A Rapid New Approach for the Quality Evaluation of the Folk Medicine *Dianbaizhu* Based on Chemometrics

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Dianbaizhu, a folk medicine from *Gaultheria leucocarpa* BLUME var. *yunnanensis* (FRANCH.) T. Z. HSU & R. C. FANG (Ericaceae) used as an antirheumatic, has multiple plant origins and officinal parts. A rapid high-performance liquid chromatography with diode array detector (HPLC-DAD) method was established for the simultaneous determination of the characteristic ingredient methyl benzoate-2-*O*- β -D-glucopyranosyl(1 $\rightarrow$ 2)-[*O*- β -D-xylopyranosyl(1 $\rightarrow$ 6)]-*O*- β -D-glucopyranoside and seven bioactive constituents in eight *Gaultheria* species. This chromatographic method is precise, accurate, and stable. Kruskal–Wallis analysis, hierarchical cluster analysis, and factor analysis were used to analyze the content of reference compounds in different *Gaultheria* species and officinal parts. The analyses showed significant differences ($p < 0.05$) in *Gaultheria* species but few differences ($p > 0.05$) in their medicinal parts. *G. leucocarpa* var. *yunnanensis* appeared to be the best among the *Gaultheria* species tested for the treatment of rheumatic diseases. Taken together, the results show that this simultaneous quantification of multiple active constituents using HPLC-DAD combined with chemometrics can be reliably applied to evaluate the quality of *Dianbaizhu*.

Key words *Dianbaizhu*; Chinese folk medicine; quality evaluation; chemometrics; HPLC coupled with diode array detector

Dianbaizhu, the whole plant or root or aerial part of *Gaultheria leucocarpa* BLUME var. *yunnanensis* (FRANCH.) T. Z. HSU & R. C. FANG (Ericaceae), is widely used for the treatment of rheumatic disease by ethnic minority people.¹⁾ Some other plants of *Gaultheria* genus, *Gaultheria fragrantissima*, *Gaultheria leucocarpa* var. *pingbienensis*, and *Gaultheria griffithiana*, are also regarded as *Dianbaizhu* by many minority nationalities to treat rheumatism in clinical practice.^{1,2)} The whole plant of the above-mentioned species is used as *Dianbaizhu* in the Bai ethnic minority. The aerial part is deemed to be *Dianbaizhu* in the other minority nationalities.^{2–4)} In addition, the leaves of *G. fragrantissima* are sold as *Dianbaizhu* in Chuxiong medicine market.

The *G. leucocarpa* var. *yunnanensis* is a research focus in the genus *Gaultheria*. Our project team and others reported salicylic acid derivatives, flavonoids, terpenoids, lignins and organic acids were the primary ingredients of this plant.^{1,2,5)} Methyl benzoate-2-*O*- β -D-glucopyranosyl(1 $\rightarrow$ 2)-[*O*- β -D-xylopyranosyl(1 $\rightarrow$ 6)]-*O*- β -D-glucopyranoside (MSTG-B), together with salicylic acid and methyl salicylate with favorable anti-inflammatory activities were confirmed as non-steroid anti-inflammatory drugs (NSAIDs).^{6–8)} Furthermore, the quercitrin,^{9,10)} hyperoside,¹¹⁾ cynaroside,¹²⁾ chlorogenic acid^{12–14)} and vanillic acid¹⁵⁾ in *G. leucocarpa* var. *yunnanensis*, possess reducing the inflammation abilities. Our group current study also supported that the above-mentioned compounds were principle anti-rheumatic constituents by anti-rheumatism fingerprint-efficacy relationship on the basis of *G. leucocarpa* var. *yunnanensis*.

To the best of our knowledge, now, the evaluation to the quality of genus *Gaultheria* always takes one or a type of index ingredient without anti-inflammatory effect as the standard compound.^{16–20)} And it is just only concentrated on the

whole plant of *G. leucocarpa* var. *yunnanensis*.^{21–23)} Here, a new approach was established to evaluate the quality on *G. leucocarpa* var. *yunnanensis* with multiple active constituents prepared by our group as index compounds. And it can be used for some other species in *Gaultheria* together with various officinal parts. So to sum up, by using the established method and several chemometric methods, the medicinal plant resources of *Dianbaizhu* were assessed well.

