The mixture was vigorously shaken, allowed to stand at a fixed volume for 30 min, subjected to ultrasonication for another 30 min, and finally centrifuged at 10,000 rpm for 10 min (at a temperature of 4 °C). The resulting supernatant was used as the test solution with an appropriate amount.

2.3.2. Preparation of the reference solution

The appropriate amounts of chlorogenic acid, rutin, catechin, and epicatechin were accurately weighed and transferred into a 10-mL volumetric flask. Subsequently, a 90 % methanol solution was added to prepare mixed reference solutions with concentrations of 0.5108 mg/mL, 0.2368 mg/mL, 0.1764 mg/mL, and 0.15525 mg/mL respectively.

2.3.3. Chromatographic conditions and optimization of HPLC

To enhance sample separation and achieve stable HPLC chromatographic peaks, the chromatographic conditions for HPLC were initially optimized. Methanol and acetonitrile were employed as organic phases, while 0.1 % trifluoroacetic acid aqueous solution (TAAS) and diammonium hydrogen phosphate (DHP, pH 2.38) served as mobile phase A, respectively. The optimized mobile phase system was selected as the mobile phase for FLBL.

2.3.4. Chromatographic conditions

The chromatographic column used in this study was a YMC-Triart C18 (4.6 mm × 250 mm, 5 μm) column. After optimization, the mobile phase system selected for analysis consisted of a flow rate of 0.8 ml/min and a diode-array detector (DAD) operating at a column temperature of 30 °C with detection wavelength set at 240 nm. A sample loading amount of 10 μL was employed, and the gradient elution program is presented in Table 2.

2.4. Method

2.4.1. Examination of linear relationships

The mixed reference solution mentioned above was extracted and loaded in volumes of 1, 4, 6, 8, 10, 15, and 20 μl respectively based on the chromatographic conditions outlined in section "2.4.4″ for detection purposes. The quantity of the reference samples served as the independent variable (X), while the peak area (Y) was utilized as the dependent variable for conducting linear regression analysis.

2.4.2. Precision

The intra-day precision.

According to the chromatographic conditions described in section 2.4.4, the sample solution was subjected to six replicate analyses on the same day to determine the peak area values of catechin, chlorogenic acid, epicatechin, and rutin; RSD% was calculated.

2.5. The inter-day precision

According According to the chromatographic conditions described in section 2.4.4, the same sample solution was subjected to six replicate analyses on the following day to determine the peak area values of catechin, chlorogenic acid, epicatechin, and rutin; RSD% was calculated.

2.5.1. Stability

The same sample solution was collected, and the relative peak areas of catechin, chlorogenic acid, epicatechin, and rutin were

Table 1
Sample informations of FLBL.

number	samples name	picking date	number	samples name	picking date
S1	FLBL	5/15	S2	FLBL	5/30
S3	FLBL	6/15	S4	FLBL	6/28
S5	FLBL	7/15	S6	FLBL	7/31
S7	FLBL	8/15	S8	FLBL	8/31
S9	FLBL	9/8	S10	FLBL	9/15

Note: S1~S10 (Samples 1~Samples 10), FLBL:fruitless *Lycium barbarum* leaves, m/d (month/day).

Table 2
Protocol for gradient elution.

(time) min	Mobile phase A (%)	Mobile phase B (%)
0	98	2
5	88	12
18	70	30
43	52	48
45	50	50
49	98	2
50	98	2

determined at 0, 2, 4, 8, 12, and 24 h after preparation using the aforementioned chromatographic conditions described in section "2.4.4". Subsequently, the RSD% was calculated.

2.5.2. Repeatability

Six samples were collected on July 31 and weighed accurately. The tested solution was then prepared following the method described in section 2.4.1. Subsequently, a volume of 10 μL of the prepared solution was subjected to testing under the chromatographic conditions outlined in section 2.4.4 to determine and calculate the average concentrations of catechin, chlorogenic acid, epicatechin, and rutin."

2.5.3. Recovery rate test

For the recovery rate test, nine samples of FLBL powder with known content in "2.5.4″ were taken and grouped into three pieces, each weighing precisely 0.1 g. The reference solution was added to each group and the average recoveries of catechin, epicatechin, chlorogenic acid, and rutin were calculated."

