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不同来源丹参药材酚酸类成分的分析研究

盛东峰*, 李俐俐
(周口师范学院生命科学系, 河南 周口 466001)

摘要: 为比较不同来源的丹参(*Salvia miltiorrhiza*)药材的酚酸类成分,采用化学指纹图谱和定量分析的方法,对不同来源丹参药材中的酚酸类成分进行了系统分析。结果表明:产地、采收期、病害、根色、根的粗细以及药材部位等因素尽管对丹参酚酸类成分绝对含量的影响比较大,但对各成分相对含量的影响较小;不同来源丹参药材酚酸类成分指纹图谱相似性较高;8 月份采收的药材,丹酚酸 B 含量较高;病害能够显著降低丹酚酸 B 的积累;与白根和褐色根相比,砖红色根中的丹酚酸 B 含量较高;根越粗,丹酚酸 B 含量也越高。这为丹参药材的品质评价和资源利用提供了依据。

关键词: 丹参; 酚酸类成分; 高效液相色谱; 指纹图谱; 质量评价
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Comprehensive Investigation of Phenolics in *Salvia miltiorrhiza* by HPLC Fingerprinting and Marker Compounds

SHENG Dong-feng*, LI Li-li
(*Department of Life Science, Zhoukou Normal University*, Zhoukou 466001, China)

Abstract: In order to compare the phenolics in *Salvia miltiorrhiza* with different sources, the phenolics extracted from different sources were studied by chemical fingerprinting method, and salvianolic acid B as marker molecule was quantitatively analyzed. The results showed that the absolute amounts of phenolics in *S. miltiorrhiza* roots were seriously affected by different factors, such as origin, harvest time, root disease, root color, root diameter, and the part of plant. However, these factors had little influence on relative amounts of phenolics. The chemical fingerprinting of phenolics derived from different sources had high similarity. The content of salvianolic acid B harvested in August was the highest, and which was significantly reduced by diseases, such as root rot and nematode. Contents of phenolics in the brick red roots were the highest, compared to puce and white roots. The bigger the diameter, the higher contents of phenolics were. So, these could provide the basis for quality evaluation and resource utilization of *S. miltiorrhiza*.

Key words: *Salvia miltiorrhiza*; Phenolics; High performance liquid chromatography; Chemical fingerprinting; Quality assessment

Salvia miltiorrhiza has been widely used for prevention and treatment of coronary heart disease[1]. Studies on chemical constituents of *S. miltiorrhiza* were mainly focused on lipophilic compounds. However, more attention has been paid to phenolics in recent years due to their pharmacological activities[2–3], of which some phenolics have been identified[2], such as salvianolic acid A – K, danshensu and rosmarinic acid. Chemical fingerprinting developed in these years has been widely used in quality control of

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* Corresponding author. E-mail: shengdongfeng@126.com

traditional Chinese medicine (TCM)[4]. Quality assessments of *S. miltiorrhiza* have been reported with the method of fingerprinting[5–10] and quantitative analysis of marker compounds[11]. Whereas, most of them focused on the roots of *S. miltiorrhiza* and analyzed by HPLC[5], high-speed counter-current chromatography (HSCCC)[6], thin layer chromatography (TLC)[7], capillary electrophoresis (CE)[8], coulometric electrode array detector (CEAD)[9] and mass spectrography (MS)[10–11]. These methods were considered to be feasible for quality assessment. However, the quality of *S. miltiorrhiza* is difficult to control, because it is usually influenced by climate, disease, geography, morphological character and harvest time. Fingerprinting is insufficient for quality assessment of TCM since it is not quantitative, while quantitative analysis is necessary to evaluate the amounts of the main active compounds. The method of chromatographic fingerprint together with quantitative analysis of marker compounds was confirmed as a useful approach for quality assessment of TCM[12]. By this method, quality of lipophilic components in *S. miltiorrhiza* has been assessed and the influences on the quality has been comprehensively investigated by impact factors including origin, harvest time, root disease, root diameter, root color and different part of *S. miltiorrhiza*. In this paper, based on phenolic components in *S. miltiorrhiza*, the quality of *S. miltiorrhiza* will be assessed and the factors will be investigated.

1 Materials and methods

1.1 Chemicals and Materials

HPLC grade methanol was purchased from Fisher Scientific (Fairlawn, NJ, USA). Analytical grade ethanol was from Ante Biochemistry (Suzhou, China). HPLC-quality water was generated from a UPW ultrapure water system purchased from Shanghai Ultrapure Technology (Shanghai, China).

