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ORIGINAL ARTICLE

Distinguishing the Rhizomes of *Atractylodes japonica*, *Atractylodes chinensis*, and *Atractylodes lancea* by Comprehensive Two-Dimensional Gas Chromatography Coupled with Mass Spectrometry Combined with Multivariate Data Analysis

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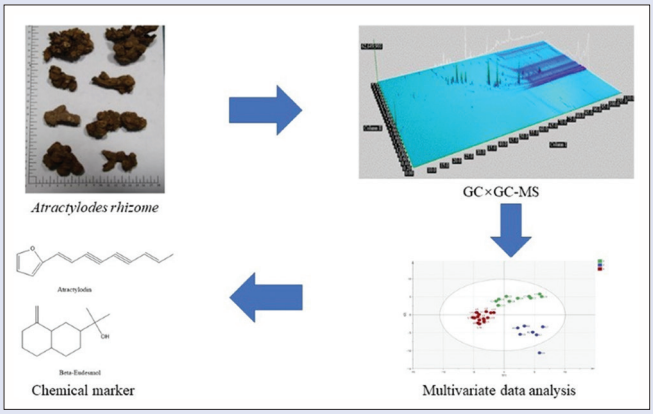
ABSTRACT

Background: In clinical practice, the species of *Atractylodes* are difficult to identify based on their morphological and chemical features which often leads to confusion. In addition, the composition of volatile components may influence the clinical efficacy of rhizomes of *Atractylodes*. **Materials and Methods:** In this study, a comprehensive two-dimensional gas chromatography with mass spectrometry coupled with multivariate data analysis was employed to investigate the differences in the volatile components of the rhizomes of three species of *Atractylodes*, namely *Atractylodes lancea* (Thunb.) DC, *Atractylodes japonica* Koidz. et Kitam, and *Atractylodes chinensis* (DC.) Koidz. **Results:** A total of 119 compounds were tentatively identified and confirmed based on the NIST database. Thirty-three samples were well distinguished and the results of two different analytical methods using principal component analysis and partial least-squares discriminant analysis were in satisfactory agreement with one-way analysis of variance. Atractylodin and β-eudesmol can be used to reveal the chemical differentiation and distinguish different species of *Atractylodes*. **Conclusion:** The results may provide a reliable reference to quality control and product grade of rhizomes of *Atractylodes*.

Key words: *Atractylodes* rhizome, comprehensive two-dimensional gas chromatography, multivariate-data analysis, partial least-squares discriminant analysis, principal component analysis

SUMMARY

- 119 compounds were identified based on comprehensive two-dimensional gas chromatography coupled with mass spectrometry between the 33 samples of *Atractylodes* rhizome
- According to the multivariate data analysis, Atractylodin and β-eudesmol could be used to distinguish different kinds of *Atractylodes* rhizome.



Abbreviations used: GC × GC-MS: Comprehensive two-dimensional gas chromatography coupled with mass spectrometry; GC-MS: Gas chromatography coupled with mass spectrometry; PCA: Principal Component Analysis; PLS-DA: Partial least-squares discriminant analysis; one-way ANOVA: One-way analysis of variance.

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INTRODUCTION

Rhizomes of *Atractylodes* species have long been used in the preparation of traditional Chinese medicine to treat cold and diarrhea. The history of use of *Atractylodes* rhizome in patients can be dated back to the Han dynasty, when it was first recorded in the first Chinese pharmacopeia (Shennong's Materia Medica).

According to the literature, the primary pharmacological components in the volatile oils of *Atractylodes* rhizomes include terpenoids, sesquiterpenes, lactones, and flavonoids involving β-eudesmol, hinesol, atractylon, atractydin, and atractylenolide.[1-4] Moreover, several new components have been recently reported, such as two thiophene polyacetylene glycosides, one eudesmane-type sesquiterpenoid, one

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guaiane-type sesquiterpenoid, two C_{14}-polyacetylenes, and four C_{10}-type polyacetylene glycosides.[5-7] Some of them show hepatoprotective and anti-inflammatory activities.[8] The volatile oils of *Atractylodes* rhizomes demonstrate numerous pharmacological activities such as anticancer, anti-inflammatory, antimicrobial, intestinal immune system modulating activity, and antipyretic activities.[9-14] The anti-gastritis effect was found to be associated with Akt/IκBα/nuclear factor-κB signaling pathway.[15] The bran-processed *Atractylodes* rhizome has been reported to have a greater effect than that of the crude one.[16-18] In recent years, scholars are more interested in understanding the effect of *Atractylodes* rhizome in preventing diarrhea.