2.5.4. Establishment and similarity evaluation of HPLC fingerprint of FLBL

The FLBL samples were analyzed in ten different picking times under optimized chromatographic conditions. Subsequently, the obtained HPLC fingerprints were imported into the Traditional Chinese Medicine fingerprint similarity evaluation software system (2012 version). The optimal time window width of 0.1 min was determined using the median method, and through multi-point correction, automatic matching of chromatographic peaks and generation of control charts were performed to evaluate the similarities of chromatographic peaks among the ten batches.

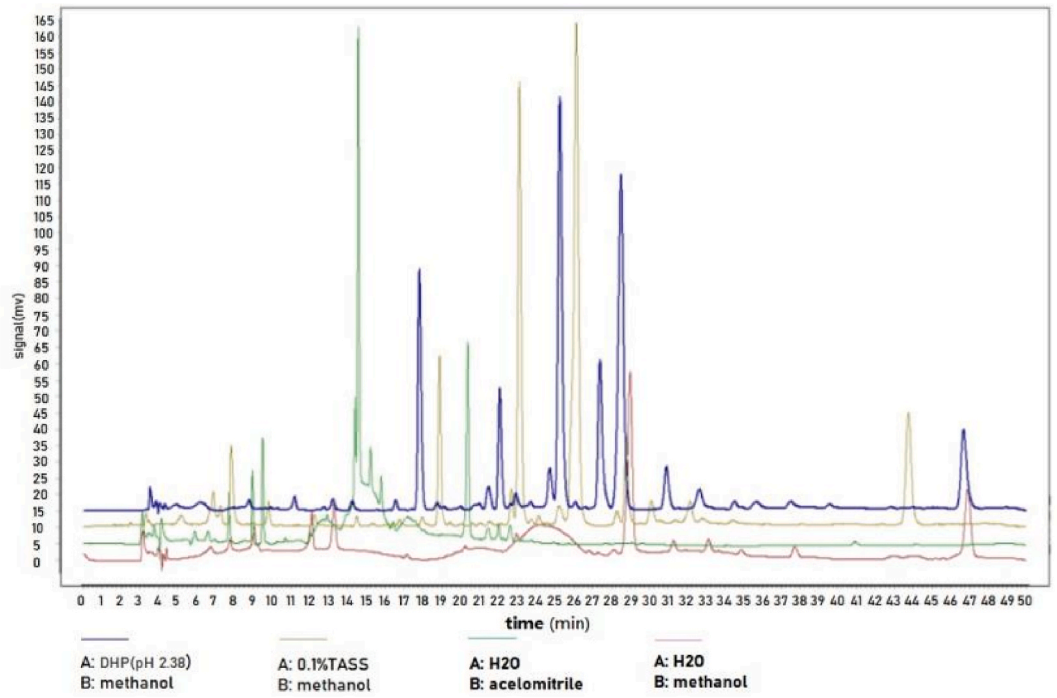

Fig. 2. Optimization graph of HPLC chromatographic conditions.

2.5.5. Statistical analysis

The analyses were performed in triplicate. The data was presented as mean ± SD, and the relative standard deviation (RSD in %) was calculated using Microsoft Excel (Microsoft Office 2013).

3. Results

3.1. Optimization of chromatographic conditions

The chromatographic conditions of HPLC were optimized, and the results showed that acetonitrile was selected as the organic phase instead of methanol. As a result, the chromatographic peaks shifted collectively and led to reduced separation between individual peaks. Consequently, methanol was ultimately chosen as the organic phase. When utilizing a 0.1 % trifluoroacetic acid aqueous solution (TAAS) as mobile phase A and methanol as mobile phase B, the chromatographic peaks in the HPLC map of the sample exhibited improved peak shape and stable baseline; however, there was inadequate separation between the main peaks." The utilization of diammonium hydrogen phosphate (DHP, pH 2.38) as mobile phase A and methanol as mobile phase B resulted in improved peak shape and separation for each chromatographic peak in the HPLC profile of FLBL samples, along with a stable baseline (Fig. 2). Consequently, this mobile phase system was selected for analyzing the HPLC fingerprint of Ningxia FLBL.

