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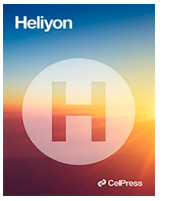

Research article

Comprehensive evaluation of chemical constituents and antioxidant activity between crude and processed Polygalae radix based on UPLC-Q-TOF-MS/MS combined with multivariate statistical analysis

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ABSTRACT

Polygalae radix (PR) is a famous herbal medicine obtained by drying the root of *Polygala tenuifolia* Willd., one of the traditional Chinese medicines (TCM) that can be used for healthy food. There are three main processed methods of PR, including removing the xylem of roots (Polygalae Cortex, PC), frying PC with licorice (LP), and frying PC with honey (HP). While processing is believed to enhance efficacy and reduce toxicity, it is crucial to understand the differences in chemical composition and biological activities between crude and processed PR. This study used ultra-performance liquid chromatography with quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS/MS) combined with multivariate statistical analysis to analyze the chemical profiles and differences between the crude and processed products. Total polyphenol contents (TPC), total flavonoid contents (TFC), total saponin contents (TSC) and antioxidant activity of the processed and crude PR were also investigated. A total of 131 chemical compounds, including 42 saponins, 44 oligosaccharide esters, 25 xanthones, 2 organic acids, 3 Carbohydrates, and 15 components detected in auxiliary materials, were detected in all samples. Notably, PC exhibited significant changes among the three processed products, with the content of 62 compounds being higher. Processing of licorice (LP) and honey (HP) decreased the content of several compounds due to temperature and moisture influences. Comprehensive comparison of the antioxidant capacity of crude and processed PR showed that the antioxidant capacity of PC was higher than that of PR, HP, and LP. Our results can provide a scientific basis for further developing and applying *P. tenuifolia* resources.

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1. Introduction

Traditional Chinese herbs are derived from nature, and the majority of them should be processed before being used in clinical settings. In China, a specialized technique for traditional medicinal herbs known as "Pao-Zhi" has shown good capacities for boosting efficacy or minimizing the toxicity of raw materials. Various technologies have been frequently used in "Pao-Zhi", including stir-heating, steaming, boiling, and roasting. Meanwhile, special auxiliary materials have been frequently employed in the processing, such as licorice juice [1], honey [2], and saltwater [3]. The processing of raw materials is the essential technological assurance in this unique processing procedure, representing a qualitative leap in the efficacy of traditional Chinese medicines (TCM). Previous research has demonstrated that processing alters the chemical compositions of herbs, resulting in an increase in active constituents or a decrease in stimulating constituents, thereby enhancing pharmacological activities and reducing side effects. Related research pointed out that the processing with licorice has been shown to significantly reduce the acute toxicity of *Tetradium ruticarpum* fruits while improving analgesic and anti-inflammatory properties [4]. Similarly, studies explored the impact of honey processing on the chemical composition of medicinal herbs and suggested that after frying with honey, the chemical composition of crude licorice changed dramatically, and the anti-acute inflammatory effect, immunomodulatory activity, and hepatoprotective effect of licorice were also significantly improved [5].

Polygala tenuifolia Willd., is a famous medicinal herb distributed throughout East Asia, mainly in China, Japan, and Korea. Polygalae radix (PR), commonly known as "Yuanzhi" in China and "Onji" in Japan is obtained by drying the roots or root cortex of *P. tenuifolia* [6]. In China, *P. tenuifolia* is also included in the health food list, designated as one of the 114 traditional Chinese medicines suitable for use in health food according to the National Health Commission. It is renowned for its tranquilizing and enlightening effects and its ability to dispel phlegm and reduce swelling. Traditional medical theories suggest that the xylem of PR contains toxic components responsible for gastrointestinal tract toxicity [7]. Thus, in regions where PR is produced, the initial processing step typically involves the removal of the xylem, resulting in the market circulation being dominated by the Polygala cortex (PC). Meanwhile, PC and its processed products but not PR are mainly used in clinical applications and producing proprietary Chinese medicines. Processing PR by removing the xylem can alleviate gastrointestinal motility disorders and enhance neuroprotective effects. The main processing methods of PR include PC, frying PC with licorice (LP), and frying PC with honey (HP) [8].

Up to now, more than 150 compounds have been isolated from PR, mainly including saponins, xanthones oligosaccharide esters, and so on. Among them, saponins are recorded as the main active constituents of PR, exhibit structures typical of oleanolic pentacyclic triterpenoid saponins, exert the functions of sedation, relieving excitement, expelling phlegm, relieving cough, and so on [9]. Xanthones, a characteristic constituent of the Polygala genus, comprise approximately 30 compounds found in PR and possess pharmacological activities such as antidepressant effects [10]. Oligosaccharide esters, present in the roots, stems, and leaves of *P. tenuifolia*, exhibit significant biological activities related to brain protection, anti-dementia, and anti-depression, making them potential candidates for the development of new anti-aging and neuroprotective drugs [11]. Although numerous chemical and pharmacological studies have been conducted for PR, the difference in chemical compounds and pharmacological activities between the root and root cortex of *P. tenuifolia* is still unclear.

In recent years, several quantitative and qualitative methods have been employed to analyze secondary metabolites in PR, including high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), direct infusion-tandem mass spectrometry (DI-MS/MSALL®), ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), gas chromatography-mass spectrometry (GC-MS) and ultra-performance liquid chromatography quadrupole/time of flight mass spectrometry (UPLC-Q-TOF-MS/MS). Among them, UPLC-Q-TOF-MS/MS is a powerful analytical tool for studying chemical composition due to its high sensitivity, resolution, accuracy, and wide scanning range [12]. However, due to the limited information available in the literature on PC and its processing products, this study aimed to identify and compare their chemical composition by UPLC-Q-TOF-MS/MS. Additionally, in vitro biological effects (total phenolic content, total flavonoid content, and total saponin content) and antioxidant capacity (ABTS, FRAP, CUPRAC, and DPPH) were assessed. These findings will help in the assessment of plant-based resources as prospective new raw materials for application in a variety of commercial industries such as food, cosmetics, and pharmaceuticals, as well as give basic data and a scientific foundation for the processing development of *P. tenuifolia*.

2. Materials and methods

2.1. Plant materials and preparation of extracts

P. tenuifolia was harvested from the standardized cultivation base of Polygalae in Yulin City, Shaanxi Province. Licorice was purchased from Sichuan Xinhehua Decoction Pieces Co., Ltd (Sichuan, China). Honey was purchased from Guanshengyuan Bee Products Co. Ltd (Shanghai, China). All processing is carried out in the laboratory.

According to the classical records of processing Polygala in China, four groups were selected, which were PR, PC, HP, and LP. The detailed processes were as follows: remove the root and sand, ash and other impurities, clean with sterile water, cut it into small sections, and finally dry it to get PR. Take the PR to the skin slightly wrinkled, rub with the hand to remove the wood core, and dry to obtain the PC. 40 g of honey were mixed with 200 g of PC at room temperature for 2 h with the required amount of water, fried at 180 °C for 1 h, then spread evenly and keep the drying temperature at 40 °C. After taking it out, let it cool to obtain frying PC with honey (HP). 6 g of licorice was added to 1 L of water steeped for 30 min, and decocted 3 times for 45 min/time over moderate heat, The juice was extracted from the residue to obtain licorice juice. After that, take out 100 g of the PC and decoct it with licorice juice until it dries, then spread it evenly and keep the drying temperature at 40 °C. After taking it out, let it cool to obtain LP.

The powder was taken from the 4 kinds of samples, passed through a No. 5 sieve, and 0.200 0 g was placed in a 10 mL EP tube, 10 mL of 70% methanol was added precisely, sonicated for 45 min, filtered through a 0.22 μM microporous membrane, and placed in a 1.5 mL liquid phase vial. Repeat 5 times, eventually, these different samples were obtained for UPLC-Q-TOF-MS/MS analysis.

