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Original Article

Differential Evaluation of Multi-Index Components in Different Parts of *Ophiopogon japonicus*

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ABSTRACT

Objectives: To establish a method for determination of 11 components content to evaluate their quality and compare the difference between tuberous root and fibrous root of *Ophiopogon japonicus* by multivariate statistical analysis. **Materials and Methods:** The contents of total saponins, total flavonoids and total polysaccharides were determined by ultraviolet-visible spectrophotometry. The mineral elements were determined by atomic absorption spectrophotometry. Cluster analysis, partial least squares discriminant analysis and principal component analysis model were used for discrimination and comprehensive quality evaluation of tuberous root and fibrous root. **Results:** The results showed that there were obvious differences among samples from different parts, the results of 3 kinds chemometrics method analysis were consistent, which indicates that multi-index component quantitative analysis combined with chemometrics can be used for plant part identification. The difference between the two type roots was obvious and the content of most components in tuberous root was significantly higher than that in fibrous root. **Conclusion:** The method is simple and efficient and can be used to distinguish tuberous root and fibrous root of *Ophiopogon japonicus*.

Keywords: Different parts, Differential evaluation, *Ophiopogon japonicus*.

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INTRODUCTION

Ophiopogon japonicus (Maidong) was a liliaceae plant; its dried tuberous root was the Chinese herbal medicine of *Ophiopogon japonicus* (Linn.f.) Ker-Gawl.[1] Originally published in Shennong's Herbal Classics,[2] it had the effect of nourishing Yin, moistening lung and clearing heart.[2] And was widely used in clinical practice, often used to treat lung dryness and dry cough, throat impediment and pharyngeal pain, dryness and constipation, etc.[2] Modern pharmacological studies had shown that it had extensive pharmacological effects such as anti-cardiovascular and immune regulation, anti-inflammation and anti-tumor.[3,4] It cultivated in Sichuan, Zhejiang, Guangdong, Guangxi, Fujian and other provinces.[5-7] Maidong contains saponins, polysaccharides, flavonoids and other types of effective components.[6] In addition, Maidong also contains amino acids,[8,9] volatile oil components,[10,11] as well as K, Ca, Na, Mg, Fe, Mn, Zn, Cu, Ni and other inorganic elements.[12-14] Maidong Tuberous Roots (TR) were used for medicinal purposes and its Fibrous Roots (FR) were usually discarded as non-medicinal parts.[15] But the dry weight ratio between the yield of FR and the yield of TR was 1.16∶1, the yield of FR was larger than that of TR.[16] There were a large number of FR urgently need to be exploited and utilized. Because of the price of FR was lower than that of TR, some unscrupulous businessmen mixed Non-medicinal parts into medicinal materials, mixed with medicinal materials and crushed to increase the weight of medicinal materials for financial gain.[17] TR and FR are similar in color and smell, there was no significant difference in powder characteristics,[15] however, to ensure the efficacy and safety of clinical drugs, whether the fibrous root can replace the root tuber for medicinal purposes remains to be studied. At present, there were only a few researches on the content of the active ingredients of FR. Existing studies have proved that the components and contents of flavonoids and volatile oil in ophiopogon tubers and its fibrous roots are similar,[18,19] the total saponin content of FR is higher than that of TR,[20] and the polysaccharide content of FR is slightly lower than that of TR.[21] However, the content of a single component cannot accurately evaluate the overall quality of medicinal materials and whether the FR can replace the TR for medicinal purposes remains to be studied.

At present, the comprehensive quality evaluation and discrimination of TR and FR was still lacking based on multi-index components and mineral elements. In order to make full use of the resources of Maidong, in this study, a method was established to determine the contents of main 8 mineral elements, total saponins, total flavonoids and total polysaccharides in TR and FR of Maidong. Multiple comparisons were made by Principal

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Component Analysis (PCA) and Clustering Analysis (CA) and Partial Least Squares Discriminant Analysis (PLS-DA). Through these analysis models to analyze the differences of compounds in TR and FR, which provided theoretical basis to identify the different type roots and evaluate the comprehensive quality and for the rational development and application of fibrous root in China. It also can enrich the understanding of chemical constituents of different parts of *Ophiopogon*.

MATERIALS AND METHODS

Reagents and Instruments

The standards of ruscogenin and rutin were purchased from Chengdu Master Biotechnology Co., Ltd., Sodium nitrite, aluminum nitrate, sulfuric acid, glucose, etc all were analytically pure (Chengdu Kelon Enterprise, Chengdu, China). Standard reserve solution of all mineral elements (National Metal Materials Analysis and Testing Center). Spectrophotometer (Beijing spectral analysis instrument factory); Atomic absorption spectrophotometer (Beijing Puyang General Instrument Co., Ltd.,); Ultrasonic cleaners (KQ-250DB, Chang Zhou Jabosen Instrument Co., Ltd.).