Salvia miltiorrhiza was purchased from markets (Table 1), in which DB-1 to DB-10 were collected from GAP base in five counties of Shangluo City. Salvianolic acid B were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

1.2 Sample preparation

Samples of *S. miltiorrhiza* were dried at 60℃, comminuted into powder, and sieved through a 0.45 mm screen. The powder (0.5 g) was extracted in water bath at 90℃ for 4 h with 25 mL deionized water. 1 mL of water-extract was added into 4 mL ethanol, and then the extract was centrifuged at 12000 ×*g* for 15 min and finally filtered through a 0.45 μm millipore filter.

Stock solutions of salvianolic acid B were prepared in methanol and were diluted to the desired concentration.

1.3 Chromatography

HPLC was performed with a Waters (Milford, MA, USA) binary pumpand photodiode array detector (PAD). The column was a Waters SunFire C_{18} (250 mm × 4.6 mm, 5 μm particle). Data were acquired and processed by use of Empower 2 Software. Separation was achieved by eluting with a linear gradient prepared from methanol and water (containing 0.01% H_3PO_4). The gradient was: t = 0 min 15% methanol; t = 30 min, 40% methanol; t = 40 min, 55% methanol; t = 60 min, 75% methanol. The flow rate was 1 mL min^{-1}, the column temperature 30℃, and the sample size 20 μL. The effluent was monitored between 190 nm and 500 nm by use of the PDA.

2 Results and discussion

2.1 Method validation

The extract of sample 1# was injected five times. The repeatability of the method was assessed by analysis of five independently prepared extracts of sample 1#. The precision of the analysis was determined by replicate analysis of the same extract of sample 1# over a period of 48 h. The relative standard deviation (RSD) values of retention time and peak area of salvianolic acid B were calculated. The results revealed the method performed well. The detector

Table 1 Samples tested

No.	Sample code	Source	Sample description	Collection time (M / Y)
1	DB-1	Shangluo, Shaanxi	Whole root	11 / 2006
2	DB-2	Shangluo, Shaanxi	Whole root	11 / 2006
3	DB-3	Shangxian, Shaanxi	Whole root	11 / 2006
4	DB-4	Shangxian, Shaanxi	Whole root	11 / 2006
5	DB-5	Luonan, Shaanxi	Whole root	11 / 2006
6	DB-6	Luonan, Shaanxi	Whole root	11 / 2006
7	DB-7	Zhashui, Shaanxi	Whole root	11 / 2006
8	DB-8	Zhashui, Shaanxi	Whole root	11 / 2006
9	DB-9	Danfeng, Shaanxi	Whole root	11 / 2006
10	DB-10	Danfeng, Shaanxi	Whole root	11 / 2006
11	DA-1	Shaanxi	Whole root	11 / 2006
12	DA-2	Shanxi	Whole root	11 / 2006
13	DA-3	Hebei	Whole root	11 / 2006
14	DA-4	Jiangsu	Whole root	11 / 2006
15	DA-5	Shandong	Whole root	11 / 2006
16	DA-6	Beijing	Whole root	11 / 2006
17	DA-7	Anhui	Whole root	11 / 2006
18	DP-1	Shangluo, Shaanxi	Leaf	05 / 2006
19	DP-2	Shangluo, Shaanxi	Petiole	05 / 2006
20	DP-3	Shangluo, Shaanxi	Stem	05 / 2006
21	DP-4	Shangluo, Shaanxi	Petal	05 / 2006
22	DP-5	Shangluo, Shaanxi	Receptacle	05 / 2006
23	DP-6	Shangluo, Shaanxi	Rachis	05 / 2006
24	DP-7	Shangluo, Shaanxi	Seed	05 / 2006
25	DP-8	Shangluo, Shaanxi	Root periderm	05 / 2006
26	DP-9	Shangluo, Shaanxi	Root xylem	05 / 2006
27	DP-10	Shangluo, Shaanxi	Root phloem	05 / 2006
28	DT-1	Shangluo, Shaanxi	Root velamen*	05 / 2006
29	DT-2	Shangluo, Shaanxi	Root velamen	06 / 2006
30	DT-3	Shangluo, Shaanxi	Root velamen	07 / 2006
31	DT-4	Shangluo, Shaanxi	Root velamen	08 / 2006
32	DT-5	Shangluo, Shaanxi	Root velamen	09 / 2006
33	DT-6	Shangluo, Shaanxi	Root velamen	10 / 2006
34	DT-7	Shangluo, Shaanxi	Root velamen	11 / 2006
35	DT-8	Shangluo, Shaanxi	Root velamen	12 / 2006
36	DD-1	Shangluo, Shaanxi	Whole root suffered from nematodiasis	05 / 2006
37	DD-2	Shangluo, Shaanxi	Whole root suffered from root rot	05 / 2006
38	DC-1	Shangluo, Shaanxi	Sable root	05 / 2006
39	DC-2	Shangluo, Shaanxi	White root	05 / 2006
40	DJ-1	Shangluo, Shaanxi	Root head	11 / 2006
41	DJ-2	Shangluo, Shaanxi	Root diameter > 0.8 cm	11 / 2006
42	DJ-3	Shangluo, Shaanxi	Root diameter at 0.5 – 0.8 cm	11 / 2006
43	DJ-4	Shangluo, Shaanxi	Root diameter at 0.3 – 0.5 cm	11 / 2006
44	DJ-5	Shangluo, Shaanxi	Root diameter at 0.2 – 0.3 cm	11 / 2006
45	DJ-6	Shangluo, Shaanxi	Root diameter at 0.05 – 0.2 cm	11 / 2006
46	DJ-7	Shangluo, Shaanxi	Fibrous roots diameter < 0.05 cm	11 / 2006