Experimental

Herb Materials Thirty-six *Dianbaizhu* samples from eight species, collected in different producing areas in Yunnan province of China were authenticated by Dr. Shengli Wei, School of Beijing University of Chinese Medicine, Beijing (shown in Table 1).

Instrumentation and Reagents A Waters HPLC system consisting of a quaternary HPLC pump (Waters Model 2695), an auto injector (Waters 2695 separation module), a 100 μ L loop, a Waters 2695 column oven and a photodiode array detector (Waters 2998) were employed with a data system (Empower-2) in this study. An ultrasonic cleaner (KQ-250DE, Kunshan, Jiangsu, China) was used for extraction. Water for HPLC was purified by a Millipore Synergy UV system (18.2 M Ω cm, Billerica, MA, U.S.A.), and then used for all the solutions and dilutions. HPLC grade acetonitrile (ACN) and methanol (MeOH) of Fisher Scientific (Fairlawn, NJ, U.S.A.), together with chromatographic grade acetic acid and phosphoric acid from Beijing Chemical Works (Beijing, China) were employed.

The 70% ethanol extract of *G. trichoclada* was chromatographed on silica gel column, AB-8, MCI-gel CHP20P and ODS-A to give chlorogenic acid (1), vanillic acid (2), MSTG-B (3), hyperoside (4), cynaroside (5), salicylic acid (6), quercitrin (7) and methyl salicylate (8), respectively. And their purity all reached more than 98%. They were used as index compounds

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Table 1. The Samples Information

Batches	Species	Sample source	Harvesting time	Medicinal parts ^{a)}
A	<i>G. trichoclada</i>	Chuxiong, Yunnan	June 2011	<i>a, c, d</i>
B	<i>G. semi-infera</i>	Dali, Yunnan	August 2011	<i>a, b, c, d</i>
C	<i>G. griffithiana</i>	Baoshan, Yunnan	December 2011	<i>a, c, d</i>
D	<i>G. leucocarpa</i> var. <i>yunnanensis</i>	Dali, Yunnan	December 2011	<i>a, c, d</i>
E	<i>G. leucocarpa</i> var. <i>hirsuta</i>	Baoshan, Yunnan	December 2011	<i>a, b, c, d</i>
F	<i>G. hookeri</i>	Baoshan, Yunnan	December 2011	<i>a, b, c, d</i>
G	<i>G. leucocarpa</i> var. <i>pingbienensis</i>	Qujin, Yunnan	August 2012	<i>a, b, c, d</i>
H	<i>G. fragrantissima</i>	Dali, Yunnan	August 2012	<i>a, b, c, d</i>
I	<i>G. fragrantissima</i>	Dali, Yunnan	October 2012	<i>a, b, c, d</i>
J	<i>G. fragrantissima</i>	Dali, Yunnan	December 2012	<i>a, b, d</i>

a) *a*: Aerial parts, *b*: Whole plant, *c*: Leaf, *d*: Branch.

Fig. 1. Structures of the Eight Active Ingredients

in this study and their chemical structures were shown in Fig. 1.

Preparations of Sample and Standard Solution All the dried samples of *Dianbaizhu* were crushed into powder in a grinder, and then passed through a 20-mesh sieve. Accurately weigh 1.0 g powder, in 20 mL ethanol–water (1:1, v/v), extract by dint of sonication (power: 250 W, frequency: 99 kHz) at 25°C for 60 min. Then the extract was replenished to 20 mL with extraction solvent, shook well and filtered with a syringe filter (0.22 μ m, Alltech, Beerfield, IL, U.S.A.) prior to injecting an aliquot of 10 μ L solution into the HPLC system.

Standards (1–8) were weighed accurately and dissolved in ethanol–water (1:1, v/v) to prepare the stock solutions at the concentrations of 214, 192, 230, 510, 204, 250, 202 and 204 μ g/mL, respectively. The stock solution was appropriately diluted with ethanol–water (1:1, v/v) to obtain series of working standard solutions. All solutions were filtered through 0.22 μ m membrane filter before HPLC analysis.