Comprehensive two-dimensional gas chromatography (GC × GC) is a popular choice for the separation of complex biomolecules. It yields superior separation efficiency by enhancing resolution and increasing peak capacity, in addition to improving the limit of detection.[19,20] This technique is usually combined with mass spectrometry (MS), which provides effective separation chromatogram and comprehensive mass spectrum for the analyses of complex sample matrixes. The GC × GC-MS is a robust separation method, with a superior resolution and separation efficiency compared with GC × GC or MS alone. Contended with GC-MS, the chemical profiling information revealed by a GC × GC-MS chromatogram after secondary separation is markedly more dispersed and rich.[21-23] Moreover, GC × GC-MS can furnish with the lower detection limit compared to other methods.[24] With the development of MS, GC × GC-MS has developed into a significant method for the rapid identification of constituents in Chinese herbs.

At present, there are three kinds of *Atractylodes* rhizomes available in the market: *Atractylodes lancea* (Thunb.) DC, *Atractylodes japonica* Koidz. et Kitam, and *Atractylodes chinensis* (DC.) Koidz. However, due to the low content of volatile components, *A. japonica* Koidz. et Kitam is no more considered as a medicine in recent times,[25] and has not been adopted in the Chinese Pharmacopoeia. This has led to a serious confusion about the clinical efficiency of *Atractylodes* rhizomes. At present, the standard identification and quantification of *Atractylodes* rhizome in the Chinese Pharmacopoeia is confined to atractydin.[26] Moreover, this single compound cannot fundamentally distinguish the three species of *Atractylodes* that we intend to research in this study. A previous study showed that fructooligosaccharides can be applied for the authentication of *Atractylodes* rhizome and that it can distinguish *A. chinensis* from *A. lancea*.[27] In addition, four sesquiterpenoids were determined by GC in the rhizome of *Atractylodes*.[28] Another study analyzed the chemical composition of *A. japonica*, *A. chinensis*, and *A. lancea* through high-performance liquid chromatography (HPLC)/GC and multivariate data analysis and showed that different species of *Atractylodes* rhizome significantly differed in the chemical composition.[29,30] This shows that there are still some deficiencies in the quality control of *Atractylodes* rhizome, which needs to be further elaborated.

Therefore, in this study, we employed GC × GC-MS approach to investigate and compare different compounds in the rhizomes of *A. japonica*, *A. chinensis*, and *A. lancea*. To this end, 33 samples were tested, and the components in each sample were analyzed. Multivariate data analysis was used to classify three species of *Atractylodes* rhizome. The results of this study may be beneficial to perform quality control analysis of *Atractylodes* rhizome. The developed method can be reliably used in the analysis of compounds in *Atractylodes* rhizome in addition to distinguishing different species of *Atractylodes*. Furthermore, the developed method may be valuable as a reference method for analyzing other Chinese herbal medicines.

MATERIALS AND METHODS

Reagents and chemicals

In this study, 33 *Atractylodes* rhizome samples obtained from different geographical locations were purchased or collected from vendors. The samples are classified and numbered as C1–C10, K1–K7, and L1–L16. All the samples were authenticated by Professor Zhili Zhao (Shanghai University of Traditional Chinese Medicine, Shanghai, China). HPLC-grade *n*-Hexane and methanol (SCRC, Shanghai, CN) were used for the sample preparation. Table 1 presents the information on the samples.

Preparation for essential oil and samples

Each sample of *Atractylodes* rhizome was powdered and passed through 50 mesh sieves to obtain a fine powder. Next, 1 g of each powder was precisely weighed and soaked in 10 mL of *n*-hexane in a flask, which was weighed and recorded. Then, the material was extracted in the ultrasonic bath at 40 kHz for 30 min under room temperature. Adding *n*-hexane to make up for the weight loss during the extraction is a necessary step. Then, the solvent was collected after centrifugation (12,000 rpm, 10 min, 4°C). The supernatant was filtered through a 0.45 μm microporous film before the analysis.

Table 1: Information on samples

Sample number	Species	Sources	Sample number	Species	Sources
C1	*A. chinensis*	Bozhou, Anhui	L1	*A. lancea*	Chengde, Hebei
C2	*A. chinensis*	Bozhou, Anhui	L2	*A. lancea*	Liaoyuan, Jilin
C3	*A. chinensis*	Bozhou, Anhui	L3	*A. lancea*	Bozhou, Anhui
C4	*A. chinensis*	Bozhou, Anhui	L4	*A. lancea*	Suolun, Inner Mongolia
C5	*A. chinensis*	Chengdu, Sichuan	L5	*A. lancea*	Xinbing, Liaoning
C6	*A. chinensis*	Chengdu, Sichuan	L6	*A. lancea*	Yingshan, Hubei
C7	*A. chinensis*	Chengdu, Sichuan	L7	*A. lancea*	Yingshan, Hubei
C8	*A. chinensis*	Chengdu, Sichuan	L8	*A. lancea*	Yingshan, Hubei
C9	*A. chinensis*	Chengdu, Sichuan	L9	*A. lancea*	Suizhou, Hubei
C10	*A. chinensis*	Bozhou, Anhui	L10	*A. lancea*	Yingshan, Hubei
K1	*A. koreana*	North Korea	L11	*A. lancea*	Bozhou, Anhui
K2	*A. koreana*	Bozhou, Anhui	L12	*A. lancea*	Chengdu, Sichuan
K3	*A. koreana*	North Korea	L13	*A. lancea*	Chengdu, Sichuan
K4	*A. koreana*	North Korea	L14	*A. lancea*	Chengdu, Sichuan
K5	*A. koreana*	Bozhou, Anhui	L15	*A. lancea*	Chengdu, Sichuan
K6	*A. koreana*	Bozhou, Anhui	L16	*A. lancea*	Anhui
K7	*A. koreana*	Bozhou, Anhui			