Materials

Eighteen batches Maidong, wild growing in the Wutong, Muchuan, Jiajiang, Emei and Shizhong 5 different districts of Leshan city (Sichuan, China). The collected radix ophiopogon were washed and fibrous roots separated from tubers, TR1~TR18 were the TR samples and FR1~FR18 were the corresponding FR samples. Roots were dried at 40°C, crushed into powder, passed through a sieve and then seal preservation.

Determination of polysaccharide

Preparation of the test solution

The sample powder 1.0000 g was accurately weighed in a conical bottle and then a certain amount of purity water was accurately removed to the conical bottle for ultrasonic extraction for 40 min. Centrifuged and then the centrifugal supernatant was concentrated too nearly dry. Then transferred to a beaker and added a small amount of water to dissolve, then added 4 times the volume of anhydrous ethanol and then setting in a refrigerator at 4°C for 12 h. Centrifuged then discarded the supernatant, dissolved in 10 mL hot water after drying the solvent. The solution of extracting crude polysaccharide from ophiopogon was obtained.

Making standard curve

The standard curve was prepared by phenol-sulfuric acid method.[22,23] The glucose standard solution (0.1500 mg/mL) 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80 mL was accurately transferred into a test tube, then 0.5 mL 5% phenol aqueous solution and 3.0 mL sulfuric acid were added respectively, filled full of distilled water to 5 mL. Heated in a 70°C water bath for 15 min, cooled to room temperature, Absorbance (A) was measured at 490 nm wavelength. The value of A as the horizontal coordinate and the mass Concentration (C) as the ordinate and the standard curve was drawn to obtain the regression equation.

Content determination

Accurately removed 1.00 mL crude polysaccharide solution and dilute it with appropriate amount of distilled water to 50.00 mL to obtain the test solution. 0.20 mL of test solution was accurately transferred to a 10.00 mL colorimetric tube with a pipet and 1.00 mL 5% phenol solution was added to each colorimetric tube, then added 6.00 mL concentrated sulfuric acid, added distilled water and set the volume to the scale. The A value was determined after cooling.

Flavonoid content determination

Making standard curve

Accurately removed different volume rutin standard solution (1.00 mg/mL) to the different colorimetric tube, respectively. Added 0.7 mL of 5% sodium nitrite solution into the test tube successively and place for 10 min, then add 0.7 mL of 10% aluminum nitrate solution, mix well and place for 5 min, then add 5 mL of sodium hydroxide solution with a concentration of 1 moL/L shake well and place for 20 min. The absorbance of each solution was determined at the wavelength of 510 nm and the standard curve linear regression equation was calculated with absorbance (Y) as the vertical coordinate and rutin solution concentration (X) as the horizontal.

Total flavonoids determined

The samples were accurately weighed (1.00 g) to a conical flask with cover and 20.00 mL of 70% ethanol was added, ultrasonic extraction for 50 min. After centrifugation, the supernatant was the sample solution. Accurately added 0.50 mL of the sample solution into a 5.00 mL colorimetric tube, the color reaction is performed according to the method of drawing the standard curve, the absorbance was determined at the wavelength of 510 nm. According to the standard curve of to calculate the total flavonoid content of a sample.

Determination of inorganic element content

Preparation of test solution

The samples were digested by wet method.[24,25] Nitric acid-perchloric acid was used as digestion solution and 1.0000 g samples were accurately weighed for digestion. After complete resolution, then washed the inner wall and lid of the crucible with 0.2% nitric acid solution and then transfer all the sample liquid into a 100.00 mL Volumeter bottle, constant volume and shake well. The absorbance was determined by atomic absorption

spectrophotometer, according to the standard curve to calculate the content of each element.

Element standard curve

The standard storage solution of each element was diluted with 0.1 mol/L nitric acid to prepare the standard solution of different concentration gradients. The standard solution was absorbed, its absorbance value was determined, and the standard curve of each element was drawn.

Determination of total saponins

Test solution preparation

Take 0.1 g powder of samples, place it in a conical flask with cover, add 20.00 mL methylalcohol, ultrasonic treatment for 10 min and cool it. After filtering, accurately measure the filtrate 10.00 mL into a 50 mL volumetric bottle, add methanol and dilute it to the scale, shake well and then obtain the test solution.