*: Root velamen including periderm and xylem.

response was linearly correlated with concentration of salvianolic acid B with a range of 55.0 – 220.0 μg mL^{-1}. The regression equation was $y = 1020000x + 74200$ ($r = 0.9997$).

2.2 Assessment of *S. miltiorrhiza* roots from the same origin

Shangluo was an important GAP origin of *S. miltiorrhiza*. In this study, ten batches of *S. miltiorrhiza* from Shangluo in Shaanxi Province were collected and analyzed. Fifteen common peaks of phenolic components at 288 nm were obtained according to the consistency of retention time and spectra of the peaks (Fig. 1). A standard fingerprinting representing the mean values of the RRT and RPA of all the samples was constructed according to SFDA (State Drug Administration of China 2000). Correlation coefficient was employed to evaluate the values of similarity between the chromatograms of samples and the standard fingerprinting. The results showed the values of correlation coefficient were all more than 0.99. It indicated that the relative contents of phenolic components in the samples from GAP base of *S. miltiorrhiza* were consistent.

Salvianolic acid B is the main phenolic component in *S. miltiorrhiza*. Peak area of salvianolic acid B takes up more than 60% of total peak areas. Its contents were inconsistent in 10 batches of *S. miltiorrhiza* from GAP base. The highest content reached to 4.851%, and the lowest content was just 2.106%. Our previous report showed that all the ten batches of samples had good quality on lipophic components[13]. However, Table 2 showed that samples DB-6, 7, 8 had bad quality on phenolic components. It demonstrated that quality of *S. miltiorrhiza* should be assessed according to their usage and components.

2.3 Assessment of *S. miltiorrhiza* roots from different origins

The roots of *S. miltiorrhiza* were collected from seven provinces in China and analyzed. The quantitative analysis showed that the contents of salvianolic acid B in seven samples were inconsistent. The highest content reached to 6.594%, and the lowest was only 3.891%. However, All of them were more than 3%, which was the lowest statute content. The similarity values among seven samples were more than 0.99, which indicated the origin had more influence on the absolute amounts than the relative contents of phenolics components.