*lack of *A. lancea*: *Atractylodes Lancea*

Comprehensive two-dimensional gas chromatography coupled with mass spectrometry analysis

In this study, the GC × GC-MS analysis was conducted using GC/MS-QP2010 Ultra (SHIMADZU, Tokyo, Japan) equipped with a rail autosampler (AOC-20i, SHIMADZU, Japan) and fitted with a two-dimensional column set consisting of an Inter Cap Pure Wax (30 × 0.25 × 0.25) as the first column, followed by a BPX-5 (2.5 × 0.1 × 0.1) as the second column. The volume of the sample injection was 1 μL. The split ratio of the sample was 20:1 and the injector temperature was 300°C. The oven temperature was held at 40°C for 4 min and then changed to 256°C by an increase with 3°C/min. The oven temperature was held at 256°C for 35 min. Hydrogen was used as the carrier gas at a constant flow rate of 0.93 mL/min. The modulation period was 5 s. The mass transfer line temperature was 250°C, ion source temperature was 200°C, and the detector was operated in a scan mode with a mass range of 45–339 *m/z*.

Multivariate data analysis

Data processing

In this study, GC image software was employed to acquire total ion chromatograms. For peak identification, there is a forward searching in the NIST Mass Spectral Database (NIST 11) for the resulted peaks. A forward match score of at least 800 was achieved for putative compound identification. The data were then exported to excel files, which included compound identification and peak volume.

Principal component analysis

Principal component analysis (PCA) is a multivariate statistical method that examines correlations among variables. Instead of dealing with a considerable number of variables, PCA identifies fewer principal components to describe both correlations and differences between samples, without losing any significant information. The similarities among samples can be assessed by the score plot. To carry out the PCA analysis, it is necessary to normalize peak volumes among different chromatograms. The chromatograms of 33 samples were handled and 119 peaks were generated, in which a 33 × 119 data matrix, including the peak volumes from GC × GC-MS, was used to discriminate 33 samples and find out the compounds with significant differences. We used SIMCA 14.1 software (Umetrics, Umea, Sweden) for performing PCA.

Partial least-squares discriminant analysis

Partial least-squares discriminant analysis (PLS-DA) is generally used for the supervised classification which is a variant of the multivariate calibration method PLS. The PLS-DA model can be utilized to reveal the inner connection and key makers. In this study, PLS-DA was adopted to enhance the authenticity of discriminating the samples according to their geographical origins. The discriminative compounds were identified by the analysis of variable importance in projection (VIP). In this study, PLS-DA was used to differentiate the geographical origins and chemical compositions of *Atractylodes* rhizome samples and found the key makers. PLS-DA was analyzed using SIMCA 14.1 software (Umetrics, Umea, Sweden).

One-way analysis of variance and boxplots

Based on the PLS-DA analysis, the components with VI*P* value, which were >1, were selected by PLS-DA analysis and the sample category was used as the independent variable. The peak volumes of these components in the samples were the dependent variable for one-way analysis of variance (ANOVA) ($P < 0.05$ was considered statistically significant). All raw data for the probable maker were used for boxplots. SPSS 25.0 software (IBM, New York, NY, USA) was utilized to conduct one-way ANOVA and boxplots.

RESULTS AND DISCUSSION

Tentative identification of volatile components by using GC×GC-MS

Figure 1 shows the GC × GC-MS contour plots of the volatile oils in *Atractylodes* rhizome samples. Based on GC × GC-MS with NIST 11,