HPLC and Data Analysis All separations were carried out on a Thermo Hypersil Gold C₁₈ column (150 mm $\times$ 4.6 mm, 5 μ m) by HPLC system (Waters 2695) with diode array detection (DAD). The optimum detection wavelength was set at 290 nm according to the result of wavelength scanning from 200 to 400 nm. The mobile phase comprised 0.5% acetic acid in water (solvent A) and acetonitrile (solvent B). A linear gradient elution was performed as follow: 0–12 min, 10% B; 12–25 min, 10–20% B; 25–40 min, 20% B; 40–50 min, 20–60% B; 50–55 min, 60–75% B. The flow rate was 1.0 mL/min and the injection volume was 10 μ L.

In this study, all the data analyses were performed by the SPSS 20.0 (SPSS Science, Chicago, IL, U.S.A.).

Results and Discussion

Sample Analysis Based on Medical Statistics The whole plant or the aerial-part of some *Gaultheria* species is used as *Dianbaizhu* in several minority nationalities.^{24,25)} And some

Table 2. Content of Eight Compounds (mg/g, $n=3$)

Samples	Active ingredients							
	1	2	3	4	5	6	7	8
Aa	0.23	0.04	0.07	21.19	0.03	0.78	0.24	1.26
Ac	0.13	0.05	0.05	9.17	—	0.43	0.16	2.12
Ad	0.44	0.04	0.07	44.76	0.11	0.56	0.47	3.94
Ba	0.20	0.06	—	1.87	0.15	0.06	0.35	1.14
Bb	0.29	0.03	—	2.05	0.10	0.19	0.23	1.41
Bc	0.21	0.02	0.08	1.04	0.04	0.10	0.04	1.52
Bd	0.97	0.03	—	4.00	4.39	0.44	0.22	2.63
Ca	2.39	0.05	0.05	2.37	2.00	0.19	6.64	1.01
Cc	3.13	0.03	—	0.73	0.40	0.47	0.56	1.24
Cd	0.77	0.03	—	1.20	0.06	0.09	0.16	0.16
Da	11.19	0.10	0.05	341.99	0.98	1.00	5.07	0.13
Dc	9.11	0.11	0.72	253.33	0.24	0.77	2.19	0.05
Dd	17.14	0.70	—	356.73	2.83	3.79	14.34	0.06
Ea	0.49	0.09	0.17	46.15	0.06	0.29	1.01	0.24
Eb	0.38	0.02	0.27	14.22	—	0.13	0.43	0.48
Ec	0.08	0.05	0.06	6.99	0.01	0.18	0.45	1.62
Ed	3.33	0.04	0.08	131.32	0.05	0.49	3.49	0.94
Fa	0.2	0.04	—	14.56	—	0.15	0.10	1.59
Fb	0.29	0.05	0.28	35.60	0.09	0.26	0.13	2.38
Fc	0.06	0.03	—	9.89	—	0.16	0.09	3.18
Fd	1.31	0.11	—	31.34	0.45	0.35	0.09	2.59
Ga	0.18	0.06	0.09	12.32	0.01	0.27	0.38	1.15
Gb	0.18	0.06	0.1	11.44	0.03	0.31	0.35	1.21
Gc	0.05	0.05	0.12	0.60	—	0.14	0.01	1.01
Gd	0.82	0.07	0.07	71.62	—	0.41	1.96	0.61
Ha	0.28	0.04	0.05	15.44	0.01	0.15	0.21	3.42
Hb	0.22	0.03	—	3.88	0.27	0.14	0.37	1.68
Hc	0.11	—	—	—	0.12	0.28	—	1.68
Hd	0.10	0.03	—	—	1.48	0.17	0.27	0.63
Ia	0.54	—	0.01	0.43	0.48	0.14	0.04	5.14
Ib	0.23	—	0.03	3.23	0.59	—	0.18	2.44
Ic	0.15	0.02	0.02	0.16	0.01	—	0.01	3.00
Id	0.16	0.03	0.02	3.59	0.44	—	0.39	10.52
Ja	0.31	0.02	—	8.33	0.28	0.08	0.08	3.57
Jb	0.30	—	—	0.47	1.12	0.15	0.08	3.60
Jd	0.78	0.02	—	3.58	0.50	0.10	0.30	8.67