3.2. HPLC chromatogram of reference and FLBL samples

The HPLC chromatograms of both the reference solution and tested solution were obtained under the chromatographic conditions specified in Table 2, as depicted in Fig. 3. By comparing the chromatogram of a single reference product with that of four reference samples, it was possible to identify peaks 1, 2, 3, and 4 as catechin, chlorogenic acid, epicatechin, and rutin respectively in the mixed reference samples (Fig. 3A). Based on the elution positions and peak areas observed for these four chromatographic peaks in the reference products, we hypothesized that peaks No. 1–4 in FLBL sample corresponded to catechin, chlorogenic acid, epicatechin and rutin respectively (Fig. 3B). However due to interference from miscellaneous peaks present in FLBL samples, there was a slight backward shift observed for positions of peaks No. 1–4.

3.3. Methodology investigation

3.3.1. Linear relationship analysis

The results of the linear relationship analysis are presented in Table 3, demonstrating high linearity (R^2 values of 0.9999, 0.9999, 0.9998, and 0.9999) for catechin, epicatechin-catechin, chlorogenic acid, and rutin respectively within the detection range of the reference samples.

3.3.2. Precision experiment

3.3.2.1. Intraday precision. The intraday precision of the sample was assessed, and the peak area values for catechin, chlorogenic acid, epicatechin, and rutin were determined with corresponding RSDs calculated as 0.08 %, 0.06 %, 0.08 %, and 0.09 % respectively.

3.3.3. Interday precision

The interday precision of sample was tested, and the peak area values of catechin, chlorogenic acid, epicatechin and rutin were determined with corresponding RSDs calculated as 0.39 %, 1.24 %, 0.41 % and 0.40 % respectively.

3.3.4. Stability experiment

The stability experiment results of the samples demonstrated that the RSDs of catechin, chlorogenic acid, epicatechin, and rutin were 0.33 %, 0.79 %, 0.32 %, and 0.77 % respectively.

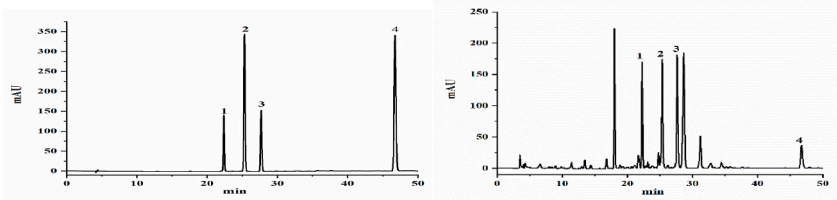

Fig. 3. HPLC chromatogram of FLBL sample and the mixed reference substance (1. catechin, 2. chlorogenic acid, 3. epicatechin, 4. rutin).

Table 3
Linear relationship of 4 types of phenolic acids.

reference solution	regression equation	R^2
catechin	Y = 1005.5X+0 .2447	0.9999
chlorogenic acid	Y = 1864.1X+2.8877	0.9999
epicatechin	Y = 1138.7X+7.4012	0.9998
rutin	Y = 1234.8X+0.9702	0.9999

3.3.5. Test of repeatability

The repeatability test yielded an average content of 16.08, 11.86, 21.80 and 5.78 mg/g for catechin, chlorogenic acid, epicatechin and rutin respectively; the RSD values for the relative peak areas of these four common peaks were found to be 2.91 %, 2.12 %, 2.50 % and 2.58 %.

3.3.6. Recovery rate test

The average recovery rates and RSD of catechin, epicatechin, chlorogenic acid and rutin in FLBL were calculated, the results were presented in Table 4.

3.4. Establishment of HPLC fingerprint and similarity evaluation of FLBL

The chromatograms of 10 batches of samples were recorded successively according to the optimized chromatographic conditions, and imported into the Traditional Chinese Medicine fingerprint similarity evaluation software system (2012 Edition), set the S1 map as the reference map, the median method was used to determine the optimal time window width of 0.1 min, by multi-point correction, chromatographic peaks were automatically matched and reference maps were generated. A total of 17 characteristic peaks were determined through chromatographic peak matching. By comparing reference substances and the full-wavelength scanning map of DAD, one of the components was identified as catechin (NO. 10). Peak 10 was designated as the reference peak for calculating the

Table 4
Recovery rates and RSDs of catechin, epicatechin, chlorogenic acid and rutin in FLBL (n = 9).