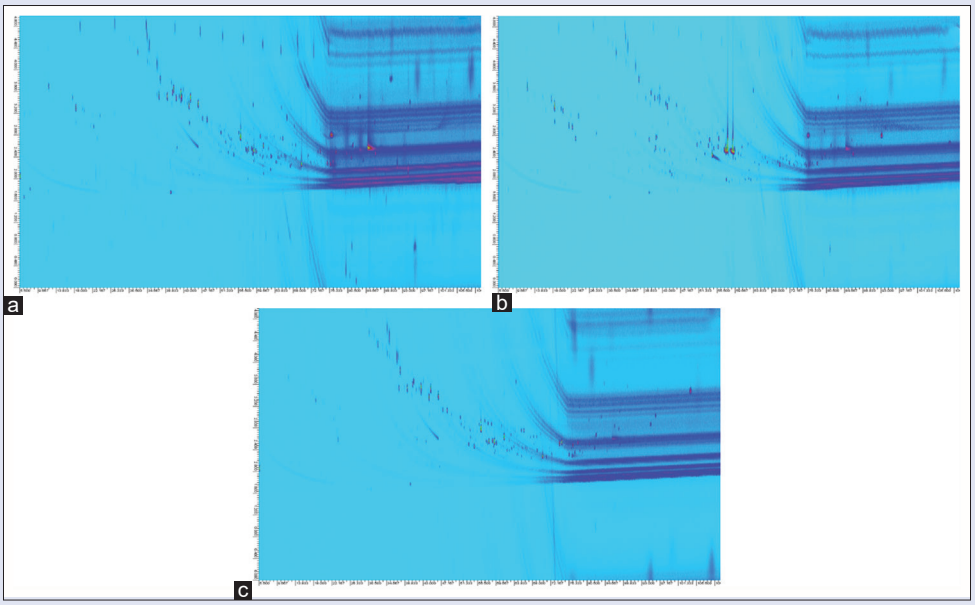

Figure 1: The comprehensive two-dimensional gas chromatography coupled with mass spectrometry contour plots of volatile oils in *Atractylodes rhizome*; the picture a is the sample form *Atractylodes chinensis*; the picture b is from *Atractylodes japonica*; the picture c is from *Atractylodes lancea*. Where dark blue means there is a component eluted, and where the darker color means a higher component content

Table 2: Tentative identifications of components in *Atractylodes rhizome* by gas chromatography × gas chromatography-mass spectrometry