—: Below the LOD.

southern ethnic minorities directly use the leaves and branches as internal or external medicine for expelling wind-damp.^{4,24} Here, our investigated subjects included 10 batches of 36 samples, which consisted of the aerial-part (*a*), whole plant (*b*), leaf-part (*c*) and branch-part (*d*) of *Dianbaizhu* (Table 1). The content of eight active ingredients in all samples was shown in Table 2. Obviously, there was a wide variance at the content of all analytes in the 10 batches. It was due to various kinds of factors, such as plant origins, geographical conditions, climates, and some other hidden factors.

Optimization of Extraction Conditions To obtain the largest quality of the chemical constituents from *Dianbaizhu*, two extraction methods were tried, ultrasonic extraction (setting temperature: 25°C) and heating reflux extraction. The former method was slightly better than the latter. Thus, ultrasonic extraction was chosen and the extraction optimization was executed. Three main extraction factors of the ultrasonic extraction were optimized through a $L_9(3^3)$ orthogonal experiment. Table 3 demonstrated the factors and levels of the or-

thogonal experiment. The sum of peaks area of eight analytes (**1–8**) was chosen as the norm to assess the optimal extraction condition. Three factors affected extraction ability were in the order: $B>A>C$, according to the range analysis of the orthogonal experiment (Table 3). Consequently, the optimal extraction condition was that 1.0g powder of dried sample extracted once with 20mL 50% ethanol by ultrasonic extraction for 60min.

Optimization of HPLC Conditions In this section, the main chromatographic optimization program included selecting the composition and gradient of mobile phase, column temperature as well as detection wavelength. Several mobile phases, including MeOH–water, ACN–water, ACN–0.1% phosphoric acid, ACN–0.5% acetic acid and ACN–1.0% acetic acid were screened. Finally, ACN–0.5% acetic acid was regarded as the optimal mobile phase, for it giving better separation and lower column pressure. The most appropriate temperature was set at 30°C. The following two points were thought out, (i) the maximum UV absorption wavelengths

Table 3. The Orthogonal Design of Extraction Conditions

Run no.	A ^{a)}	B ^{b)}	C ^{c)}	A _Σ ^{d)}
1	20	30	30	1476791
2	20	50	60	1768716
3	20	70	90	1430540
4	40	30	90	1323386
5	40	50	30	1514868
6	40	70	60	1523278
7	60	30	60	1448059
8	60	50	90	1591427
9	60	70	30	1576916
K ₁ ^{e)}	4676047	4248236	4591496	
K ₂	4361532	4875011	4669018	
K ₃	4616402	4530734	4393467	
k ₁ ^{f)}	1558682	1416078	1530498	
k ₂	1453844	1625003	1556339	
k ₃	1538800	1510244	1464489	
Range	104838	208925	91850	
Optimized scheme	A1	B2	C2	
Primary and secondary order	2	1	3	

a) A: Solvent volume (mL). b) B: Ethanol concentration (%). c) C: Sonication time (min). d) A_Σ: The area sum of 8 peaks (from analytes 1 to 8). e) K: The total value of the same level of the same factor. f) k: The average value of the same level of the same factor.

of compounds (1–8) being 326, 220, 289, 350, 255, 204, 256 and 290nm, respectively, and (ii) the strong UV absorption of ACN presents approximately 210nm. Then, the 290nm was found to be optimal, since the eight analytes revealed adequate absorptions. The favorable separation of eight mixed standards presented within 55min under the optimal HPLC chromatograms conditions, as well as that of the samples (Fig. 2). Eight peaks in the chromatogram of the tested samples were corresponded with those of the eight standards under the same conditions, and eight standards were identified in the tested samples. In addition, the standard-addition method was also a way to confirm the peaks of analytes.