成分		amount of measurement/mg	background dose/mg	addition amount/mg	recovery rate/%	RSD%
catechin	high1	3.9750	1.6080	2.4120	98.13	
	high2	4.0110	1.6080	2.4120	99.63	
	high3	3.9940	1.6080	2.4120	98.92	0.75
	medium1	3.2110	1.6080	1.6080	99.69	
	medium2	3.1640	1.6080	1.6080	96.77	
	medium3	3.1720	1.6080	1.6080	97.26	1.60
	low1	2.3980	1.6080	0.8040	98.26	
	low2	2.3960	1.6080	0.8040	98.01	
	low3	2.4050	1.6080	0.8040	99.13	0.60
chlorogenic acid	high1	2.9950	1.1860	1.7790	101.69	
	high2	2.9980	1.1860	1.7790	101.85	
	high3	3.0220	1.1860	1.7790	103.20	0.81
	medium1	2.3435	1.1860	1.1860	97.60	
	medium2	2.3435	1.1860	1.1860	97.60	
	medium3	2.3120	1.1860	1.1860	94.94	1.59
	low1	1.8320	1.1860	0.5930	108.94	
	low2	1.7990	1.1860	0.5930	103.37	
	low3	1.8250	1.1860	0.5930	107.76	2.75
epicatechin	high1	5.2640	2.1800	3.2340	95.36	
	high2	5.2470	2.1800	3.2340	94.84	
	high3	5.2560	2.1800	3.2340	95.11	0.28
	medium1	4.2900	2.1800	2.1560	97.87	
	medium2	4.2600	2.1800	2.1560	96.47	
	medium3	4.2300	2.1800	2.1560	95.08	1.44
	low1	3.1180	2.1800	1.0900	86.06	
	low2	3.1000	2.1800	1.0900	84.40	
	low3	3.1140	2.1800	1.0900	85.69	1.02
rutin	high1	1.6756	0.5781	0.8905	123.25	
	high2	1.6390	0.5781	0.8905	119.14	
	high3	1.6640	0.5781	0.8905	121.94	1.73
	medium1	1.2430	0.5781	0.5781	115.01	
	medium2	1.2550	0.5781	0.5781	117.09	
	medium3	1.2050	0.5781	0.5781	108.44	3.98
	low1	0.9516	0.5781	0.2891	129.22	
	low2	0.9376	0.5781	0.2891	124.37	
	low3	0.9323	0.5781	0.2891	122.54	2.75

similarity among 10 batches of FLBL samples. The overlay map of feature maps and the results of similarity are presented in Fig. 4 and Table 5, respectively. Comparison with the reference sample revealed that peak No. 10 corresponded to catechin (22.8 min), peak No.12 to chlorogenic acid (25 min), peak No.13 to epicatechin (27.2 min), and peak No.17 to rutin (37.48 min).

3.5. Determination of the contents of four phenolic acid bioactive substances in 10 batches FLBL samples

The samples of Ningxia FLBL were weighed in 10 different picking times, with each sample weighing 0.5 g. The tested solutions were prepared according to the procedure described in section "2.4.1″, and the samples were analyzed using the chromatographic conditions mentioned in section "3.1". The contents of catechin, chlorogenic acid, epicatechin, and rutin were determined using the external standard method, and the results were presented in Fig. 5.

4. Discussion

The HPLC fingerprint technique is widely employed in Traditional Chinese Medicine research due to its rapidity, accuracy, and excellent repeatability [17]. When performing HPLC analysis, the appropriate selection of mobile phase can enhance the separation of individual chromatographic peaks in the sample and improve response values. Study demonstrated that an acidic system can prevent catechin oxidation in FLBL while also inhibiting residual silica hydroxyl group activity on the chromatographic column [11,17–19], thereby reducing tailing and improving peak shape as well as separation. In our study, after optimization, methanol was chosen as mobile phase B while diammonium hydrogen phosphate (pH 2.38) was selected as mobile phase A, This resulted in improved peak shape and enhanced separation for each chromatographic peak within the HPLC profile of FLBL samples with a stable baseline. Consequently, diammonium hydrogen phosphate (pH 2.38) was utilized as mobile phase A alongside methanol as mobile phase B for HPLC fingerprint analysis of FLBL samples.