n	Compound name	Peak I (min)	Peak II (sec)	Volume	CAS#	Formula	R. match
1	α-Pinene	12.17	3.7	10,923,673	7785-70-8	$C_{10}H_{16}$	927
2	Nonane, 2,6-dimethyl-	12.17	0.76	21,707,241	17302-28-2	$C_{11}H_{24}$	892
3	Decane, 4-methyl-	14.00	0.88	13,325,082	2847-72-5	$C_{11}H_{24}$	931
4	2-Hexanone	14.50	2.1	6,784,671	591-78-6	$C_6H_{12}O$	950
5	4,7-dimethyl-Undecane	15.50	1.24	19,021,304	17301-32-5	$C_{13}H_{28}$	874
6	α-Phellandrene	18.42	3.28	17,533,491	99-83-2	$C_{10}H_{16}$	919
7	Undecane	19.58	1.98	14,616,690	1120-21-4	$C_{11}H_{24}$	891
8	3-Hexanol	19.75	1.92	6,032,839	623-37-0	$C_6H_{14}O$	882
9	Dodecane	20.58	1.36	17,187,823	112-40-3	$C_{12}H_{26}$	911
10	2,6-Dimethylundecane	20.67	1.78	22,796,999	17301-23-4	$C_{13}H_{28}$	903
11	2-Hexanol	20.83	1.9	5,666,151	626-93-7	$C_6H_{14}O$	884
12	Tridecane	21.67	1.86	13,214,259	629-50-5	$C_{13}H_{28}$	887
13	2,7,10-trimethyl-Dodecane	23.00	2.04	59,650,370	74645-98-0	$C_{15}H_{32}$	873
14	2-Methyltridecane	27.92	1.5	10,779,153	1560-96-9	$C_{14}H_{30}$	891
15	Silphiperfol-5-ene	30.58	4.56	13,580,822	138752-24-6	$C_{15}H_{24}$	864
16	2,6,10-Trimethyltridecane	31.67	1.92	28,018,543	3891-99-4	$C_{16}H_{34}$	890
17	Tetradecane	32.00	2.2	58,251,531	629-59-4	$C_{14}H_{30}$	856
18	α-Guaiene	32.50	4.32	33,846,919	3691-12-1	$C_{15}H_{24}$	846
19	Pentadecane	34.08	1.26	14,438,387	629-62-9	$C_{15}H_{32}$	910
20	Modephene	34.67	4.16	32,274,932	68269-87-4	$C_{15}H_{24}$	874
21	Cyperene	34.92	4.38	17,525,420	2387-78-2	$C_{15}H_{24}$	894
22	Isocomene	35.17	4.48	101,714,023	65372-78-3	$C_{15}H_{24}$	868
23	β-Isocomene	36.92	4.06	107,249,197	71596-72-0	$C_{15}H_{24}$	898
24	β-Elemene	37.33	3.54	164,337,452	515-13-9	$C_{15}H_{24}$	881
25	Caryophyllene	37.67	3.84	136,352,710	87-44-5	$C_{15}H_{24}$	883
26	Aciphyllene	40.25	3.76	19,366,793	87745-31-1	$C_{15}H_{24}$	880
27	β-Famesene	40.33	3.38	14,350,304	18794-84-8	$C_{15}H_{24}$	916
28	Octadecane	40.33	1.96	22,303,640	593-45-3	$C_{18}H_{38}$	863
29	Humulene	40.50	3.58	92,626,641	6753-98-6	$C_{15}H_{24}$	922
30	2-Isopropenyl-4a, 8-dimethyl-1,2,3,4,4a, 5,6,7-octahydronaphthalene	40.75	3.64	178,296,121	103827-22-1	$C_{15}H_{24}$	886
31	*trans*-Geranic acid methyl ester	41.25	2.52	7,122,272	1189-09-9	$C_{11}H_{18}O_2$	921
32	Isoborneol	41.33	2.16	12,334,756	10385-78-1	$C_{10}H_{18}O$	857
33	1-Methyl-4-(6-methylhept-5-en-2-yl) cyclohexa-1,3-diene	41.33	3.48	105,527,229	451-55-8	$C_{15}H_{24}$	885
34	Germacrene D	42.00	3.5	43,177,351	23986-74-5	$C_{15}H_{24}$	947
35	β-selinene	42.42	3.58	640,473,655	17066-67-0	$C_{15}H_{24}$	919
36	2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)-, cis-	43.00	2.14	6,529,253	16721-38-3	$C_{10}H_{18}O$	888
37	β-Curcumen	43.17	3.38	10,566,983	28976-67-2	$C_{15}H_{24}$	906
38	Guaia-1 (10),11-diene	43.25	3.52	6,301,843	3691-11-0	$C_{15}H_{24}$	911
39	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	43.58	2.52	8,353,488	16409-44-2	$C_{12}H_{20}O_2$	947
40	Citronellol	43.75	2.1	8,731,217	106-22-9	$C_{10}H_{20}O$	886
41	γ-Cadinene	43.83	3.38	27,738,999	39029-41-9	$C_{15}H_{24}$	916
42	Methyl salicylate	44.08	2.1	7,993,298	119-36-8	$C_8H_8O_3$	916
43	β-Sesquiphellandrene	44.17	3.36	288,827,234	20307-83-9	$C_{15}H_{24}$	938
44	α-Curcumene	44.33	3.02	41,547,105	644-30-4	$C_{15}H_{22}$	940
45	Eremophilene	44.58	3.54	427,607,806	10219-75-7	$C_{15}H_{24}$	891
46	Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-,(1α,3α,5α)-	45.25	2.08	17,439,227	3310-2-9	$C_{10}H_{16}O$	883
47	γ-Elemene	46.33	3.4	419,874,538	29873-99-2	$C_{15}H_{24}$	923
48	Nerol	46.58	2.06	15,333,130	106-25-2	$C_{10}H_{18}O$	921
49	dehydro-Aromadendrene	47.33	3.12	85,315,630	0-00-0	$C_{15}H_{22}$	817
50	β-Vatirenene	48.33	3.1	212,459,700	0-00-0	$C_{15}H_{22}$	883
51	Heptadecane, 9-hexyl-	48.75	2.22	20,253,841	55124-79-3	$C_{23}H_{48}$	821
52	Butylated hydroxytoluene	49.00	2.62	7,250,842	128-37-0	$C_{15}H_{24}O$	890
53	Cedrene epoxide	49.33	3.02	15,939,813	29597-36-2	$C_{15}H_{24}O$	838
54	(3S,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro -1H-cyclopenta[1,3]cyclopropa[1,2]benzen-3-ol	51.50	2.58	10,132,470	23445-02-5	$C_{15}H_{26}O$	892
55	*trans*-Longipinocarveol	52.58	2.96	15,946,605	547-61-5	$C_{15}H_{24}O$	837
56	Nerolidol	53.25	2.48	96,461,595	142-50-7	$C_{15}H_{26}O$	928
57	Humulene epoxide II	53.33	2.78	31,558,786	19888-34-7	$C_{15}H_{24}O$	903
58	Epicubenol	53.83	2.72	36,973,399	19912-67-5	$C_{15}H_{26}O$	921
59	5-Azulenemethanol, 1,2,3,4,5,6,7,8-octahydro-α,α,3,8-tetramethyl-	54.83	2.56	24,686,404	13822-35-0	$C_{15}H_{26}O$	913
60	1 (2H)-Naphthalenone, octahydro-4a, 8a-dimethyl-7-(1-methylethyl)-,[4aR-(4aα,7ß,8aα)]-	55.58	2.84	36,997,946	1803-39-0	$C_{15}H_{26}O$	929
61	Atractylon	56.08	2.92	2,162,038,476	6989-21-5	$C_{15}H_{20}O$	877
62	Gammaeudesmol	57.17	2.54	186,377,831	1209-71-8	$C_{15}H_{26}O$	929

Contd...