Method Validation The stock solutions containing eight components were further diluted to five appropriate concentration levels. The each calibration curve was established with six series concentration injected in triplicate, and plotted on the basis of the corresponding relation between peak area and concentration. For all analytes, good linearities ($r>0.9998$) were achieved within the wide concentration range. The results of linear regression were given in Table 4.

As the standard stock solutions continuously diluted, the limit of detection (LOD) and the limit of quantity (LOQ) of the eight components could be determined respectively, when

Fig. 2. HPLC Chromatograms of (A) Mixed Standards and (B) 36 Tested Samples

(A) Peak 1 to peak 8 are chlorogenic acid, vanillic acid, MSTG-B, hyperoside, cynaroside, salicylic acid, quercitrin and methyl salicylate, respectively.

the signal-to-noise ratio (S/N) was 3:1 and 10:1. The results can be seen in Table 5.

The precision of the chromatographic method was constituted of intra- and inter-day precision. The mixed standard solutions of three different concentrations were analyzed six times within one day. Repeat this action twice per day for three days, and the RSDs were intra- and inter-day precision, respectively. The precision was based on RSD. Here, the precision RSD values of the retention times and peak areas were less than 2.21%, which demonstrated the good reproducibility of the analytical method (Table 5). To determinate method repeatability, we made an assay, the six different working solutions of the same sample. The RSD value of the retention times was within the range of 0.01 to 0.58% and that of peak areas was in the range from 2.18 to 3.49%. It illustrated the method had good repeatability. The sample solution was repositioned at room temperature to study sample stability in triplicate at 0, 2, 4, 8, 12 and 24 h. The RSD value within the range of 0.36 to 2.19% revealed that the method was stable. To investigate the accuracy of the method, recovery determination was applied by adding the eight standards into 1.0 g *G. hookeri* sample (whole plant), and making their content become approximately 80, 100 and 120% in the sample, respectively. And the spiked samples were extracted and analyzed in triplicate as described above. The test was repeated three times for the calculation of recovery at each added level. The recoveries of all eight compounds were between 97.59 and 105.96%, together with the maximal RSD value of 3.24% (Table 6). It indicated the analysis method possessing good accuracy. The above results illustrated this analytic method being accurate, sensitive, and stable, which revealed a well accuracy for the determination of the eight analytes.

Statistical Studies In order to investigate whether active

components content was affected by different plant origins or medical parts, a non-parametric method, the Kruskal–Wallis analysis (KWA), was employed. It is the non-parametric analogue of a one-way ANOVA.²⁶⁾ This method was often applied in the quality control of traditional Chinese medicine (TCM) and used to test the difference between multiple samples with an abnormal distribution.^{27,28)} The KWA result was given in Table 7. Interestingly, the values of H statistic in plant origins analysis were greater than those in medical parts. The p values of the former ($p < 0.05$) were far less than those of the latter ($p > 0.05$). It meant that the components content had significant differences among various plant origins. The opposite result was present in the medical parts. Particularly, the content of compounds **2**, **4**, **6** and **8** had greatly significant differences ($p < 0.01$) among the eight *Gaultheria* species. Therefore, they can be considered as the index components more scientifically to control the quality of *Gaultheria* species. The approximate anti-rheumatism effects ($p > 0.05$) of different medical parts were shown in Table 7. It explained the phenomenon, various medicinal parts used to treat rheumatism by many ethnic minorities.

To further search the resemblances (in some sense or another) and differences among plant origins, another chemometric technique, the hierarchical cluster analysis (HCA) was carried out. HCA is a multivariate analysis technique, and it can divide samples into several groups in light of samples' similarities. It is usually used in the taxonomy of TCM. In this study, it was also applied to classification of 36 *Dianbaizhu* samples. The content of 8 constituents in 36 samples from various plant species formed a matrix of 36×8 (Table 2). As Fig. 3 displayed, the quality characteristics of all samples became more clear by using HCA. Upon on the squared Euclidean distance among samples, all 36 samples were classified into two broad categories, clusters I and II. The eight ingredients had different content distribution in the two clusters. It was very interesting that cluster II only contained one species, *G. leucocarpa* var. *yunnanensis* (batch D). This suggested that its quality may differ from the others'. Combined with the results of KWA, it further revealed that *G. leucocarpa* var. *yunnanensis* possessed the highest quality in all tested plant origins. And it is a good explanation for the fact, *G. leucocarpa* var. *yunnanensis* being as the dominant plant species in the treatment of rheumatism.