2.2. Liquid chromatography

Waters ACQUITYUP-LCBEHC18 (100 mm × 4.6 mm, 1.7 μm), volume flow rate 0.3 mL/min, column temperature 40 °C, injection volume 2 μL, gradient elution program time (min) 0.1% formic acid (A) acetonitrile (B) - gradient elution (0–20 min 92% B, 20–40 min 70%–68% B, 40–45 min 68%–50% B, 45–50 min 50%–0% B, 50–51 min 0%–95% B, 51–55 min 95% B).

2.3. Liquid chromatography coupled with mass spectrometry

The ion source is an electrospray ion source (ESI) in negative ion mode; the scanning range is the mass-to-charge ratio (m/z) 50 to 2000. The drying gas and cone gas flow rates are 600 and 50 L/h, respectively; desolvation gas and ion source temperatures are 250 °C and 100 °C, capillary and cone hole voltage is 2.5 kV and 40 V.

2.4. Estimation of antioxidant activity

2.4.1. Total phenolics, total flavonoids, total saponins

The determination of TPC was performed based on [13] with appropriate adjustments. The total phenolic content was determined by the Folin-Ciocalteu method. Gallic acid was used as the standard. The absorbance value was detected at 765 nm using a microplate reader (A-5082, Tecan Austria Gmbh Untersbergetr), and the magnitude of the absorbance value was proportional to the content. The TPC was expressed as Gallic acid equivalents (GE)/g DW.

The TFC was determined using the aluminum nitrate colorimetric method of [14] with appropriate adjustments. The absorbance of the mixture was measured at 420 nm, and the content of total flavonoids was calculated using Rutin as the standard, and the TFC was expressed as Rutin equivalents (RE)/g DW.

Based on the determination of total saponins content [15]. The sample to be tested was precisely absorbed 200 μL, and then 200 μL of 5 % vanillin-glacial acetic acid solution and 800 μL of perchloric acid were added to the sample. The sample was bathed in 60 °C water for 15 min, removed, cooled for 5 min, diluted with 5 mL of glacial acetic acid, shaken, stood for 15 min, and the absorbance was measured at 576 nm. The content of total saponins was calculated using Tenuifolin as the standard, and the TSC was expressed as Tenuifolin equivalents (TE)/g DW.

2.4.2. Determination of antioxidant activity in vitro

The ABTS free radical scavenging activity (ABTS), cupric reducing antioxidant capacity (CUPRAC) [16], and Ferric ion-reducing antioxidant power of 2,20-amino-bis (3-ethylbenzothiazoline) were performed according to (Ji et al., 2023), with appropriate adjustments.

DPPH was measured as the method described by (Yu. et al., 2023) [3], with slight modifications. 10 μL of plant extracts were added to the well and mixed 290 μL of DPPH was. The hen plate was incubated the in dark for 20 min. Absorbance was measured at 517 nm. The free radical scavenging activity of each fraction was determined by comparing its absorbance with that of a blank solution (DPPH without sample). Ascorbic acid was used as the positive control. The ability to scavenge the DPPH radical was expressed as percentage inhibition. Optical Density (OD) is a commonly used expression of absorbance, the absorbance is often converted to OD values to conveniently represent the concentration or concentration change of a substance.

$$\text{DPPH scavenging activity}(\%) = [(\text{OD1-OD2}) / \text{OD1}] \times 100$$

Where, OD1 = Optical density of control, OD2 = Optical density of test samples.

2.5. Statistical analysis

All experiments used a completely randomized design, for each experiment, the samples were analyzed with 3 repetitions. Means differing significantly were compared using the Duncans' multiple range test (DMRT) at the $P \leq 0.05$ probability level. A biological activities dataset was adjusted, Pearson correlation coefficient and heatmap between antioxidant activity and the sample's phenolic and flavonoid levels were analyzed using SPSS. Finally, SIMCA 14.1 was used for multivariate statistical analysis such as PCA, PLS-DA, and HCA. SPSS software (version 25.0, USA) was used for statistical analysis and Original 2021 software (version 9.8, USA) was used for graphing.

3. Results and discussion

3.1. Identification of chemical constituents by UPLC-Q-TOF-MS/MS

UPLC-Q-TOF-MS/MS is a powerful analytical technology with excellent resolution and high separation efficiency, which was

Table 1
Identification of components in crude and processed *Polygalae radix* and Change indexes of ion peak area before and after processing.

Peak No.	tR (min)	Identified Compounds	Class	PR vs. PC	PC vs. HP	PC vs. LP	HP vs. LP
1	0.457	Sucrose	Carbohydrate	0.44**	1.06*	1.36***	1.28***
2	0.651	Hydroxy-dihydromangiferin	Xanthone	2.04**	0.65**	0.68***	1.05
3	1.242	Sibiricose A3	Oligosaccharide ester	0.44*	1.04	1.35**	1.30*
4	1.441	Polygalasaponin XIV or Isomer	Saponin	3.34***	0.52***	0.80*	1.54**
5	2.250	Ferulic acid	Organic acids	0.12***	6.27***	7.45***	1.19*
6	2.253	Sibiricose A5	Oligosaccharide ester	3.00***	0.36***	0.61**	1.70**
7	2.372	Unknown	Oligosaccharide ester	14.33***	1.00**	/	/
8	2.566	Sibiricose A6	Oligosaccharide ester	0.31***	0.67**	0.86	1.28*
9	2.732	Tenuifiolin	Saponin	1.73***	10.32***	3.49**	0.34***
10	3.086	1,5-Dihydroxy-2,3-dimethoxyxanthone/1,6-Dihydroxy-3,7-Dimethoxyxanthone/3-Hydroxy-2,8-dimethoxyxanthone	Xanthone	2.06**	0.81*	0.77***	0.95
11	3.093	1,7-Dihydroxy-2,3-dimethoxyxanthone	Xanthone	1.51**	5.08**	17.02***	3.35**
12	3.434	6,8-Dihydroxy-1,2,4-trimethoxyxanthone	Xanthone	1.19**	/	0.75**	/
13	3.520	Sibiricose A2	Oligosaccharide ester	✓	/	/	/
14	3.904	2-(3,4-Dimethoxyphenyl)-3,5,7-Trihydroxychroman-4-one	Xanthone	1.84***	4.30**	0.63***	0.15**
15	3.952	6-Hydroxy-1,2,3,7-tetramethoxyxanthone	Xanthone	11.79***	0.08***	0.08***	1.02
16	4.353	1,3-Dihydroxyl-2-methoxanthone/1,7-Dihydroxy-3-methoxy Xanthone	Xanthone	1.11	0.80**	0.73**	0.90*
17	4.36	Lancerin	Xanthone	1.03	0.86	0.9	1.06
18	4.367	Wubangziside C	Xanthone	2.35***	0.52***	0.61**	1.19
19	5.179	Polygalaxanthone III	Xanthone	1.02	1.07	1.29*	1.21
20	5.257	Sibiricaxanthone A	Xanthone	1.19	0.31**	0.53*	1.69*
21	5.278	Sibiricaxanthone B	Xanthone	0.84	0.35**	0.48**	1.38**
22	6.005	7-O-Methylmangiferin	Xanthone	2.77***	2.17**	0.94	0.44*
23	6.026	Maltotriose	Carbohydrate	5.05***	0.14***	0.23***	1.59**
24	6.467	Polygalasapion XXIV	Saponin	13.28***	0.61***	0.84*	1.39*
25	7.393	Tenuifoliside B	Oligosaccharide ester	0.36**	0.95	0.87	0.92
26	7.592	Sibiricaxanthone F	Xanthone	2.94***	0.48***	0.54***	1.13
27	7.919	Onjixanthone II	Xanthone	1.41***	0.73***	0.86**	1.18*
28	7.919	1,6,7-Trihydroxoy-2,3-dimethoxyxanthone	Xanthone	6.67***	/	/	/
29	7.969	1,3,7-Trihydroxy-2,6-dimethoxyxanthone	Xanthone	1.18*	0.89***	1.07	1.20*
30	8.532	Reiniose F	Oligosaccharide ester	1.34**	0.67**	0.79**	1.19*
31	8.763	Glomeratose B	Oligosaccharide ester	1.54***	/	/	/
32	8.763	3-(3,4-Dimethoxyphenylpropionyl) fructose (2 → 1) 6-(4-methoxycinnamyl) glucose	Oligosaccharide ester	1.14	0.68**	0.79***	1.16*
33	9.121	Polygalaxanthone V	Xanthone	1.27***	0.60***	0.78**	1.31**
34	9.363	Sibiricose A4	Oligosaccharide ester	2.55***	0.63***	0.92	1.48**
35	9.690	Polygalaxanthone VII	Xanthone	1.40**	0.71***	0.81**	1.14**
36	10.442	Sibiricose A1	Oligosaccharide ester	3.42***	0.46***	0.52**	1.14
37	10.557	Arillanin A	Oligosaccharide ester	2.82*	0.23	0.18**	0.79*
38	10.735	3′-Sinapoyl-6-benzoylsucrose	Oligosaccharide ester	0.91	0.68**	0.67***	0.97
39	11.674	Reiniose B	Oligosaccharide ester	1.63***	0.64**	0.59***	0.92
40	11.814	Reiniose C	Oligosaccharide ester	0.17***	0.26***	0.39***	1.50**
41	11.900	Tenuifoliside A	Oligosaccharide ester	1.44	1.08	0.50**	0.47**
42	12.607	Reinioside F	Saponin	3.37**	0.00***	0.48**	/
43	13.048	Tenuifoliose M	Oligosaccharide ester	1.34***	0.37***	0.25***	0.70**
44	13.063	Tenuifoliose Q	Oligosaccharide ester	✓	/	/	/
45	13.098	Tenuifoliose G	Oligosaccharide ester	11.06*	0.39*	0.9	2.29*