Table 2: Contd...

n	Compound name	Peak I (min)	Peak II (sec)	Volume	CAS#	Formula	R. match
63	8,14-Cedranoxide	57.25	2.7	38,610,593	18319-31-8	$C_{15}H_{24}O$	830
64	Thymol	57.42	1.94	5,892,609	89-83-8	$C_{10}H_{14}O$	845
65	Agarospirol	57.50	2.52	136,273,170	1460-73-7	$C_{15}H_{26}O$	942
66	*trans*-Valerenyl acetate	57.58	2.86	38,531,788	101527-74-6	$C_{17}H_{26}O_2$	828
67	Hinesol	57.83	2.54	391,632,867	23811-08-7	$C_{15}H_{26}O$	958
68	Aristol-1 (10)-en-9-ol	58.33	2.84	37,798,199	1372763-27-3	$C_{15}H_{24}O$	817
69	2-methyl-5-(1-methylethyl)-Phenol	58.33	1.94	10,038,654	499-75-2	$C_{10}H_{14}O$	866
70	α-Bisabolol	58.58	2.54	89,101,938	515-69-5	$C_{15}H_{26}O$	895
71	Atractylol	58.75	2.52	181,619,344	473-16-5	$C_{15}H_{26}O$	903
72	β-Eudesmol	59.08	2.6	1,207,012,801	473-15-4	$C_{15}H_{26}O$	917
73	3,7-dimethyl-6-Octenoic acid	59.42	1.92	8,715,360	502-47-6	$C_{10}H_{18}O_2$	920
74	α-Elemol	59.50	2.5	256,381,284	639-99-6	$C_{15}H_{26}O$	847
75	Neointermedeol	59.67	2.48	42,818,742	5945-72-2	$C_{15}H_{26}O$	883
76	Dehydrofukinone	59.75	2.64	11,489,839	19598-45-9	$C_{15}H_{22}O$	846
77	Isoaromadendrene epoxide	59.75	2.4	63,237,468	0-00-0	$C_{15}H_{24}O$	849
78	Juniper camphor	61.00	2.5	98,503,454	473-04-1	$C_{15}H_{26}O$	906
79	2,4-Di-tert-butylphenol	61.25	2.08	36,516,966	96-76-4	$C_{14}H_{22}O$	923
80	2 (1H) Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	61.25	2.64	584,875,249	0-00-0	$C_{15}H_{22}O$	829
81	Aromadendrene oxide-(1)	61.33	2.42	21,615,407	0-00-0	$C_{15}H_{24}O$	834
82	3-Decenoic acid	61.67	1.92	13,187,990	53678-20-9	$C_{10}H_{18}O_2$	897
83	Heneicosane	61.75	0.96	27,182,516	629-94-7	$C_{21}H_{44}$	907
84	Valerenol	61.92	2.36	40,908,767	101628-22-2	$C_{15}H_{24}O$	814
85	Spathulel	62.08	2.4	53,557,451	6750-60-3	$C_{15}H_{24}O$	864
86	α-Cyperone	62.42	2.54	26,485,525	473-08-5	$C_{15}H_{22}O$	823
87	Diethyl Phthalate	62.83	2.16	26,935,623	84-66-2	$C_{12}H_{14}O_4$	913
88	1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e] azulene-4,7-diol	62.92	2.4	168,183,882	1212211-43-2	$C_{15}H_{26}O_2$	895
89	α-Serinene	63.00	2.84	22,893,723	473-13-2	$C_{15}H_{24}$	887
90	Caryophyllene oxide	63.08	2.32	17,569,085	1139-30-6	$C_{15}H_{24}O$	888
91	*trans*-9-Hexadecen-1-ol	63.33	2.68	21,866,953	64437-47-4	$C_{16}H_{32}O$	955
92	Kaur-16-ene	63.83	3.62	24,855,247	562-28-7	$C_{20}H_{32}$	898
93	3-methyl-1,1'-Biphenyl	64.17	2.28	26,567,934	643-93-6	$C_{13}H_{12}$	834
94	4a, 7-Methano-4aH-naphth[1,8a-b] oxirene, octahydro-4,4,8,8-tetramethyl-	64.67	2.46	38,242,129	67999-56-8	$C_{15}H_{24}O$	853
95	(1R,7S, E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	66.00	2.42	154,668,774	81968-62-9	$C_{15}H_{24}O$	814
96	Methyl 10-trans, 12-cis- octadecadienoate	66.58	3.12	17,989,579	21870-97-3	$C_{19}H_{34}O_2$	886
97	Acetic acid n-octadecyl ester	67.00	3.54	28,042,124	822-23-1	$C_{20}H_{40}O_2$	926
98	Oplopane	67.08	2.3	39,502,508	1911-78-0	$C_{15}H_{26}O_2$	930
99	Diisobutyl phthalate	67.58	2.42	17,436,272	84-69-5	$C_{16}H_{22}O_4$	887
100	2aS,3aR,5aS,9bR)-2a, 5a, 9-Trimethyl-2a, 4,5,5a, 6,7,8,9b-octahydro-2H-naphtho[1,2-b] oxireno[2,3-c] furan	68.33	2.5	50,736,868	352457-43-3	$C_{15}H_{22}O_2$	860
101	Costol	68.75	2.36	185,454,969	515-20-8	$C_{15}H_{24}O$	833
102	1-Eicosanol	68.83	2.84	39,375,402	629-96-9	$C_{20}H_{42}O$	923
103	Valerenyl isovalerate	69.75	3.1	43,404,553	101527-75-7	$C_{20}H_{32}O_2$	800
104	Octacosane	69.92	0.96	31,063,018	630-02-4	$C_{28}H_{58}$	916
105	Atractylodin	69.92	2.28	485,653,222	3218-36-8	$C_{13}H_{10}O$	835
106	Cryptomeridiol	72.08	2.28	172,328,212	4666-84-6	$C_{15}H_{28}O_2$	898
107	Spathulenol	73.08	2.26	48,226,725	77171-55-2	$C_{15}H_{24}O$	811
108	Bicyclo[4.4.0]dec-5-ene, 1,5-dimethyl-3-hy-droxy-8-(1-methylene-2-hydroxyethyl-1)-	71.92	2.3	58,655,330	0-00-0	$C_{15}H_{24}O_2$	811
109	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-2,3-diol	73.58	2.26	54,436,898	1005284-62-7	$C_{15}H_{24}O_2$	819
110	n-Hexadecanoic acid	76.50	2.26	198,803,233	1957-10-3	$C_{16}H_{32}O_2$	894
111	2-(3,7-Dimethyl-octa-2,6-dienyl)-4-methoxy-phenol	76.92	2.78	218,905,220	0-00-0	$C_{17}H_{24}O_2$	845
112	Tetratetracontane	77.17	1.72	21,790,114	7098-22-8	$C_{44}H_{90}$	847
113	Cycloisolongifolene, 8,9-dehydro-9-formyl-	77.83	2.52	605,298,081	59820-24-5	$C_{16}H_{22}O$	803
114	Squalene	81.42	0.76	64,942,147	111-02-4	$C_{30}H_{50}$	918
115	Atractylolide	81.58	2.6	714,821,019	553-21-9	$C_{15}H_{20}O_2$	855
116	6-Octadecenoic acid	83.25	2.54	563,823,487	0-00-0	$C_{18}H_{34}O_2$	848
117	9,12,15-Octadecatrienoic acid, 2,3-dihydroxyp ropyl ester, (Z, Z, Z)-	83.33	3.5	76,542,387	18465-99-1	$C_{21}H_{36}O_4$	826
118	9,12-Octadecadienoic acid (Z, Z)-	85.17	2.58	2,486,450,292	60-33-3	$C_{18}H_{32}O_2$	909
119	Olean-12-en-3-ol, acetate, (3β)-	96.25	0.4	460,241,016	1616-93-9	$C_{32}H_{52}O_2$	879