Factor analysis (FA), another multivariate statistical technique, can reduce the data dimensionality and seek for hidden representative factors based on the differences in samples. It

Table 4. Linear Regression of Eight Compounds

Compounds	Linear regression	R^2	Range ($\mu\text{g/mL}$)
1	$y = 22904.05x - 41280.91$	0.9999	1.9–214.1
2	$y = 19495.91x - 12248.73$	0.9998	1.9–192.0
3	$y = 9302.05x + 874.47$	0.9997	2.33–153.0
4	$y = 867.46x - 491.27$	1	12.0–510.0
5	$y = 9680.34x + 10446.46$	0.9999	4.1–204.0
6	$y = 12622.27x - 22341.38$	0.9997	1.5–250.0
7	$y = 10271.84x + 1371.84$	0.9999	1.4–202.0
8	$y = 9816.20x - 2967.06$	0.9998	2.0–102.0

Table 5. Precision, Repeatability, Stability, LOD and LOQ

Compounds	Intra-day precision ($n=6$)		Inter-day precision ($n=3$)		Repeatability ($n=6$)		Stability	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)
	RSD of RT ^{a)} (%)	RSD of PA ^{b)} (%)	RSD of RT (%)	RSD of PA (%)	RSD of RT (%)	RSD of PA (%)	RSD of PA (%)		
1	0.10	1.13	0.21	1.43	0.58	2.52	0.99	0.56	1.75
2	0.09	0.63	0.12	1.08	0.33	3.49	1.92	0.31	0.98
3	0.07	0.95	0.31	1.11	0.46	3.26	0.72	0.83	2.61
4	0.12	1.81	0.16	2.21	0.07	2.26	0.71	3.21	10.70
5	0.14	0.78	0.32	1.34	0.08	2.81	2.19	0.30	1.04
6	0.20	0.71	0.25	1.71	0.06	2.97	0.36	0.42	1.41
7	0.18	0.80	0.21	1.31	0.04	2.90	0.76	0.36	1.20
8	0.08	0.53	0.12	0.94	0.01	2.18	1.02	0.21	0.62

a) RT: Retention time. b) PA: Peak-area percentage.

Table 6. Recoveries of the Eight Compounds

Compounds	Contained ^{a)} (μg)	Added (μg)	Found ^{b)} (μg)	Recovery ^{c)} (%)	Mean (%)	RSD (%)
1	293.2	354.0	661.5	104.03	101.51	2.36
		290.2	581.2	99.24		
		226.5	522.6	101.28		
2	48.1	58.3	106.0	99.31	98.60	2.11
		48.1	94.4	96.25		
		40.4	88.6	100.24		
3	280.2	312.0	594.5	100.74	98.90	2.47
		304.0	583.7	99.82		
		266.0	535.9	96.13		
4	355.9	416.2	788.2	103.86	100.14	3.24
		357.7	705.8	97.81		
		285.2	637.6	98.77		
5	86.7	104.3	199.9	108.50	105.96	2.68
		86.4	175.6	102.89		
		69.3	160.5	106.49		
6	262.8	316.5	569.2	96.80	97.59	2.58
		262.0	513.2	95.57		
		211.2	474.9	100.42		
7	131.9	157.9	284.2	96.45	98.96	2.83
		130.2	260.1	98.46		
		105.2	239.2	101.99		
8	2383.8	2865.6	5217.3	98.87	101.15	2.57
		2374.7	4773.2	100.61		
		1923.1	4383.8	103.99		

a) Contained: the actual compound's content of 1.0 g sample. b) Found: the amounts of the compound found in the sample after addition of the standard stock solutions. c) Recovery (%) = [(Found - Contained)/Added] × 100%.