Preparation standard curve

Appropriate amount of ruscogenin reference was accurately weighed and 50 μg/mL solution was prepared by adding methanol.[1] The reference solution 0.5, 1, 2, 3, 4, 5 and 6 mL were precisely measured and placed in a test tube, After volatilizing the solvent to dry, 10.00 mL perchloric acid was precisely added and then heated in hot water for 15 min, then, the solution was cooled in ice water. A value was measured, and the standard curve was drawn with A value as the ordinate and concentration as the abscissas.[1]

Determination of total saponins in samples

Determination according to the methods of the 2020 edition of Chinese Pharmacopoeia,[1] precisely measured 1.50 mL of the test solution and placed it in a 5.00 mL tube. The solvent was dried in a water bath, 5.00 mL perchloric acid was precisely added and then heated in hot water for 15 min and then the solution was cooled in ice water. A value was measured at the wavelength of 397 nm and the total saponin content in the sample is calculated from the standard curve.[1]

Data processing

SPSS 27.0 was used for PCA, t-test and CA. SIMCA-P 14.1 was used to accomplish PLS-DA and used Origin 2022 to draw a box diagram.

RESULTS AND ANALYSIS

Quantitative analysis

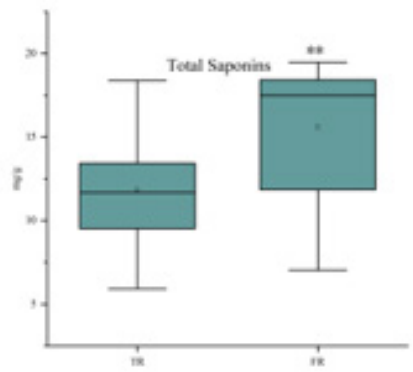
The validated method was applied to determine the eleven investigated constituents in 18 batches of TR and its FR, the contents of the investigated constituents were listed in Figure 1. The results showed that the contents of the analytes varied greatly between TR and FR. All Maidong samples were rich in saponin, the content of total saponins varied from 5.91 to 19.46 mg/g, the content of total saponins in RT and FT samples exceeded the minimum content of 0.12% prescribed by pharmacopoeia. The average total saponins content of FR (15.60 mg/g) was slightly higher than that of TR (11.83 mg/g). The ranges of total flavone were 1.01~3.86 mg/g in FR and 1.89~5.35 mg/g in TR, polysaccharide contents ranged from 61.3 to 67.20 mg/g in FR and 164.51 to 212.01 mg/g in TR, respectively. Total flavone and polysaccharide in TR were much higher than FR. In terms of mineral elements, the ranges of 4 macro elements were 60.05~2825.92 μg/g in TR and 22.85~2085.98 μg/g in FR respectively, the ranges of 4 microelements were 42.12~497.89 μg/g in TR and 6.55~368.89 μg/g in FR respectively. Figure 1 suggested that the contents of K, Ca and Mg were the relatively higher in the detected mineral elements. The content of Mn, Cu, Zn and Fe in the FR is very low. In addition, the contents of Ca, Na, Mg, Mn, Cu and Zn mineral elements in TR were obviously much higher than FR, contents of K and Fe in TR were slightly higher than that in FR. The results clearly demonstrated that the total contents of 11 chemical components in FR samples were significantly different from TR. The difference of component content between TR and FR was the result of many factors, including anatomical structure, physiological function, growth environment, genetic factors and growth stage. These differences make the TR and FR show certain differences in pharmacological action.

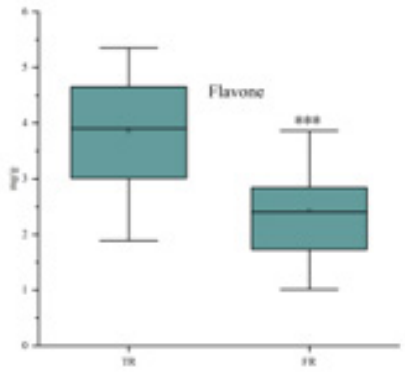
t-test of Samples

The contents of detected components were analyzed by t-test to evaluate the variation of 11 constituents in TR and FR, the $p<0.05$ was considered remarkably different. 11 constituents analyzed in this study were significantly different from two different parts. As shown in Figure 1, more than two-thirds of the constituents in TR were higher than FR ($p<0.001$). However, total saponins displayed higher level in FR than TR ($p<0.005$).

Table 1: Eigen value and cumulative contribution.