(*continued on next page*)

Table 1 (*continued*)

Peak No.	tR (min)	Identified Compounds	Class	PR vs. PC	PC vs. HP	PC vs. LP	HP vs. LP
46	13.148	Tenuifoliose D	Oligosaccharide ester	✓	/	/	/
47	14.102	Tenuifoliose S	Oligosaccharide ester	1.57**	0.9	0.60**	0.67
48	14.308	Tenuifolioside C	Oligosaccharide ester	2.02***	0.54**	0.91*	1.67**
49	14.593	Tricornose K	Oligosaccharide ester	0.21**	1.72**	0.86**	0.50**
50	14.688	Onjisaponin H	Saponin	1.38	0.80*	0.88**	1.11
51	14.956	Polygalasaponin XIX	Saponin	1.77***	0.00***	0.78***	–
52	14.992	Tenuifoliose F	Oligosaccharide ester	0.98	0.74	0.92	1.24*
53	15.065	Tenuifoliose L	Oligosaccharide ester	1.85**	0.54**	0.94**	1.75**
54	15.086	Tenuifoliose H	Oligosaccharide ester	3.52***	0.35***	0.72**	2.09**
55	15.321	Tenuifoliose K	Oligosaccharide ester	0.48***	0.54***	0.80**	1.49***
56	15.463	Myrtifolioside A1	Oligosaccharide ester	✓	/	/	/
57	15.67	Senegasaponin C	Saponin	6.84***	/	0.91	/
58	15.748	Tenuifoliose C/E	Oligosaccharide ester	✓	/	/	/
59	16.069	Telephiose C	Oligosaccharide ester	0.62*	0.85*	0.84	0.99
60	16.145	Unknown	Saponin	4.19***	0.63***	0.75**	1.18
61	16.218	Tenuifoliose P、senegose B/C	Oligosaccharide ester	0.19**	0.86*	0.86***	0.99
62	16.467	Tenuifoliose J	Oligosaccharide ester	0.07**	0.68**	0.78**	1.14*
63	16.834	Arilloside D	Saponin	2.64***	1.27	0.35***	0.27**
64	16.841	Desacylsenegin III	Saponin	✓	/	/	/
65	16.855	Polygalasaponin XXIX	Saponin	✓	/	/	/
66	16.948	Tenuifoliose B/D	Oligosaccharide ester	✓	/	/	–
67	17.84	Reiniose A	Oligosaccharide ester	4.53***	0.69	0.23***	0.34
68	18.048	Tenuifoliose O	Oligosaccharide ester	✓	/	/	/
69	18.372	Glomeratose F	Oligosaccharide ester	1.38**	0.89*	1.02	1.14
70	18.422	Onjisaponin TG	Saponin	1.88**	0.58**	0.86***	1.49***
71	18.505	Unknown	Carbohydrate	2.01**	/	/	/
72	18.857	Onjisaponion TE	Oligosaccharide ester	5.93***	0.71***	0.97	1.38**
73	19.103	Tenuifoliose A/A2	Oligosaccharide ester	6.64***	0.86**	0.78***	1.35*
74	19.331	Tenuifoliose W	Oligosaccharide ester	16.40***	1.05	0.75**	0.71*
75	19.374	Onjisaponin Y	Saponin	1.03	0.31**	0.53**	1.71**
76	19.495	Tenuifoliose N	Oligosaccharide ester	3.30**	0.27**	0.43*	1.59**
77	20.185	Tenuifoliose I	Oligosaccharide ester	0.09**	0.63***	0.81**	1.28**
78	20.382	3,4,5- Trimethoxycinnamic acid	Organic acid	1.44***	1.04*	1.38***	1.32***
79	22.698	Onjisaponin F	Saponin	1.59***	0.92*	1.01	1.09
80	23.98	1,3-Dihydroxy-2,4,7-trimethoxyxanthone	Xanthone	0.09***	0.56***	0.84*	1.50**
81	24.215	1,6-Dihydroxy-3,5,7-trimethoxyxanthone	Xanthone	1.94 ***	0.55***	0.81**	1.47***
82	24.315	1,8-Dihydroxy-2,3,6-trimethoxyxanthone	Xanthone	1.25**	/	/	/
83	24.443	Unknown	Oligosaccharide ester	1.09	0.67 **	0.86***	1.28*
84	24.585	3,6-Dihydroxy-1,2,7-trimethoxyxanthone	Xanthone	0.40***	/	/	/
85	25.083	6,8-Dihydroxy-1,2,3-trimethoxyxanthone	Xanthone	✓	/	/	/
86	30.394	Onjisaponin V	Saponin	1.53*	10.55***	0.90*	0.09***
87	30.65	Onjisaponin Vg	Saponin	1.23	0.21*	0.81*	0.95
88	31.448	Arilloside B	Saponin	✓	/	/	/
89	31.462	Arillatanoside A	Saponin	✓	/	/	/
90	31.483	Polygalasaponin XXII	Saponin	✓	/	/	/

(*continued on next page*)

Table 1 (*continued*)