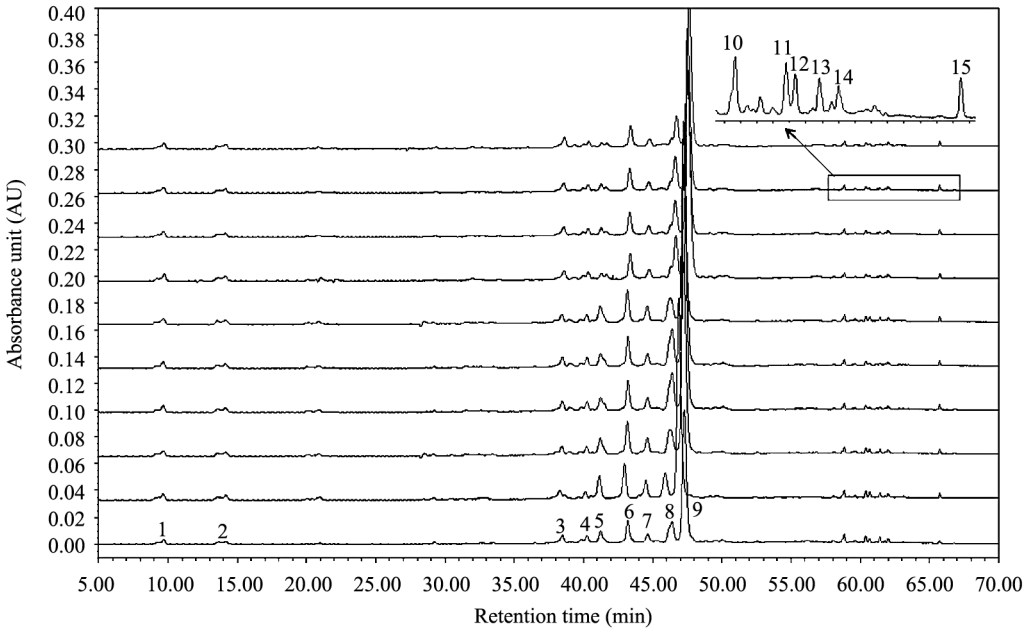

Fig. 1 Fingerprinting of the phenolics components at 288 nm in ten batches of *Salvia miltiorrhiza* from Shangluo GAP base. Peak 9 was salvianolic acid B.

Table 2 Contents of salvianolic acid B in different *Salvia miltiorrhiza* samples

Sample No.	Salvianolic acid B (%)	Sample No.	Salvianolic acid B (%)
DB-1	3.714	DA-5	6.594
DB-2	3.824	DA-6	3.891
DB-3	4.851	DA-7	4.577
DB-4	4.063	DD-1	1.734
DB-5	3.052	DD-2	2.513
DB-6	2.706	DC-1	2.772
DB-7	2.106	DC-2	4.411
DB-8	2.505	DJ-1	2.310
DB-9	3.631	DJ-2	4.253
DB-10	3.865	DJ-3	2.792
DA-1	5.178	DJ-4	2.725
DA-2	4.647	DJ-5	2.772
DA-3	6.235	DJ-6	1.920
DA-4	4.231	DJ-7	0.917

2.4 Assessment of different parts of *S. miltiorrhiza*

In the experiment, water extracts from different parts of *S. miltiorrhiza*, including petal, receptacle, rachis, seed, leave, petiole, stem, root periderm, root xylem, and root phloem, were analyzed. The results showed that the phenolic components were mainly distributed in root xylem and root phloem. However, The other parts also contained some phenolic components. These were different from lipophilic components[13]. For example, the content of danshensu (3,4-dihydroxyphenyllactic acid) was the highest in receptacle and leave, and then in root phloem, petiole, rachis, and petal. Protocatechualdehyde and salvianolic acid B contents were the highest in root phloem, and then in root periderm, root xylem, and petal. Noticeably, areas of peaks 3 – 6 were the biggest in petal and receptacle. For example, area of peak 6 in petal and receptacle was 15 and 7 times more than that in root phloem, respectively. They might be good resources for the other medicines that mainly contain components 3 – 6. The similarity analysis showed that the green parts were similar, petal, receptacle, and seed were similar, and the underground parts were similar. In origin of *S. miltiorrhiza*, only the roots were used and the other parts were discarded or burned out. Our results gave a good guidance for exploring and utilizing the other parts of *S. miltiorrhiza*.

2.5 Assessment of *S. miltiorrhiza* roots obtained at different harvest times

In the study, roots of *S. miltiorrhiza* were collected every month from May to December. The dynamic changes in three main peaks were compared (Fig. 2). Fig. 2 showed that changes in peak 6 and 8 were unapparent and the curve of peak 9 fluctuated from May to December. However, they all reached to maximum in August and the similarities among the samples were all more than 0.9. It demonstrated that the relative contents of the components in *S. miltiorrhiza* harvested at different times were consistent. These results were consistent with lipophilic component[13].

2.6 Assessment of *S. miltiorrhiza* suffered from different diseases and different colors

Roots of *S. miltiorrhiza* suffered from root rot and nematode disease were collected. The results showed that all of the 15 components could be detected in the two kinds of ill roots, but their contents were lower than those in normal roots. However, area of peak 8 in root rot was 6 times more than that in normal roots. It indicated that root rot might accelerate the accumulation of peak 8.

In this study, quality of buff, sable and brick red roots was also studied. The number of phenolic components in three kinds of roots was the same. However, their contents were different. The total peak area and areas of peak 2, 4, 8 and 9 were the highest in brick red roots. Areas of peak 5 – 7 were the highest in buff roots. Sable roots had the worst quality.