Table 7. Kruskal–Wallis Analysis of Plant Origins and Medical Parts

Components	Plant origins analysis		Medical parts analysis	
	H Statistic	p Value	H Statistic	p Value
1	16.944	0.049*	7.577	0.056
2	23.846	0.005**	3.190	0.363
3	19.293	0.023*	2.116	0.549
4	23.408	0.005**	3.918	0.270
5	18.375	0.031*	6.091	0.107
6	22.459	0.008**	1.754	0.625
7	19.177	0.024*	4.265	0.234
8	27.016	0.001**	0.252	0.969

* $p < 0.05$ and ** $p < 0.01$ indicated a statistically significant difference among the distribution of components content.

has been widely applied in the chemometrics research of active ingredients.^{29–31)} In the research on TCM, FA can be used to seek key and representative chemical components from the complex chemical system of Chinese medicine (CM). The factoring types include principal component analysis (PCA), canonical factor analysis (CFA) and others. Here, PCA was employed as the method of FA.

The FA displayed the first two principal factors ($PF1$ and $PF2$), were chosen as the representative, and they can explained nearly 77% information of eight active ingredients (Table 8). Moreover, $PF1$ and $PF2$ could be used for further quality assessment of *Dainbaizhu* samples. They could be severally expressed as follow:

Fig. 3. Dendrogram of Clustering of 36 Samples

The clustering method used Ward linkage and the calculation of sample interval used the squared Euclidean distance.

Table 8. FA and Data Distribution Analysis Results of the Eight Analytes in 36 Samples

Compounds	Total	Initial eigenvalues ^{a)}		Compound matrix		Mean	95% Confidence interval	
		% Variance	% Cumulative	PF1	PF2		Lower limit	Upper limit
1	4.75	59.36	59.36	0.959	-0.087	1.58	0.37	2.78
2	1.40	17.51	76.87	0.917	0.140	0.06	0.02	0.10
3	0.76	9.53	86.41	0.176	-0.837	0.07	0.02	0.11
4	0.62	7.73	94.14	0.891	0.249	40.71	10.49	70.93
5	0.27	3.43	97.56	0.500	0.552	0.48	0.17	0.79
6	0.13	1.66	99.23	0.950	0.113	0.37	0.15	0.58
7	0.03	0.42	99.65	0.934	0.145	1.14	0.23	2.05
8	0.03	0.35	100.00	-0.372	0.522	2.17	1.42	2.92

a) Extraction method: principal component analysis.

Table 9. Rotated Component Score and Component Score Coefficient

Compounds	Rotated component matrix ^{a)}		Component score coefficient matrix ^{b)}	
	PF1	PF2	PF1	PF2
1	0.917	0.293	0.184	0.105
2	0.925	0.063	0.210	-0.055
3	-0.011	0.855	-0.094	0.591
4	0.816	0.437	0.144	0.215
5	0.608	-0.430	0.188	-0.362
6	0.952	0.096	0.213	-0.035
7	0.943	0.062	0.214	-0.058
8	-0.250	-0.590	0.005	-0.381

a) Rotation method: varimax with kaiser normalization. b) Extraction method: principal component analysis. The authors declare no conflict of interest.

$$PF1 = 0.959X_1 + 0.917X_2 + 0.176X_3 + 0.891X_4 + 0.5X_5 + 0.95X_6 + 0.934X_7 - 0.372X_8$$

$$PF2 = -0.087X_1 + 0.14X_2 - 0.837X_3 - 0.249X_4 + 0.552X_5 + 0.113X_6 + 0.145X_7 + 0.552X_8$$

Rotated component matrix displayed that, PF1 was most correlated with active compounds 1, 2, and 4–7, while PF2 was most correlated with compounds 3 and 8 (Table 9). The two score variables originated from PF1 and PF2 were representative of the eight compounds' content with only a 23% loss of the content information (Table 9). The content mean values of eight analytes, together with their lower and the upper limits were given in Table 8. Obviously, there were great deals of differences among the range of those limits. And each lower limit value could be used as the minimal limits to control the quality of *Dianbaizhu*. The mainstream species, *G. leucocarpa* var. *yunnanensis* could be chosen as a reference for the quality evaluation of other *Gaultheria* species.