The results of intraday precision, interday precision, stability, and repeatability were presented, all RSDs values were below 5 %, indicating a high degree of accuracy and precision in the methods employed within this study.

In our study, HPLC fingerprints of FLBL at different harvesting periods were determined, and the contents of catechin, epicatechin, rutin, and chlorogenic acid were quantified. Upon comparing the HPLC fingerprints of 10 batches of FLBL harvested at different time points, we identified 17 common peaks with similarities ranging from 0.71 to 0.976, indicating significant variations in the components of FLBL across different harvesting periods.

Therefore, it is essential to establish a unique profile of FLBL during various harvest periods and identify its active components and bioactive substances for the purposes of cultivation, harvesting, deep processing, and efficacy research. Additionally, our study

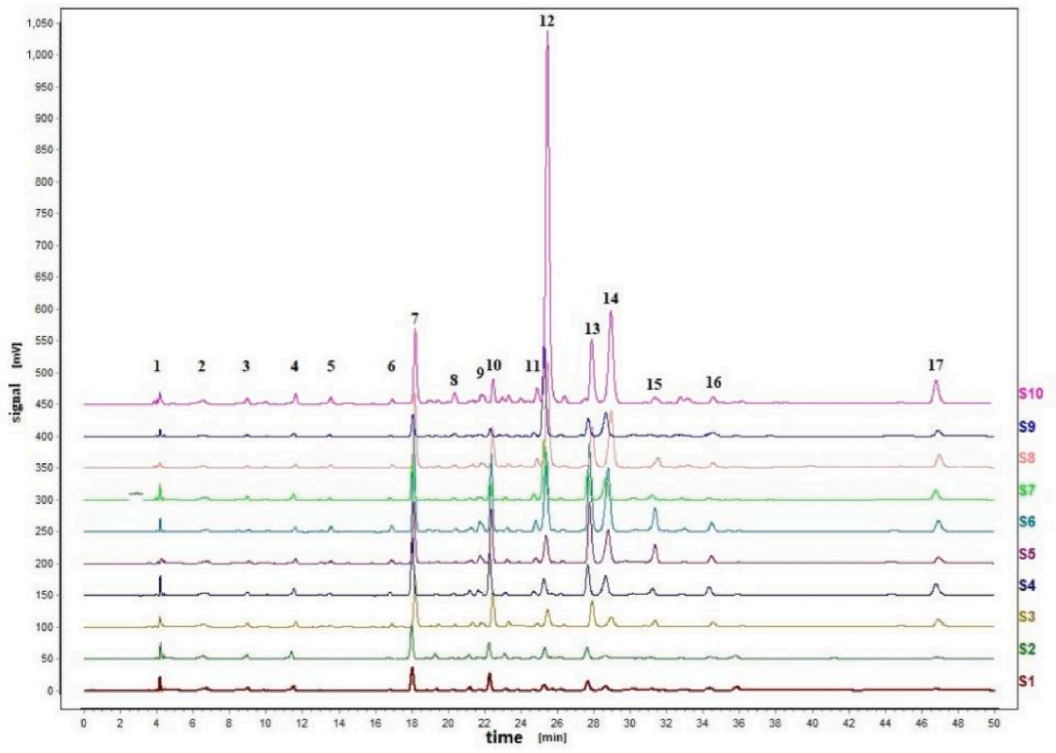

Fig. 4. HPLC fingerprints of 10 batches FLBL samples.

Table 5
Similarities of HPLC chromatogram of 10 batches FLBL samples.

picking time	5/3	5/15	6/15	6/28	7/15	7/31	8/15	8/31	9/8	9/15
similarities	0.71	0.72	0.758	0.8	0.813	0.901	0.976	0.958	0.928	0.908

Note: picking time: m/d.