2.7 Assessment of *S. miltiorrhiza* roots with different diameters

The dried roots from Shangluo GAP base were divided into seven groups according to root diameters (Table 1). The results showed that all of the 15 peaks were detected in all seven groups of roots. Similarity analysis showed that the correlation coefficient for the seven samples were all more than 0.9. It indicated that the relative contents of fifteen components in seven groups of roots were consistent. However, peak areas in seven groups were quite different (Fig. 3), only peak 9 was seriously influenced by root diameter. The quantitative analysis showed that the thicker the diameter was, the better the quality was (Table 2),

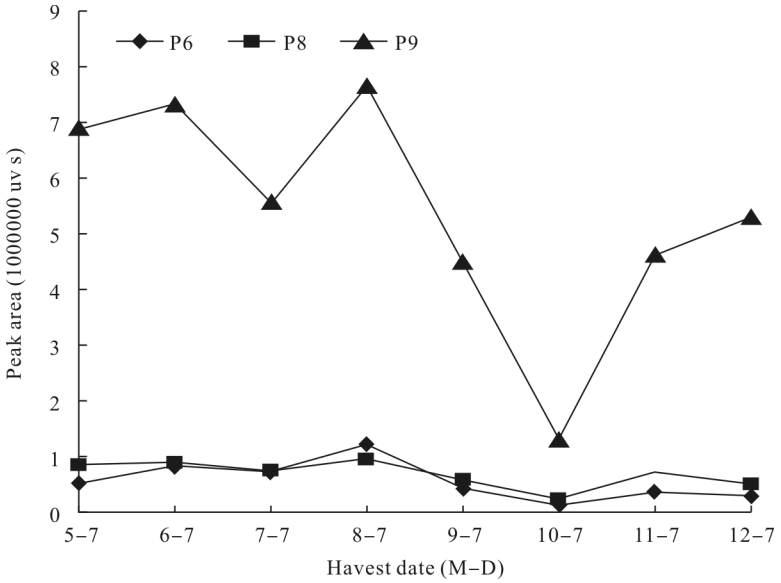

Fig. 2 Dynamic accumulation of main phenolic components in roots of *Salvia miltiorrhiza*. P6, P8 and P9 was the peak 6, 8 and 9, respectively.

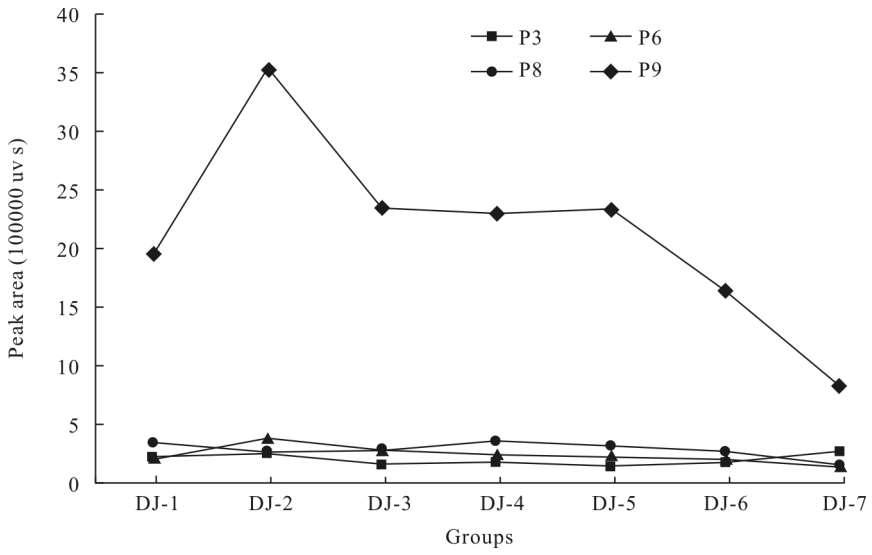

Fig. 3 Peak areas of phenolic components in different groups of *Salvia miltiorrhiza*. DJ-1 to DJ-7 were seven groups of *S. miltiorrhiza* roots according to diameter. P3, P6, P8 and P9 was the peak 3, 6, 8 and 9, respectively.

which was different from lipophilic component[13].

3 Conclusions

In our previous report, quality of lipophilic components in *S. miltiorrhiza* has been assessed by HPLC fingerprinting and quantitative analysis[13]. By the method, quality of phenolic components was also assessed in this paper. The standard fingerprinting including 15 common peaks at 288 nm was established, in which salvianolic acid B was determined. The influences of climate, disease, geography, morphological character, and harvest time on the quality of *S. miltiorrhiza* were also comprehensively investigated. The relative contents of phenolic components were consistent under different conditions as well as lipophilic components. However，the influences on the absolute amounts of phenolics and tanshinones were different. For example, contents of lipophilic components were the highest in DJ-3 and DJ-6, but those of phenolics were the highest in DJ-2.

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