The Fig. 4 showed that, eight active constituents were classified into two groups, group I and II. What's most interesting here, the group I only contains two active ingredients (3 and 8) possessing a similar chemical structure with aspirin. For all we know, MSTG-B (3) only isolated from *G. leucocarpa* var. *yunnanensis* and *Lycopersicon esculentum*, exhibited remarkable anti-inflammatory activity.^{6,32)} It states that FA is very effective for the study on bioactive components in TCM. Another group, group II, mainly included organic acids (1, 2 and 6)^{15,33–35)} and flavonoids (4, 5 and 7)^{36–40)} which possess good anti-inflammatory activities. The two groups severally

Fig. 4. Factor Plot in Rotated Space of Eight Active Ingredients

Compounds 1 to 8 are chlorogenic acid, vanillic acid, MSTG-B, hyperoside, cynaroside, salicylic acid, quercitrin and methyl salicylate, respectively.

represented two types of significant index components. Thus, the two types of index components could be considered as the primary representative ingredients of *Dianbaizhu*. Furthermore, they should be included in the quality control and evaluation of *Dianbaizhu*.

To compare intuitively the quality of eight *Gaultheria* species and four officinal parts, the score of PF1 and PF2 in all 36 samples was plotted with 2D graph (Fig. 5). In the graph, PF1 and PF2 as a whole represented all eight active compounds. Generally, the pharmacological effects of active constituents are in positive correlation with their dose within certain limits.^{41–43)} Accordingly, the score of PF1 and PF2 could reflect the quality of *Dianbaizhu* in some degrees. The higher factor score was, the better quality was (Fig. 5). Qualitatively, the species 4 (*G. leucocarpa* var. *yunnanensis*) was much better than the other tested species. The species 8 (*G. fragrantissima*) maybe had the worst quality in the eight species. And for the rest of species, they had a similar quality. Additionally, the content distributions of all eight active ingredients had less uniformity in the species than those in the medicinal parts (Fig. 5). This demonstrated that there were great quality differences among *Gaultheria* species, and the officinal parts had no significant quality differences in view of the compounds content. These inferences were consistent with the results of KWA.

Apart from the statistical analysis above, the Table 2 obviously showed the fact that, among eight active ingredients,

Fig. 5. The 2D Score Plot of (A) 8 Plant Species and (B) 4 Medicinal Parts on PF1 and PF2

(A) Species from A to J are *G. trichoclada*, *G. semi-infera*, *G. griffithiana*, *G. leucocarpa* var. *yunnanensis*, *G. leucocarpa* var. *hirsuta*, *G. hookeri*, *G. leucocarpa* var. *pingbienensis* and *G. fragrantissima*, respectively (species H, I and J are the same). (B) Medicinal parts: a: aerial parts, b: whole plant, c: leaf, d: branch.

both salicylic acid (6) and methyl salicylate (8) existed in about 92% of samples. Maybe it is because some injurious insects and pathogen infections cause accumulation of salicylic acid (6) and synthesis of volatile methyl salicylate (8) in *Dianbaizhu*.^{44–46} And then, salicylic acid (6) as a messenger transfers to other parts of plant via vascular system, resulting in increased systemic resistance to encroachment.⁴⁷ Analogously, methyl salicylate (8) as airborne messenger floats to other parts of the plant and neighboring plants and enhanced the ability to resist external invasion.⁴⁷

Conclusion

A quality evaluation procedure of *Dianbaizhu* was successfully established using one distinctive ingredient MSTG-B and seven principal ingredients as reference compounds. The optimized procedure based on the eight active constituents was accurate, reliable and comprehensive. It is very suitable for the quality control of several *Gaultheria* species.

From multivariate statistical results of KWA, HCA and FA, we found that species origins had a significant effect on the

Dianbaizhu quality, while the medicinal parts' did not exhibit. In addition to *Gaultheria* species origins and medicinal parts as well as their growing environment may also affect the quality of medicinal material. Among eight species, *G. leucocarpa* var. *yunnanensis* is not only the uppermost plant species, but also the best one for the treatment of rheumatic diseases according to the comprehensive analysis.

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