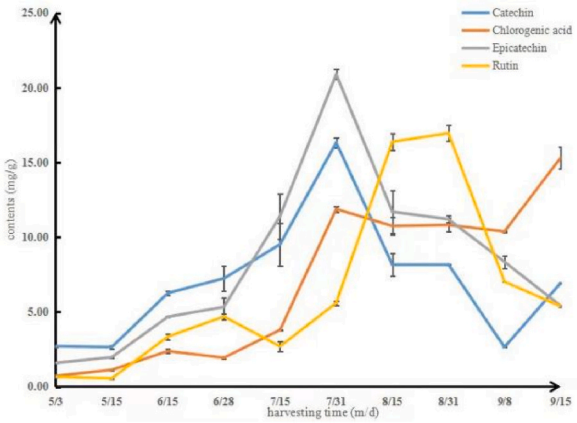

Fig. 5. The contents of 4 kinds of phenolic acid bioactive substances in 10 batcheses FLBL (n = 4).

identified substantial fluctuations in the concentrations of four phenolic acid bioactive compounds (catechin, epicatechin, rutin, and chlorogenic acid) across ten batcheses of FLBL harvested at distinct time points. The contents of catechin and epicatechin in FLBL exhibited a gradual increase from 2.71 mg/g and 1.5 mg/g in May to 16.33 mg/g and 20.9 mg/g, respectively, by late July, followed by a gradual decline after reaching their peak values, ultimately dropping to the lowest level of 2.63 mg/g and 5.41 mg/g by late September; meanwhile, the content of rutin increased gradually from 0.66 mg/g in May to its highest value of 16.96 mg/g in late August before decreasing to 5.38 mg/g by late September; on the other hand, chlorogenic acid content displayed a continuous increase throughout the harvesting period, rising from an initial value of 0.72 mg/g in May to reach its peak at 15.31 mg/g by late September. The analysis of phenolic acid bioactive substances across ten batches of FLBL revealed that catechin, epicatechin, and rutin contents were higher during June–August period while chlorogenic acid content was higher during July–September.

The Chinese pharmacopoeia specifies that Ningxia *Lycium barbarum* is the exclusive variety suitable for medicinal purposes, owing to the unique geographical conditions of Ningxia, particularly Zhongning County. Positioned at 105.67° east longitude and 37.48° north latitude, Zhongning County lies in the transitional zone between the Inner Mongolia Plateau and the Loess Plateau, in close proximity to the Tengeli Desert towards its westward direction. This region experiences a temperate continental monsoon climate with a northerly aspect, characterized by ample sunlight exposure, low precipitation levels, high rates of evaporation, and significant accumulated effective temperature.

The unique geographical environment and microclimate provide the most favorable natural conditions for the growth of *Lycium barbarum*. Numerous studies have been conducted on the impact of different regions, years, fertilizer efficiency, and other factors on the composition and effects of *Lycium barbarum* [19–25]. The results indicate that Ningxia Zhongning county's distinctive geographical position gives Chinese *Lycium barbarum* its own nutritional profile and efficacy compared to other countries and regions [12,24,25]. As a result, it has gained a reputation as "the world's best *Lycium barbarum* from China," "China's best *Lycium barbarum* from Ningxia," and "the world's best *Lycium barbarum* from Zhongning County." Fruitless *Lycium barbarum* tree is a new species in Ningxia that does not flower or bear fruit. Instead, all nutrients are stored in its stems and leaves. We refer to its leaves as Ningxia FLBL. Its various nutrients and bioactive substances surpass those found in Ningxia *Lycium barbarum* fruits and leaves; however, its nutritional value, medicinal properties, economic potential remain largely unexplored due to limited understanding usage, utilization, excavation and study. This greatly hinders its cultivation planting development product development. Kruczek [22] discovered that both the leaves and fruits of *Lycium barbarum* possess nutritional value, with the leaves exhibiting higher polyphenol contents compared to the fruits. The fruits are known for their health-promoting properties, while the leaves exhibit antibacterial characteristics. Wang [23] observed a significant negative correlation between altitude, relative humidity, light intensity, and most metabolites of *Lycium barbarum*. This suggests that high altitudes and strong light intensity are not conducive to optimal fruit quality in terms of nutritional content. Soil water content displayed a highly negative correlation with vitamins, organic acids, and carbohydrates but showed moderate positive correlations with other metabolites. Conversely, air and soil temperatures exhibited significant positive correlations with most metabolites. These findings indicate that conditions such as high soil moisture and air temperature, low altitude, low light intensity, and moderate soil moisture are favorable for producing *Lycium barbarum* fruits rich in nutrient metabolite content. Chen [24] reported that the soil in the Zhongning area exhibited poor quality; however, there was a high abundance of arbuscular mycorrhizal fungi (AMF) community.