a total of 119 compounds with reverse match factors were found to be >800, mainly including terpenoids and benzene derivatives. Table 2 lists 119 compounds that match well. These 119 compounds were retrieved by MS library and were verified by reference reports.[31-35] In this study, We identified 52 compounds in *A. japonica*, *A. chinensis*, and *A. lancea*. Due to the fact that the species and disparate habitats might cause significant changes in the volatile compounds in *Atractylodes* rhizome samples, we identified 67 compounds in *A.*

japonica, *A. chinensis*, and *A. lancea* samples. By optimizing the chromatographic conditions, we identified 119 compounds with a reverse match factor >800. The results revealed that the number of peaks identified by GC × GC was remarkably higher than that of GC-MS, further indicating that the full two-dimensional GC has higher resolution and sensitivity.

Multivariate data analysis

Principal component analysis

To further evaluate *Atractylodes* rhizome samples collected from China and North Korea, PCA was undertaken to explore the diversities among the chemical nature of *Atractylodes* rhizome samples and make further efforts to find out the key components. The total variance explained by the two principal components was 43.19% and the PCA score plot showed a separation of *Atractylodes* rhizome without any specific order [Figure 2]. In addition, in the *A. japonica* group, two batches of samples from North Korea were separated from other batches of samples, which might be attributed to the differences in their origin. The three-dimensional PCA score plot showed a distance separation of *Atractylodes* rhizome from diverse species [Figure 3]. Thus, three principal components were found to be appropriate. The primary confusion emerged from the samples of *A. chinensis* and *A. lancea*. Sample C10 originated from *A. chinensis*, whereas it was closer to the group of *A. lancea*. The results indicated that *A. chinensis* and *A. lancea* resembled in their chemical composition. Although the volatile oil contents in *A. chinensis* had no special features, it is possible that similar contents existed in higher quantities than that of other volatile compounds. The aforementioned results indicated that inherent causes such as place of origin and species could affect the volatile components

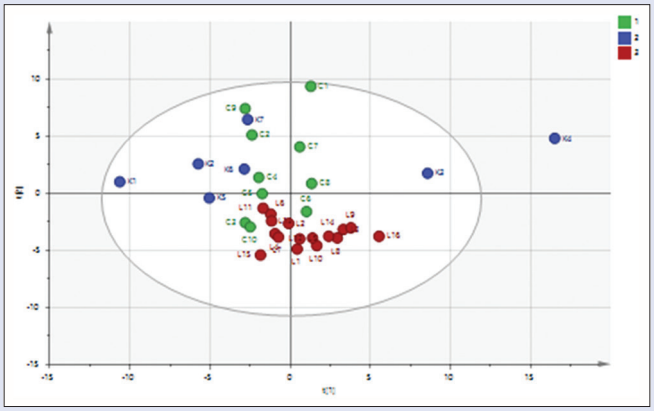

Figure 2: The principal component analysis result of essential oil of *Atractylodes rhizome*. The green point means the samples from *Atractylodes chinensis*; the blue point means the samples from *Atractylodes japonica*; the red point means the samples from *Atractylodes lancea*. The closer the points on the graph are, the more similar their chemical composition is