Peak No.	tR (min)	Identified Compounds	Class	PR vs. PC	PC vs. HP	PC vs. LP	HP vs. LP
91	32.266	Onjisaponin TH	Saponin	0.40***	0.89	0.94	1.06
92	32.401	SeneginII/onjisaponin G	Saponin	0.34**	1.04	1.04	1
93	32.665	Onjisaponin S	Saponin	2.29***	0.75**	0.89*	1.19*
94	33.250	Unknown	Saponin	0.26***	0.25**	0.44**	1.79**
95	34.267	Onjisaponin T	Saponin	4.08***	0.64***	0.59***	0.92
96	34.362	Polygalasaponin XLV	Saponin	22.27***	1.01	0.74*	0.73**
97	34.786	Onjisaponin Gg/K	Saponin	1.52***	0.68**	0.73*	1.08
98	34.822	Onjisaponin K/Gg	Saponin	0.43**	0.65**	0.77***	1.19*
99	38.441	Onjisaponin R	Saponin	0.06***	1.59*	3.05**	1.91**
100	38.915	Senegin IV	Saponin	0.77**	0.9	0.94	1.04
101	39.086	Onjisaponin A	Saponin	0.86	0.8	0.89	1.1
102	41.322	Onjisaponin B	Saponin	0.59*	0.81*	0.99	1.22*
103	43.291	Polygalasaponin XXXII	Saponin	1.17	0.85	0.94*	1.11
104	43.366	Tenuifoside A	Oligosaccharide ester	0.28***	2.79*	0.74	0.26*
105	43.431	Onjisaponin Ng	Saponin	28.61***	0.80**	0.91**	1.14*
106	43.532	Onjisaponin J	Saponin	35.12***	0.76**	0.86**	1.13
107	43.810	Onjisaponin Wg	Saponin	9.58***	1.07	1.17**	1.09
108	43.996	Onjisaponin W	Saponin	0.08***	11.55***	0.87**	0.08***
109	44.046	Onjisaponin Fg	Saponin	34.10***	0.91	1.06	1.17*
110	44.360	Onjisaponin Sg	Saponin	22.40***	0.17***	0.45**	2.61**
111	45.045	Unknown	Saponin	3.28***	0.84	0.87	1.03
112	46.809	Polygalaxanthone IV	Xanthone	0.68*	0.88*	0.99	1.12
113	52.513	Tenuifoliose X	Oligosaccharide ester	3.59**	0.6*	0.60**	0.91
114	52.899	Senegose D	Oligosaccharide ester	1.31**	4.37**	0.84**	0.19**
115	53.453	Senegasaponin A	Saponin	✓	/	/	/
116	54.001	Unknown	Saponin	✓	/	/	/
117	2.096	Diosmetin	Other compounds	/	/	✓	/
118	1.858	Sucrose	Other compounds	/	/	✓	/
119	2.740	Abscisic acid	Other compounds	/	/	✓	/
120	4.356	Luteolin	Other compounds	/	/	✓	/
121	5.473	Cinnamic acid	Other compounds	/	/	✓	/
122	6.783	4-Hydroxybenzoic acid	Other compounds	/	/	✓	/
123	4.423	Dihydrokaempferol	Other compounds	/	/	✓	/
124	13.844	D-(−)-glutamine	Other compounds	/	/	✓	/
125	6.430	Liquiritin	Other compounds	/	/	/	✓
126	6.444	Liquiritin apioside	Other compounds	/	/	/	✓
127	12.701	Isoliquiritin	Other compounds	/	/	/	✓
128	11.555	Liquiritigenin	Other compounds	/	/	/	✓
129	23.088	Licoricesaponin G2	Other compounds	/	/	/	✓
130	2.096	Licoflavono	Other compounds	/	/	/	✓
131	1.858	Licoisoflavone B	Other compounds	/	/	/	✓

(**Note:** Detailed information is provided in Table 1 of the supplementary. The peak area change index was calculated as the mean value of the peak areas of the 5 samples.
"√" means only detected in PR or auxiliary materials. "/" indicates that only one or none is detected and cannot be calculated. The change index >1 indicated that the content of the component increased after processing, the change index <1 indicated that the content of the component decreased after processing, and the change index = 1 indicated that the content of the component did not change after processing. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

successfully used for qualitative and relative quantitative analysis of the chemical constituents of medicinal plants [17]. In the present study, the PR and processed products were identified, by the mass spectrometric information such as retention time, mass-to-charge ratio, and secondary fragmentation ions as well as cleavage pattern of the compounds and combined with the relevant literature data. The contents of crude and processed PR were compared by peak area change index. A total of 131 compounds were identified or characterized in this study (Table 1), the total ion chromatogram is shown in Fig. 1.

The Venn diagram is a useful, powerful, and versatile tool that can quickly analyze a large set of data and convert it into simple and digestible information. It can visually observe the number of compounds in different processed products of *P. tenuifolia*. The Venn diagram (Fig. 2A) represents the number of compounds in samples. It shows that PC has a greater variety (12.98) of unique compounds (17) as compared to other samples, in which only 8 (6.11%) and 7 (5.34%) unique compounds were identified as HP and LP, respectively. A total of 90 (68.70%) compounds overlapped in all four samples, further depicting a similar type of compound in the four samples. The changes in the chemical composition of the samples were mainly attributed to the differences in processing methods, honey and licorice can introduce new chemical components. 131 compounds were detected by UPLC-Q-TOF-MS/MS. These consisted of 41 (31.30%) saponins, 45 (34.35%) oligosaccharide esters, 25 (19.08%) xanthones, 2 (1.53%) organic acids, 3 (2.29%) carbohydrates and 15 (11.45%) exogenous introductions, with 8 compounds were introduced by honey, and 7 compounds were introduced by

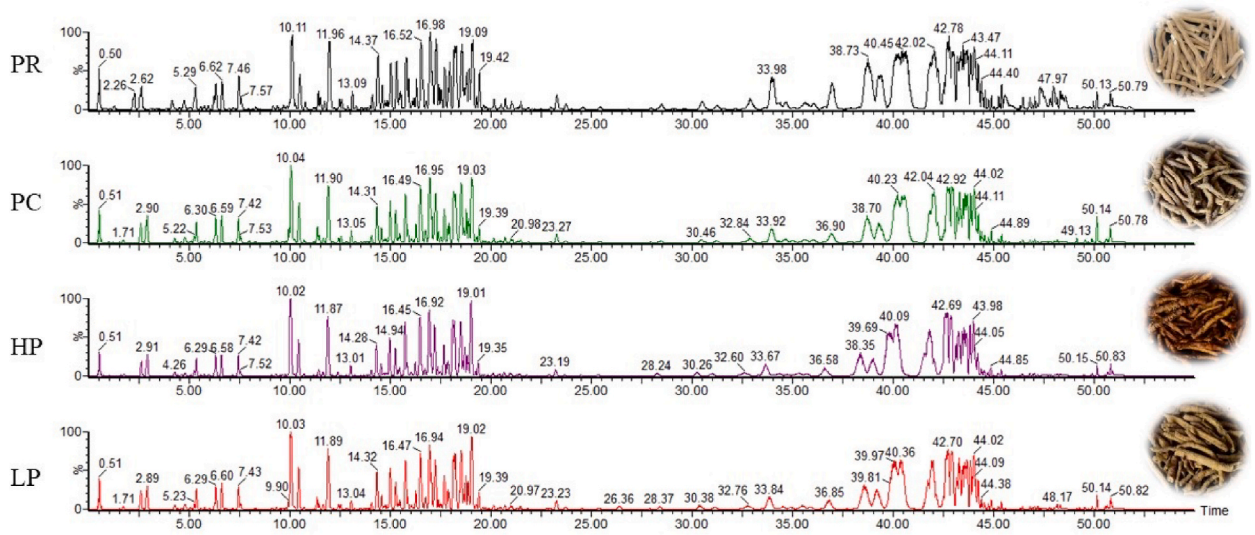

Fig. 1. Total ion chromatograms of different processed products of *Polygalae Radix* (negative ion mode).