Component	Initial eigen values			
	Characteristic value	Variance contribution (%)	Weight (%)	Accumulated contribution (%)
1	5.808	52.799	0.726	52.799
2	1.124	10.221	0.141	63.02
3	1.064	9.674	0.133	72.694


Total Saponins
**
TR
FR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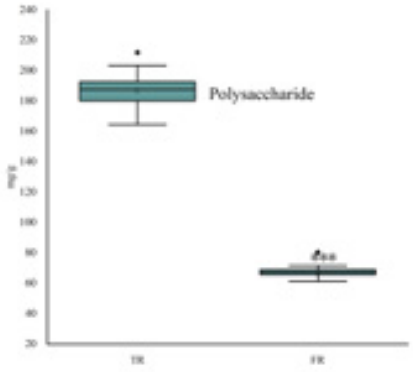
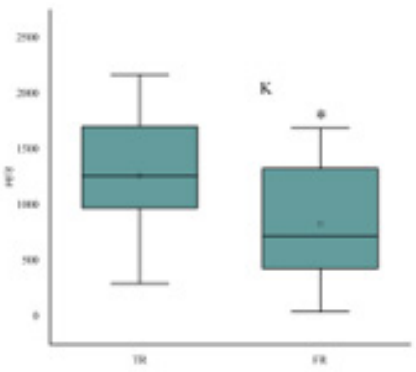
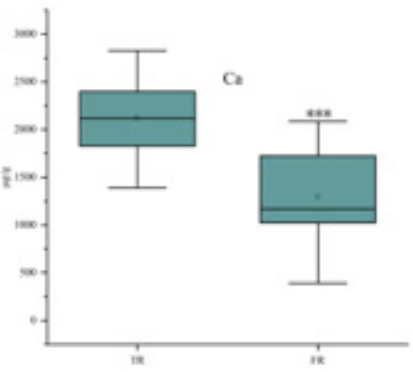
***
TR
FR


Polysaccharide
TR
FR


K
*
TR
FR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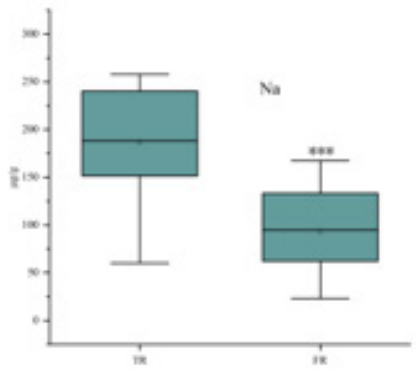
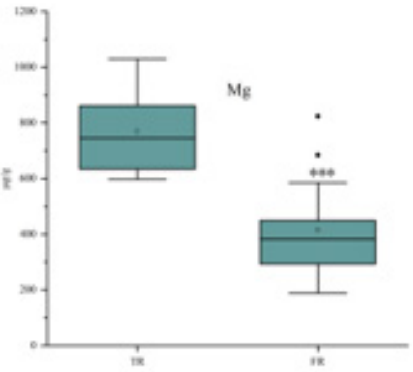
***
TR
FR


Na
***
TR
FR


Mg
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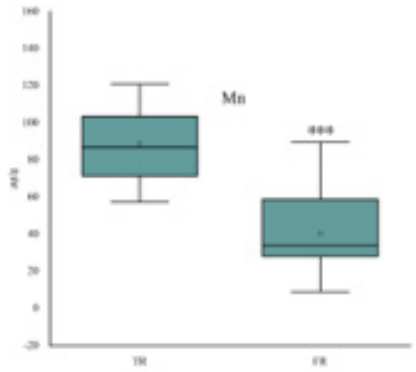
TR
FR