Differences in geographical environments resulted in variations in AMF communities, which subsequently influenced the active components of *Lycium barbarum* fruits. The study conducted by Li [25] revealed that *Lycium barbarum* fruits sourced from inner Mongolia exhibited relatively higher levels of vitamins and naringin, while those from Xinjiang displayed elevated polysaccharide content. Additionally, the protein content was found to be higher in fruits obtained from Ningxia. Through analysis of the four chemical components and ecological environmental factors, it was found that chemical components with higher percentage contents were more affected by climate factors, while those with lower contents were more influenced by soil factors. Therefore, the quality of *Lycium barbarum* from different regions should be evaluated comprehensively using multiple indicators. Liu [26] employed HPLC to analyze the phenolic constituents, antioxidant properties, flavonoids, and phenolic acids in the leaves and stems of three *Lycium barbarum* varieties collected at different time points; the levels of total phenols, total flavonoids, and condensed tannins in both leaves and stems were found to be higher during months with elevated temperatures. Furthermore, a significant correlation was observed between antioxidant activity and the content of flavonoids and phenolics." The study conducted by Wei [27] revealed that the fertilization level exerted a significant influence on both the yield and nutrient content of *Lycium barbarum*. Specifically, it was observed that higher phosphorus levels were associated with lower yields of *Lycium barbarum* fruits. Furthermore, there were significant correlations between the contents of amino acids, flavonoids, polysaccharides, betaine and fertilization levels. Notably, the impact of P fertilizer level primarily affected the biosynthesis of flavonoids.

The components of FLBL were tested by the Dairy Testing Center, Ministry of Agriculture of China, commissioned by Ningxia Wolfberry Sprout Food Technology Co., Ltd. The results revealed that per 100 g, FLBL contained the following substances: protein (44 g), crude fiber (10.22 g), calories (1466.2 kJ), thiamine (0.35 mg), carotene (683.2 μg), catechin (7064 mg), flavonoid (2491 mg), niacin (33.12 mg), folic acid (1.15 mg), riboflavin (0.574 mg), ascorbic acid (34.8 mg). Additionally, it contained total sugar content of 4.71 g and high levels of calcium (2030 mg), P (717 mg), Fe (36 mg), Zn (12.4 mg) and Se (14 mg). These contents were significantly higher compared to *Lycium barbarum* fruits and leaves [28–32]. Moreover, FLBL not only serves as a nutritional supplement but also promotes health due to its excellent source of functional raw materials with broad application prospects in food, nutraceuticals, and healthcare.

However, insufficient attention has been given to its nutritional value, pharmacological effects, quality control standards, and other related studies, which significantly impede the cultivation, product development, promotion, and economic income of farmers.

5. Conclusion

Although numerous studies have been conducted on the efficacy and application of *Lycium barbarum* and *Lycium barbarum* leaves, our study is the first to report the establishment of a fingerprint and determination of the contents of four phenolic acids in Ningxia FLBL at different picking periods. In this study, we successfully established HPLC fingerprints for Ningxia FLBL harvested at various time points, revealing 17 common peaks. Additionally, significant differences were observed in the levels of catechin, epicatechin, rutin, and chlorogenic acid. These findings not only serve as a foundation for quality control and cultivation practices but also provide valuable insights for developing and producing Ningxia FLBL as an important industrial crop with applications in tea production, vegetable farming, food processing, and traditional Chinese medicine. Furthermore, we hope that our research will raise awareness about Ningxia FLBL among a wider audience while exploring the potential value of its by-products and associated economic advantages.

Funding statement

This work was supported by R & D Plan Project of Ningxia Autonomous Region of China (Grant No. 2019BEG03047).

Data availability statement

All data can be available.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Lianxiang Zhang: Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Yanting Li:** Software, Methodology. **Qin Yan:** Resources. **Yu Ning:** Methodology. **Yanping Wang:** Methodology. **Kunmei Liu:** Resources. **Yuanyuan Qiang:** Methodology. **Xueqing Ma:** Supervision, Methodology. **Xiangping Sun:** Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

All the authors declare no competing interests.

Abbreviations

FLBL	fruitless Lycium barbarum leaves
HPLC	high performance liquid chromatography
DAD	diode-array detector
RSD	relative standard deviation

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