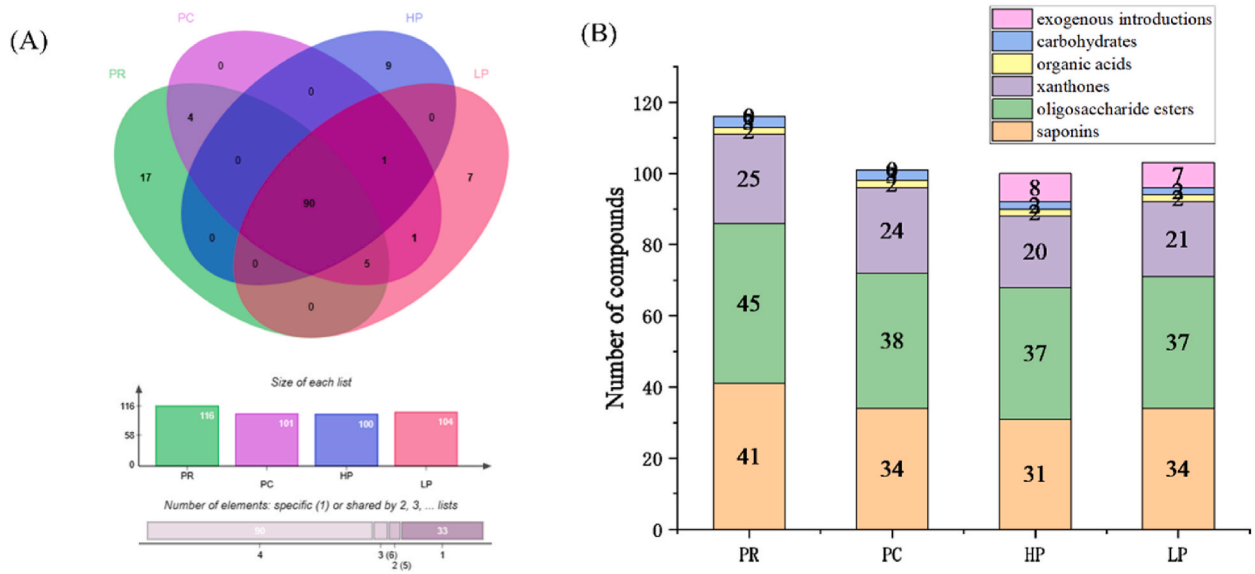

Fig. 2. (A) Venn diagram of chemical constituents in crude and processed PR, (B) The classification and distribution of compounds.

licorice. To have a more intuitive observation, we summarized the number of compounds of 4 kinds of samples, and made a bar graph as shown in Fig. 2B. The results show that the quantity of PR chemicals dropped to varying degrees during processing. After processing, compared with PC and LP, the saponins in HP were reduced by 10 compounds. In oligosaccharide esters, PC decreased by 7 compounds, while HP and LP decreased by 8 compounds. The overall proportion of xanthone compounds was less than that of the first 2 compounds, there were up to 5 compounds of reduction in HP, only 1 compound of reduction in PC, and 4 compounds of reduction in LP. The number of organic acid compounds remained constant. In carbohydrates, HP and LP decreased by 1 compound each. Relevant research shows that the saponins contained in PC decreased after honey processing, in addition, the related compounds of the auxiliary honey are increased, which improved the efficacy of tranquilizing the mind while maintaining the benefits of clearing phlegm and relieving cough [18]. Licorice can reduce toxicity, and has obvious neuroprotective, improves learning and memory and has antidepressant effects. Liquiritin, liquiritigenin and other components will be introduced after processing [19]. Traditional Chinese medicine believes that the PR processed with licorice juice can get rid of the numb taste and increase the sedative effect [20].

3.1.1. Saponins

Saponins have been identified as the primary pharmacological components of PR, which are rich in the roots and stems of PR.

Pharmacological studies indicate that saponins exert the functions of neuroprotective properties [21]. Diagnostic fragment ions of *m/z* 455 and 425 were employed to screen out saponins, the offset as 10 mDa was tolerated, then, confirmed the precursor ion. The neutral loss was applied to pursue those components bearing featured substitution. Finally, a total of 41 saponins were identified in the samples. In which $[M-H]^-$ ion at *m/z* 679.4432 (*Rt* = 2.732 min) was considered as Tenuifolin (molecular formula of $C_{36}H_{56}O_{12}$), glucose at position 3 and a carboxyl group at position 23 was broken under coupling, and one molecule of glucose, H_2O and CO_2 was removed to obtain the characteristic fragment ion *m/z* 455.3655 $[M\text{-}H\text{-}Glc\text{-}H_2O\text{-}CO_2]^-$. On this basis, the CH_2OH at C-14 was broken and CH_2O was removed to obtain the characteristic fragment ion *m/z* 425.0530 $[M\text{-}H\text{-}Glc\text{-}H_2O\text{-}CO_2\text{–}CH_2O]^-$, fragment ions at *m/z* 425

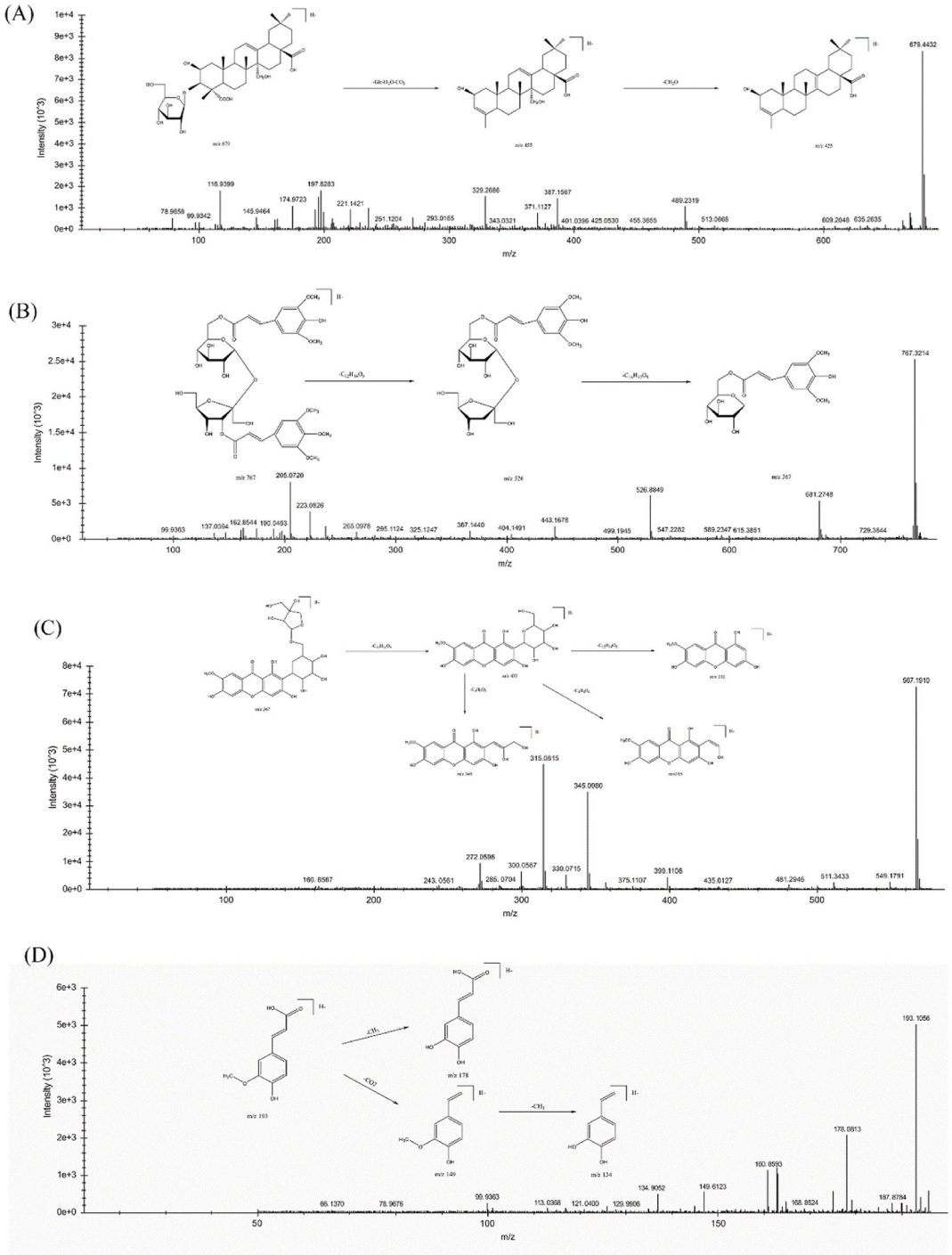

Fig. 3. The fragmentation pathways of Tenuifiolin (A)、Tenuifolioside C (B)、Polygalaxanthone III (C) and Ferulic acid (D).

and 455 could be considered as the diagnostic ions for this type of triterpene saponins regularly [22]. The cleavage pathway is shown in Fig. 3A.