Mn
***
TR
FR

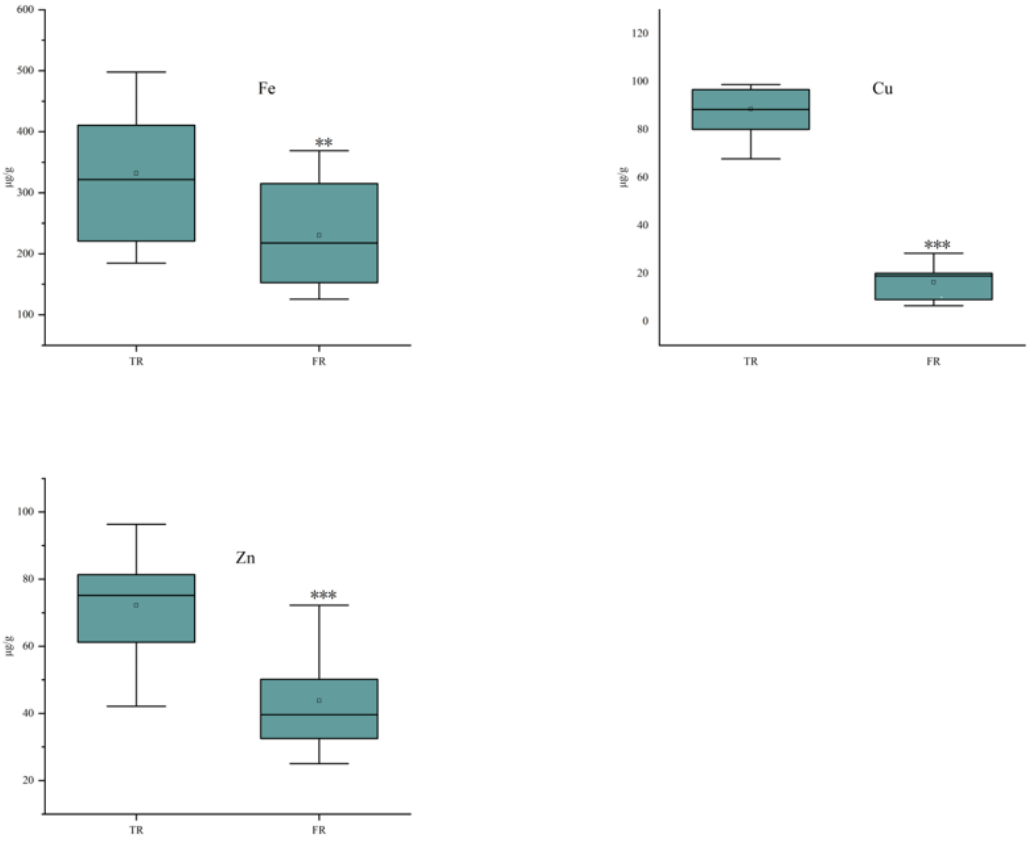


Figure 1: Box plot of 11 components contents in tubers and corresponding fibrous roots (*p<0.05, ** p<0.005, *** p<0.001).

Cluster analysis

The contents of 11 components in RT and FR were analyzed by Between-Groups Linkage method and Euclidean distance square measure and the results were shown in Figure 2. When the clustering distance is 15, the 36 batches of samples were obviously divided into two groups. The first groups were TR1-TR18 samples, all of which were TR of Maidong. In first group the contents of polysaccharides, flavonoids and mineral elements were higher than the average level. Another group was FR1-FR18 samples, all of which were FR of corresponding to the tuberous roots, in this group the contents of total saponins was higher than the average level. The results of cluster analysis showed that the composition of FR was different from that of TR.

PCA and comprehensive evaluation

The content of 11 components were standardized (As a heavy metals, Cu element is a negative index in quality evaluation, so its reciprocal value of content was used) and the feature value greater than 1 was used as the extraction standard to obtain the first three principal components, with a cumulative contribution rate of 72.694% (Table 1), which can be used for the quality identification of the TR and FR. Based on the principal component coefficient of the above 11 indexes, the scores of each principal component were calculated (Table 2), From eigenvectors (Table 1) a comprehensive evaluation function for quality of different types of roots was established $F=1.307F_1+0.141F_2+0.133F_3$. According to the above comprehensive evaluation model, the comprehensive quality scores of different roots and their ranking were calculated, and the results were shown in Table 3. As can be seen from Table 3, in terms of the comprehensive quality ranking of samples, the quality scores of all TR were higher than those of FR samples except TR2, that is, the comprehensive quality of TR is the better. The comprehensive quality scores of FR were negative and the ranking was lower, indicating that the comprehensive quality of these FR was poor.

The PCA scatter plot for principal component analysis is shown in Figure 3. The PCA model could clearly distinguish the samples of Radix Ophiopogon from different plant part. It can be seen that there were differences in the samples of TR and FR. The results are consistent with those of cluster analysis.

PLS-DA and differential markers screening

Content data of 11 components in 36 batches of samples were used to PLS-DA. The results of the score chart and Variable Importance Projection prediction (VIP) were shown in Figures 4 and 5. The cumulative interpretation capacity parameters of PLS-DA model $R^2X=0.689$, $R^2Y=0.983$, prediction capacity parameter $Q^2=0.939$, the above model parameters are all greater than 0.5, indicating

Table 2: Factor scores.

No.	F_1	F_2	F_3	No.	F_1	F_2	F_3
TR1	0.222	0.915	2.372	FR1	-0.321	-1.166	-0.576
TR2	-0.693	2.562	-0.068	FR2	0.370	-1.403	-1.112
TR3	1.489	-0.286	0.532	FR3	-0.245	-1.124	1.647
TR4	1.324	-0.355	-0.459	FR4	-0.135	-1.298	-1.019
TR5	0.162	1.401	0.446	FR5	-1.578	-0.445	0.625
TR6	0.280	0.584	0.046	FR6	-2.192	0.727	-0.356
TR7	-0.649	1.946	1.216	FR7	-1.139	-0.892	-0.807
TR8	1.501	0.481	0.069	FR8	-1.685	0.166	-0.252
TR9	0.624	0.742	0.057	FR9	-0.631	0.465	-1.239
TR10	0.782	0.465	0.252	FR10	-1.161	0.556	-1.130
TR11	1.613	-0.479	-0.214	FR11	-1.237	0.536	0.489
TR12	0.741	1.152	-0.514	FR12	-0.684	-1.032	-1.650
TR13	1.162	-0.022	1.604	FR13	0.068	-1.267	1.902
TR14	1.044	0.416	-1.557	FR14	-0.355	-0.541	0.701
TR15	0.528	0.389	-0.377	FR15	-0.420	-0.934	0.778
TR16	1.317	-0.328	-1.456	FR16	-0.436	-1.624	0.908
TR17	0.143	1.391	-1.261	FR17	-0.955	-0.861	0.270
TR18	1.449	-0.121	0.155	FR18	-0.306	-0.716	-0.022