3.1.2. Oligosaccharide esters

Oligosaccharide ester is a class of biologically active compositions of *P tenuifolia*, which has neuroprotective and antidepressant effects [23]. Recent studies have shown that oligosaccharide esters have significant effects on neuroprotective and antioxidant systems. They also cause improvements in the function of the central cholinergic system, with a structure that has sucrose as the common mother nucleus, oligosaccharide esters connect glucose or rhamnose with various forms of glycosidic bonds to form oligosaccharides and then form esters with organic acids. 45 oligosaccharide esters were identified in the samples by comparing with the literature. As one of the diagnostic compounds for *P. tenuifolia,* Polygala oligosaccharide C was used as an example for analysis., In detail, *m/z* 767.3214 ($C_{35}H_{44}O_{19}$) was assigned as the deprotonated molecular ion, and *Rt* = 14.308 min. The primary fragment ion species were observed at *m/z* 526.8849 $[M\text{-}H\text{-}C_{12}H_{14}O_5]^-$ and 367.1440 $[M\text{-}H\text{-}C_{12}H_{14}O_5\text{-}C_6H_{12}O_5]^-$ [19]. The cleavage pathway is shown in Fig. 3B.

3.1.3. Xanthones

Xanthones are one of the important components in PR, which have good efficacy in the treatment of gout [24]. Among them, 25 xanthones were identified in the samples. Xanthones in PR mostly have similar chemical skeletons, generally, the substituents are methoxy, hydroxyl, methylene dioxy, monosaccharides and disaccharides. When fission occurred for the linkage between glucosyl and xanthone skeleton, the neutral loss of 162 Da (Glc), 132 Da (Api), and 146 Da (Rha) was generated firstly, then the C ring of xanthone aglycones broken, subsequently gave birth to A and B ring fragments ions. Taking PolygalaxanthoneIII as an example, the excimer ion peak *m/z* 567.1910 $[M-H]^-$ was produced in the negative ion mode. After high energy bombardment, the sugar chain was broken, the cross-loop cleavage occurred in the sugar chain, and the neutral fragments $C_3H_6O_3$ and $C_4H_8O_4$ were lost, respectively, the following fragment ions were obtained: *m/z* 435.0127 $[M\text{-}H\text{-}C_5H_9O_4]^-$, 345.0980 $[M\text{-}H\text{-}C_5H_9O_4\text{ - }C_3H_6O_3]^-$, 315.0815 $[M\text{-}H\text{-}C_5H_9O_4\text{ - }C_4H_8O_4]^-$ and 272.0596 $[M\text{-}H\text{-}C_5H_9O_4\text{ - }C_6H_{11}O_5]^-$ [21]. The cleavage pathway is depicted in Fig. 3C.

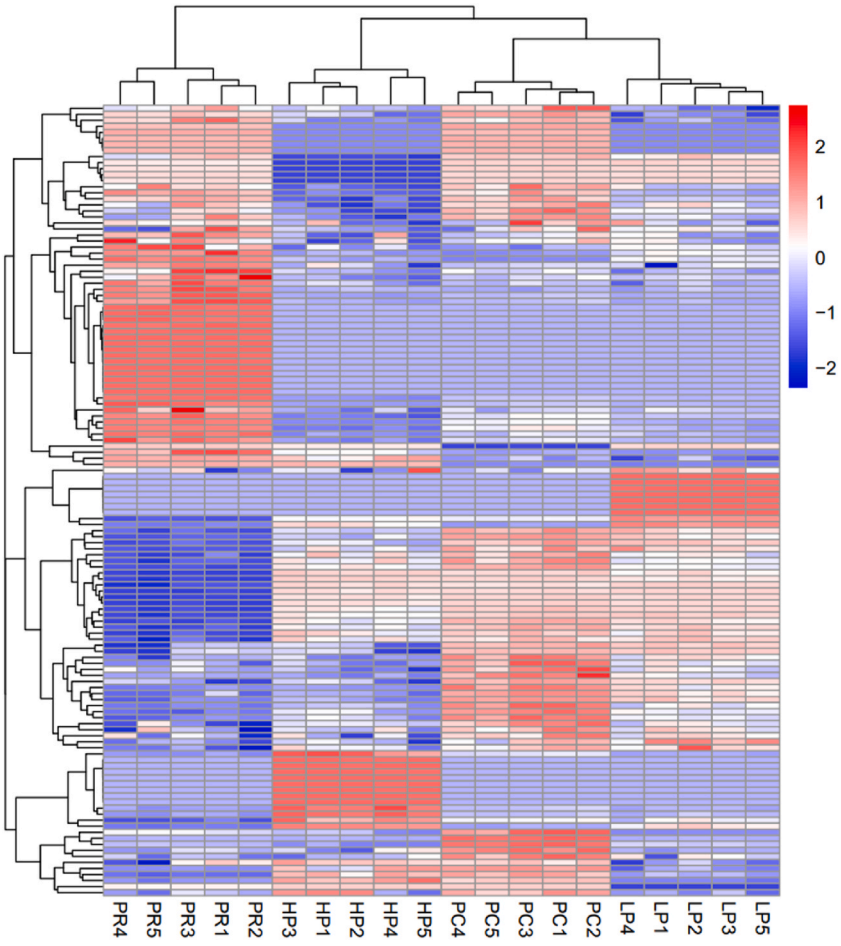

Fig. 4. Cluster heat map of 131 differential chemical constituents.

3.1.4. Organic acids

Organic acids are small molecular active substances, which often lose H_2O, CO_2, and CO to produce fragment ion peaks under high energy collisions. In this study, organic acids mainly included ferulic acid, cinnamic acid, erucic acid. Taking ferulic acid as an example, the excimer ion peak *m/z* 193.1056 $[M-H]^-$, was produced in the negative ion mode, and a molecule of CH_3 was lost, resulting in a fragment ion *m/z* 178.0813 $[M\text{-}H\text{-}CH_3]^-$, or the loss of one molecule of CO_2 and CH_3, yielding the fragment ions *m/z* 149.6123 $[M\text{-}H\text{-}CO_2]^-$ and 134.9052 $[M\text{-}H\text{-}CO_2\text{-}CH_3]^-$ [25]. The cleavage pathway is shown in Fig. 3D.

3.1.5. Carbohydrates

One of the important compounds for PR is carbohydrate. In this study, A total of 3 carbohydrates were identified. 3 carbohydrates were identified in PR and PC, however, 2 species were identified in HP and LP.

3.1.6. Other categories of compounds

A total of 15 compounds of other origins were obtained from HP and LP. HP contained 8 honey components, including sucrose and diosmetin. Meanwhile, 7 licorice components, including liquiritin, apiosylliquiritin, and isoliquiritin, were found in LP. Traditional Chinese medicine believes that processing can alleviate the gastrointestinal motility of PR [26], increase its neuroprotective impact, and foster mental tranquility and intellectual growth [27]. According to the theories of TCM, licorice processing can reduce the side effects of RP pharyngeal irritation and gastrointestinal stimulation, as well as promote peace of mind and intellectual development [8]. PR has certain toxicity, so its processed products are commonly used in clinics. Pharmacological studies have found that HP can reduce the toxicity of PR, while retaining the effect of relieving cough and expectorant and enhancing the effect of tranquilizing the mind [18].