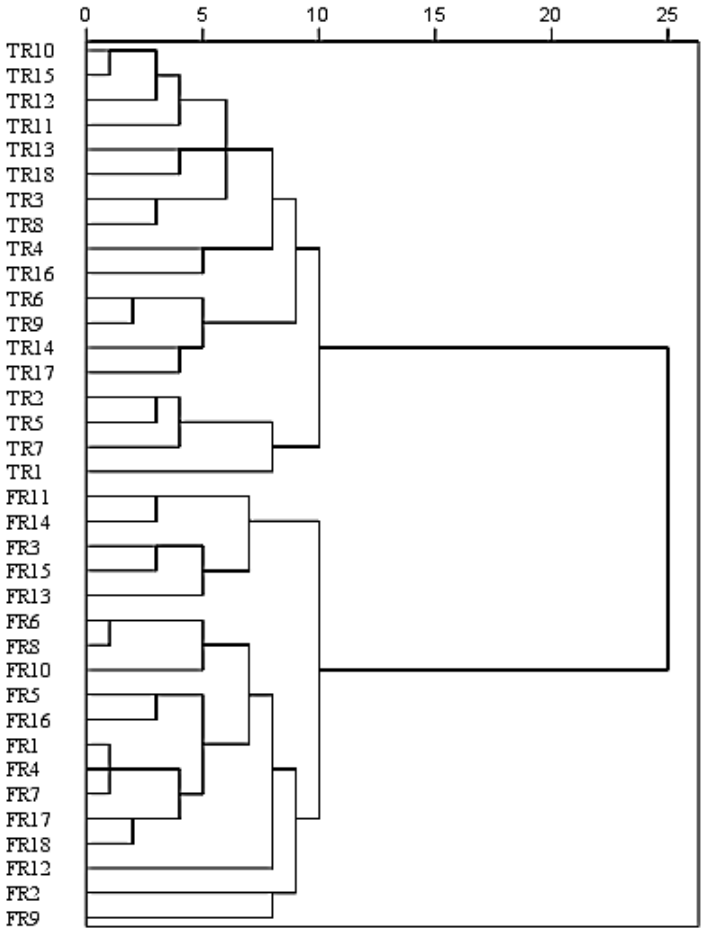


Figure 2: Cluster analysis dendrogram.

Table 3: Quality sequencing of samples.

No.	F	Ranking	No.	F	Ranking
TR1	0.599	11	FR1	-0.472	27
TR2	-0.156	21	FR2	-0.071	19
TR3	1.116	2	FR3	-0.122	20
TR4	0.857	6	FR4	-0.413	25
TR5	0.372	14	FR5	-1.133	34
TR6	0.292	15	FR6	-1.544	36
TR7	-0.043	18	FR7	-1.061	33
TR8	1.172	1	FR8	-1.239	35
TR9	0.567	12	FR9	-0.556	28
TR10	0.669	8	FR10	-0.917	32
TR11	1.082	3	FR11	-0.765	29
TR12	0.635	9	FR12	-0.858	31
TR13	1.054	5	FR13	0.120	17
TR14	0.618	10	FR14	-0.244	22
TR15	0.391	13	FR15	-0.337	24
TR16	0.726	7	FR16	-0.428	26
TR17	0.135	16	FR17	-0.783	30
TR18	1.061	4	FR18	-0.327	23

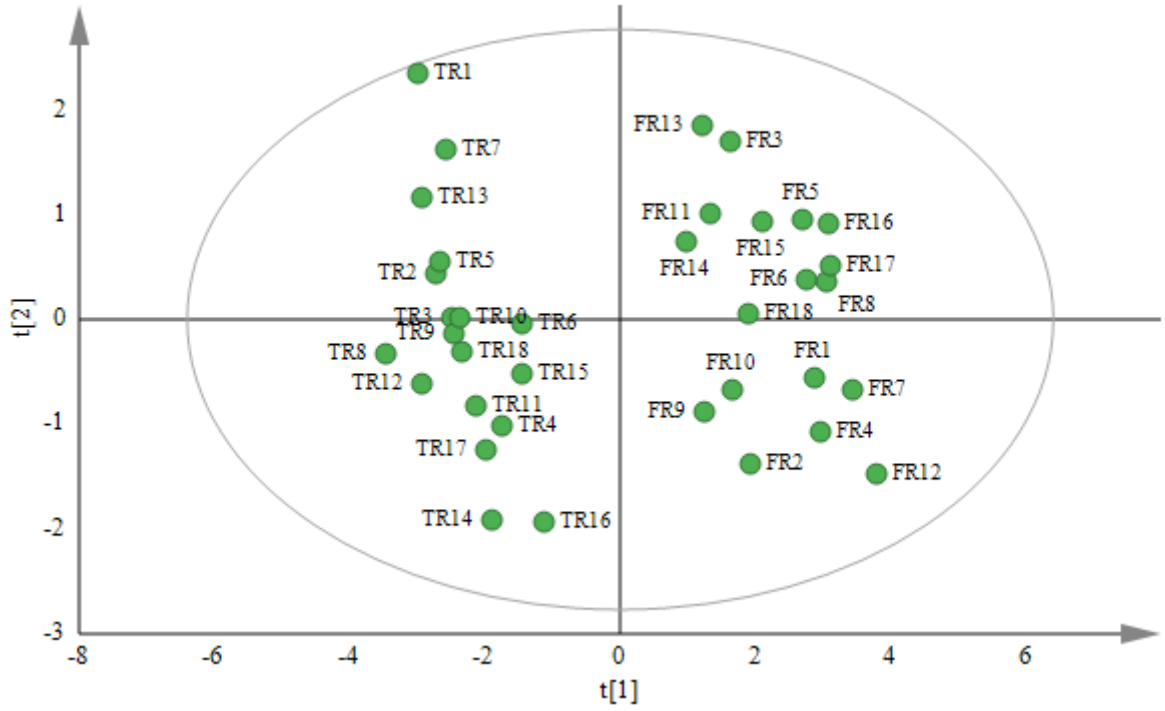


Figure 3: Loading plot of PCA.

that the model is stable and reliable with strong prediction ability,[26,27] and can be used to distinguish FR and TR of Maidong. TR and FR were obviously divided into two categories, indicating that the contents of 11 components were significantly different between them,[27] these results were consistent with cluster analysis and PCA results. Using VIP>1.0 as the standard, four different components were screened, which were total polysaccharides and Cu, Mn, Mg elements, indicating that these components can be used to distinguish the index components of TR and FR of Maidong.

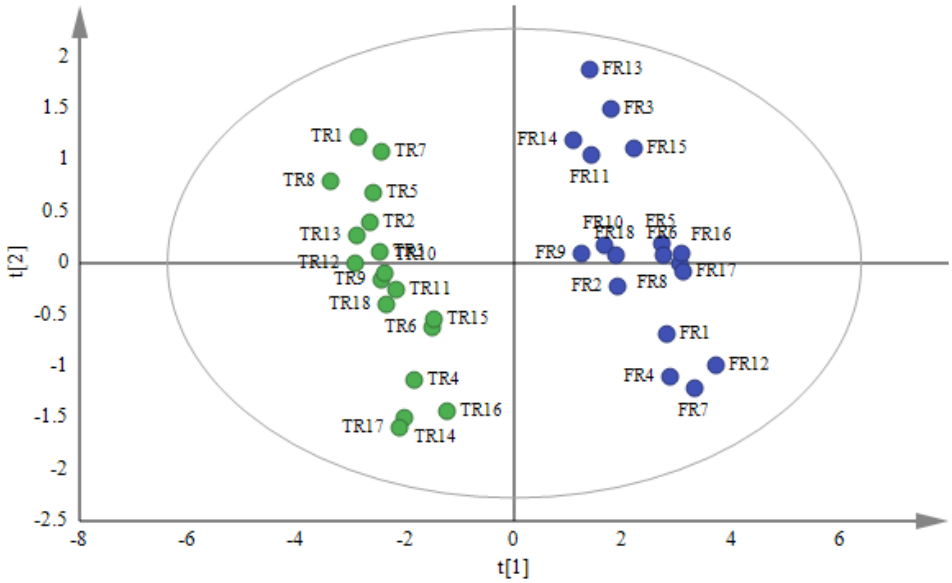


Figure 4: PLS-DA scatter plots.