3.2. Differences in chemical constituents among the crude and processed PR

Heatmap was applied to visually depict the content profile of 131 compounds present in crude and processed PR (Fig. 4). The results showed that the contents of compounds were greatly different in the crude and processed PR. Furthermore, the heatmap analysis demonstrated distinct variation trends for each chemical during the processing procedure. Color intensity ranging from green to red denoted the relative levels of individual components before and after processing, while color depth indicated the size of the Pearson correlation coefficient (r from −2 to 2). These parameters effectively distinguished different groups and showcased close relationships among PR, PC, HP, and LP samples. Importantly, the presence of compounds derived from honey and licorice did not interfere with the overall chromatogram separation for the samples. The identified compounds were divided into six clusters, which elucidated the diverse variation tendencies observed during processing. Specifically, PR exhibited higher content levels of 62 components, including Tenuifoliside B, Onjisaponin R, and Polygalaxanthone IV, etc., whereas PC displayed lower content levels of 54 components, such as Tenuifiolin, Tenuifoliside A and Onjisaponin J, etc. Notably, PC showed a relatively elevated content of 75

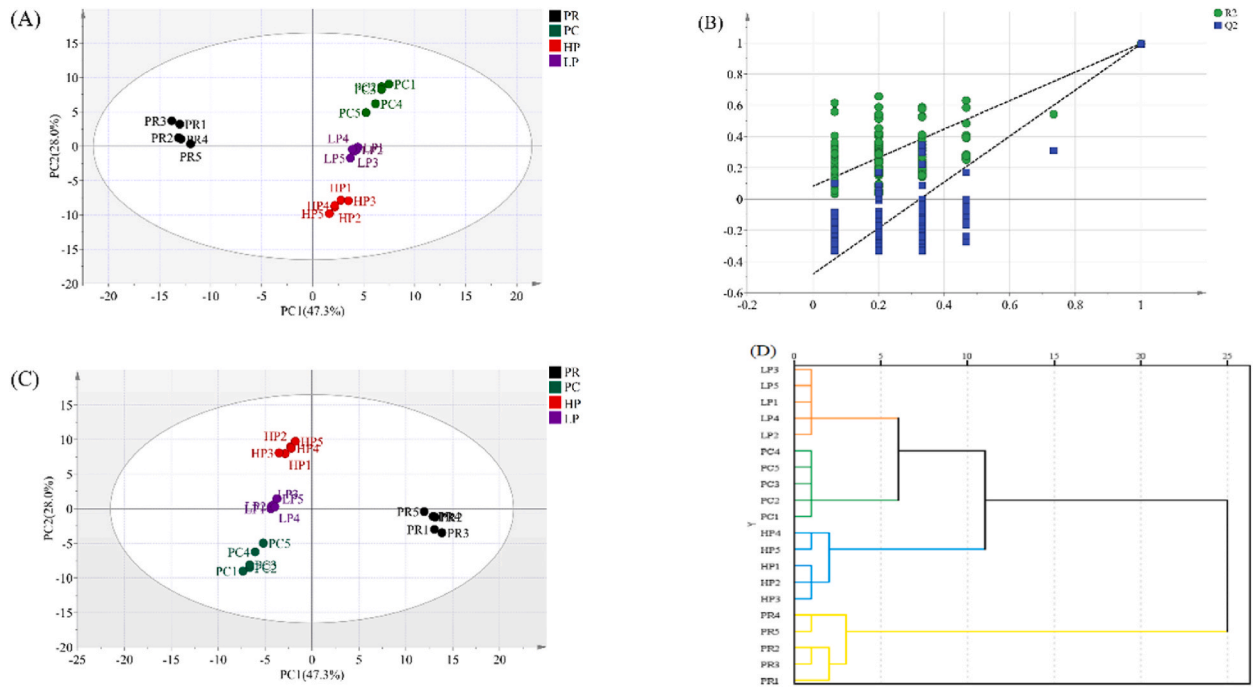

Fig. 5. Chemometric analysis of 131 compounds between crude and processed Polygalae Radix (RP, PC, HP, LP). (A) PCA-X score plot (R2X (cum) = 0.925, Q2 (cum) = 0.882); (B) PLS-DA score plot (R2X (cum) = 0.931, Q2 (cum) = 0.984); (C) Intercepts of 200 permutation tests. (D) HCA plot.

compounds, including Onjisaponin Sg, Tenuifoliose G, and Onjixanthone II, while the content of 26 components, including 10 saponins, was lower. Furthermore, HP samples demonstrated higher levels of 56 components, encompassing Tenuifiolin, Tenuifoliside A, and Onjisaponin V, etc., whereas 36 components, such as Sibiricaxanthone F, Sibiricose A5, etc. In LP samples, the content of 60 components was higher, while Tenuifiolin and Tenuifoliose W were among the 37 components found in low concentrations. In addition, Ding et al. [28] provided evidence that the heating process during PR processing resulted in the hydrolysis and breakdown of glycosidic bonds of saponins, including Onjisaponin B, into less toxic components such as Tenuifolin. As a consequence, the content of Tenuifolin decreased in HP and LP samples. These findings not only provide valuable insights for PR processing but also establish a scientific foundation for understanding the 'detoxification' effect of PR.

3.3. Influence of processing techniques on the content of compounds

To assess the similarities and differences in compound composition between different groups of samples, PCA (Fig. 5A and B), PLS-DA (Fig. 5C), and HCA (Fig. 5D) models were applied. The PCA score, PLS-DA, and HCA diagram revealed distinct clustering patterns among the analyzed samples. As a result, PR, PC, HP, and LP were found to cluster separately in the negative ion mode, with crude samples clearly distinguished from all processed groups. PCA analysis further demonstrated dynamic changes in compound profiles among crude (PR) and processed samples (PC, HP, and LP), accounting for 81.4% of the total variations through PC1 (61.1%) and PC2 (20.3%). The R2X (0.925) and Q2 (0.882) values indicated significant intergroup differences and satisfactory model fitness and predictability. These observations were held across all analyzed sample varieties. Subsequently, a PLS-DA model with three latent components was employed to classify the samples and provide a more intuitive visualization of the clustering trends. Notably, variables with VIP values > 1) (Variable Important Plot) were considered more influential in the model and could effectively screen differences before and after processing. Extracting VIP values for the 131 analyzed compounds, it was found that 42 compounds exhibited VIP values > 1, with peak No.7, 11, 86, 108, and 57 ranking as the top five contributors with VIP values of 2.5787, 2.5721, 2.3232, 2.2251, and 2.1173, respectively (Table S2, see online supplementary material). The established PLS-DA model showed good fitness ($R2X = 0.931$, $R2Y = 0.988$) and predictability ($Q2 = 0.984$). The chance permutation tests suggested the model was not overfitting ($R2 = 0.0499$, $Q2 = -0.469$). The hierarchical cluster analysis (HCA) shows that the heterogeneity of crude and processed PR samples is almost in agreement with the results of PCA, all materials can be clustered into four categories and each can be clustered into one category, which can be enough to segregate all specimens according to differences in different processing method.

3.4. Effects of processing on contents of total phenols, total flavonoids, total saponins, and antioxidant activity

With the increasing health risks, the evaluation of antioxidants in natural products is of immense importance in today's context. In this study, the TPC, TFC, and TSC in the roots of PR raw and processed products were determined, and the antioxidant activity of crude and processed PR was estimated using four in vitro assays, namely ABTS, FRAP, CUPRAC, and DPPH. These assays are widely utilized to assess the antioxidant potential of plant extracts, and their pure isolated compounds in addition to food products. The linear relationship of the related components (Table S3, see online supplementary material).

3.4.1. Total phenols, total flavonoids, and total saponins content

Polyphenols derived from medicinal plants have gained recognition as safe and effective antioxidants, offering protection against disease threats by scavenging excessive free radicals. This study focused on evaluating the TPC in crude and processed PR. PC owned the highest TPC value followed by PR, HP, and LP. The TPC values for these samples were 12.345 ± 0.107 mg/g DW, 9.459 ± 0.014 mg/g DW, 9.381 ± 0.009 mg/g DW and 7.443 ± 0.019 mg/g DW, respectively. Phenolic compounds possess redox features that enable them to act as antioxidants. The presence of free hydroxyl groups in the phenolic compounds enhanced their free radical scavenging potential. As a result, the determination of total phenolic content can serve as a primary marker for evaluating the overall antioxidant capacity of both crude and processed PR samples [29].