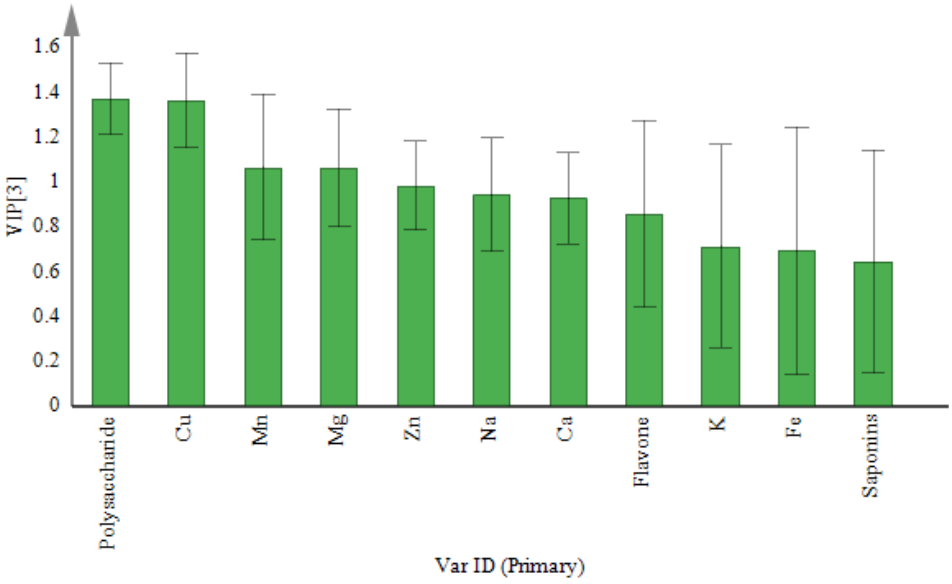


Figure 5: PLS-DA VIP value of samples.

CONCLUSION

In this study, the contents of total saponins, total flavonoids, total polysaccharides and 8 kinds of mineral elements in the TR and FR of Maidong were determined. The results show that the chemical characteristics of the two are obviously different. Both cluster analysis and principal component analysis could clearly categorize and identify TR and FR, principal component score and ranking results showed that the overall quality of TR was better than that of FR. The results of HCA, PCA and PLS-DA showed that TR and FR were obviously classified, and 4 different markers were selected, namely polysaccharides, Cu, Mn and Mg elements. The results of this study showed that only the total saponin content of FR was higher than that of TR, but flavonoids, polysaccharides and K. Ca, Na, Mg, Fe Mn, Zn, Cu are the opposite, suggesting that there may be some differences in pharmacological activity.

Flavonoids have significant antioxidant, anti-inflammatory, antiviral and anti-tumor effects. As a result, TR may be more effective in treating cardiovascular disease, diabetes and other chronic inflammatory diseases.[28] The higher content of polysaccharide, TR may be more effective in enhancing immunity and improving physique.[29] Modern studies have also shown that Fe, Mg, Zn and other elements in Ophiopogon may have a certain correlation with its use in the treatment of appetite loss,

tumor inhibition and prevention and treatment of cardiovascular diseases,[14] Zn and Cu elements are related to the effect of nourishing Yin and qi.[14] Therefore, TR may be more effective in the above pharmacological aspects.

The chemical composition content of TR and FR is very different and there is no quality uniformity between the two. From the point of view of the content of active ingredients, the quality of TR is obviously better than that of FR and whether the FR can replace the TR for medicinal purposes remains to be studied.

In conclusion, the component content determination combined with multivariate statistical analysis method established in this study revealed the differences in chemical components of TR and FR. It can be used for the quality evaluation and plant part identification of Maidong from Leshan, providing the chemical basis for the difference of drug efficacy between the two parts.

This study further explains why FR cannot completely replace TR; otherwise, it will affect the quality of medicinal materials and drug safety. To sum up, the current quality standard is difficult to complete the Maidong overall quality control and evaluation; On the other hand, TR and FR the material basis has statistical difference, indicating that the quality of the two is not one consistency, uniformity and therefore cannot be controlled by a uniform standard.

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ABBREVIATIONS

Maidong: *Ophiopogon japonicus*; **TR:** Tuberous roots; **FR:** Fibrous roots; **PCA:** Principal component analysis; **CA:** Clustering analysis; **PLS-DA:** Partial least squares discriminant analysis.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

The contents of total saponins, total flavonoids and total polysaccharides and eight kinds mineral elements in tuberous roots and fibrous roots of *Ophiopogon japonicus* from Leshan were determined. Cluster analysis, partial least squares discriminant analysis and principal component analysis model were used for discrimination and comprehensive quality evaluation of tuberous root and fibrous root of *Ophiopogon japonicus*. Result shown that all samples could be classified into two groups, which could to some extent reflect the quality differences of tuberous roots and fibrous roots. The most important marker that distinguished the two type roots were total polysaccharides and Cu, Mn, Mg elements.

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