Flavonoids derived from natural sources possess remarkable biological activity and demonstrate a wide spectrum of potential health benefits, including antioxidant properties, anti-mutagenic effects, anti-cancer activities, and blood vessel protection. The TFC values were determined for extracts obtained from crude and processed PR samples. The TFC values followed the order: PC > LP > HP > PR, with corresponding values of 78.467 ± 0.44 mg/g DW, 72.800 ± 0.608 mg/g DW, 63.052 ± 0.280 mg/g DW, and 52.338 ± 0.281 mg/g DW, respectively.

Saponins derived from PR are the main pharmacodynamic substances for the clinical treatment of anti-aging and senile dementia (Zhang et al., 2023). The results showed that PC displayed the highest TSC value, up to 19.205 ± 0.068 mg/g DW, followed by PR (18.548 ± 0.096 mg/g DW), LP (17.065 ± 0.120 mg/g DW) and HP (14.435 ± 0.012 mg/g DW), respectively. In summary, the contents of total phenols, total flavonoids, and total saponins were higher in PC compared to PR, with varying degrees of reduction observed after processing.

3.4.2. Correlation analysis of pharmacodynamic components with antioxidant ability results

The influence on the antioxidant activity of processing methods was determined using four different methods: $ABTS^{\cdot+}$, CUPRAC, FRAP, and DPPH due to the different antioxidant mechanisms used in these methods. $ABTS^{\cdot+}$ method has become a common method for the determination of antioxidant activity in the fields of food and medicine (Erel. 2004). The $ABTS^{\cdot+}$ assay demonstrated that PC exhibited the highest antiradical activity (23.983 ± 0.724 μM/g DW), while PR showed the lowest value (21.438 ± 0.310 μM/g DW), and HP is slightly larger than LP, 21.341 ± 0.081 μM/g DW and 21.224 ± 0.344 μM/g DW, respectively. Correlation analysis revealed

a significant positive correlation between $ABTS^{\cdot+}$ and TFC, with a correlation coefficient of 0.867 ($P < 0.01$). Furthermore, $ABTS^{\cdot+}$ showed positive correlations with TFC and TSC, with correlation coefficients of 0.603 and 0.585 ($P < 0.05$).

Moreover, the reduction power of crude and processed PR exhibited concentration-dependent behavior, with higher reducing activity corresponding to higher absorbance values. PC demonstrated the highest FRAP value (16.065 ± 0.065 μM/g DW), while LP displayed the lowest FRAP (6.241 ± 0.184 μM/g DW). The order of FRAP values was PC > PR > HP > LP. The higher reduction power of selected crude and processed PR indicates their higher antioxidant capacity. Correlation analysis also showed that FRAP was significantly positively correlated with TFC and ABTS ($P < 0.01$), with correlation coefficients of 0.957 and 0.787, and positive correlation with TSC, with a correlation coefficient of 0.611 ($P < 0.05$).

The cupric reducing antioxidant capacity (CUPRAC) method is widely recognized as a valuable approach for evaluating antioxidant activities. Its advantages, including pH adjustability, cost-effectiveness, rapid reaction kinetics, quick results, and stability, make it highly suitable for identifying the antioxidant potential of both natural and synthetic compounds [16]. In this study, the CUPRAC method was employed to assess the antioxidant capacities of various samples, with PC exhibiting the highest value (48.663 ± 1.293 μM/g DW), followed by PR (42.990 ± 2.335 μM/g DW), high-pressure processed (HP) (39.257 ± 0.958 μM/g DW), and low-pressure processed (LP) (32.002 ± 0.991 μM/g DW). Correlation analysis showed that CUPRAC was significantly positively correlated with TFC, FRAP, and ABTS ($P < 0.01$), with correlation coefficients of 0.942, 0.963, and 0.748, respectively.

DPPH, a stable and widely recognized organic free radical, is frequently used to evaluate the sample's levels of antioxidant activity. The results revealed significant variations in the DPPH radical scavenging ability among the samples, PC exhibited the highest scavenging efficiency (51.562 ± 3.366) %, surpassing that of HP (48.156 ± 1.066) %, PR (45.729 ± 2.688) % and LP (43.387 ± 0.627) % samples, indicating that PC has a strong scavenging ability on DPPH free radicals. Moreover, correlation analysis showed a significant positive correlation between DPPH scavenging activity and total saponin content, with a correlation coefficient of 0.713 ($P < 0.01$). Additionally, positive correlations were observed between DPPH and other antioxidant assays, including TFC, FRAP, and CUPRAC ($P < 0.05$), with correlation coefficients of 0.821, 0.714, and 0.739. Furthermore, a positive correlation with ABTS was observed (correlation coefficient: 0.634, $P < 0.05$). Correlation analysis between active components and various indexes is shown in Table 2.

4. Conclusion

In this study, the UPLC-Q-TOF-MS/MS analysis method was used to comprehensively investigate the chemical constituents between crude and processed PR in negative ion mode. A total of 131 components were identified and categorized into six classes, including 41 saponins, 45 oligosaccharide esters, 25 xanthones, 2 organic acids, 3 carbohydrates, and 15 kinds of auxiliary ingredients. Multivariate statistical analysis and heat maps revealed significant differentiation among the samples. Notably, the processing primarily affected saponins, oligosaccharide esters, and xanthones, with a marked decrease in saponins contents. This decrease is likely due to the unstable nature of saponins in Polygalae, which are prone to hydrolysis during processing. Additionally, the cleavage of glycoside bonds in processing led to a reduction in oligosaccharide esters and a consequent increase in certain organic acids, such as ferulic acid, cinnamic acid, and erucic acid. In the processed forms, HP and LP, there was a notable reduction in the contents of saponins, oligosaccharides, and xanthones compared to crude PR. Importantly, LP introduced additional components like liquiritigenin and liquiritin, known for their neuroprotective properties, and their potential to enhance learning, memory, and anti-depression effects. Simultaneously, we compared the antioxidant activity and chemical composition variations between raw and processed PR. The results showed that PC had the highest concentrations of total saponins, flavonoids, and phenols, indicating its superior antioxidant capacity. These insights present promising perspectives for developing functional food products based on PR, PC, HP, and LP. Furthermore, this study significantly contributes to the broader utilization of PR, in both its raw and processed forms, across various sectors including food, cosmetics, and pharmaceutical industries. It also offers a solid scientific foundation for the comprehensive development and application of Polygalae resources.

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CRediT authorship contribution statement

Yao Luo: Writing – original draft. **Benxiang Hu:** Investigation. **Haiyue Ji:** Investigation. **Yiyao Jing:** Investigation. **Xiaoling Dang:** Funding acquisition. **Han Zhang:** Writing – review & editing. **Bo Li:** Writing – review & editing. **Gang Zhang:** Methodology. **Yongang Yan:** Resources. **Bingyue Yang:** Writing – review & editing, Supervision. **Liang Peng:** Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 2
Correlation analysis between active components and various indexes.

	TPC	TFC	TSC	ABTS	FRAP	CUPRAC	DPPH
TPC	1.000						
TFC	0.316	1.000					
TSC	0.481	0.184	1.000				
ABTS	0.867**	0.603*	0.585*	1.000			
FRAP	0.957**	0.114	0.611*	0.787**	1.000		
CUPRAC	0.942**	0.055	0.530	0.748**	0.963**	1.000	
DPPH	0.821**	0.329	0.177	0.634*	0.714**	0.739**	1.000

Note: "*" means $P < 0.05$, significant correlation; "* *" means $P < 0.01$, highly significant correlation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27622.

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