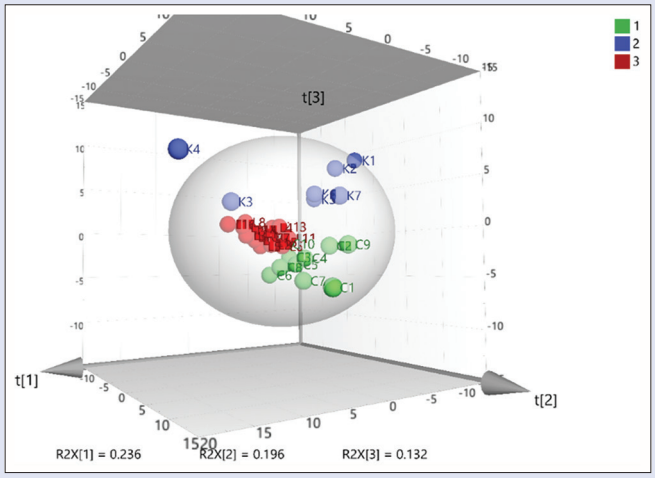

Figure 3: The three-dimensional principal component analysis result of the essential oil of *Atractylodes rhizome*. The point is the same as Figure 2. The three-dimensional principal component analysis result is the two-dimensional principal component analysis result in which a new principal component is added. The third principal component accounts for 13.2% of all the component data

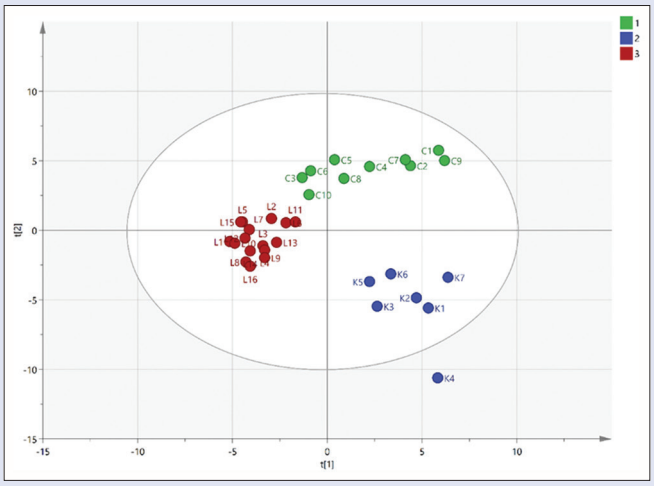

Figure 4: The partial least-squares discriminant analysis result of essential oil of *Atractylodes rhizome*. The green point means the samples from *Atractylodes chinensis*; The blue point means the samples from *Atractylodes japonica*; The red point means the samples from *Atractylodes lancea*. The abscissa means the difference between the groups, and the difference within the group is seen on the ordinate

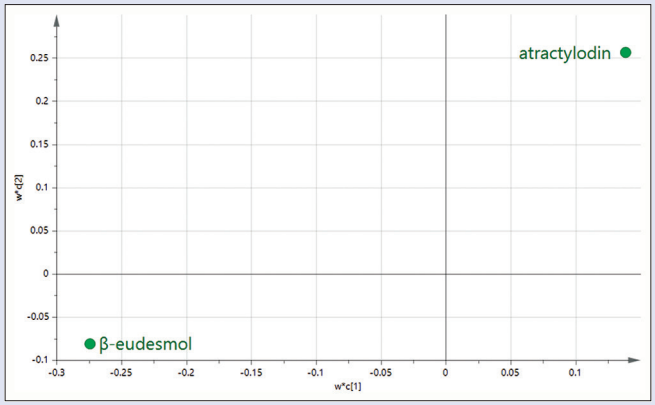

Figure 5: Loadings plot of principal component analysis for the key compounds. The abscissa indicates the correlation coefficient between the principal component and the compound, and the ordinate indicates the correlation coefficient between the principal component and the compound. The compound β-eudesmol is in the third quadrant. The compound Atractylodin is in the first quadrant. These are the components furthest from the origin

Table 3: The top ten components of variable importance in projection value

Rank	Compound name	Value	Rank	Compound name	Value
1	β-Eudesmol	2.18376	6	Agarospirol	1.66411
2	Atractylodin	2.12412	7	Epicubenol	1.5766
3	α-Curcumene	1.84511	8	β-Selinene	1.56138
4	α-Phellandrene	1.80818	9	γ-Cadinene	1.55954
5	β-Elemene	1.79072	10	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	1.54641

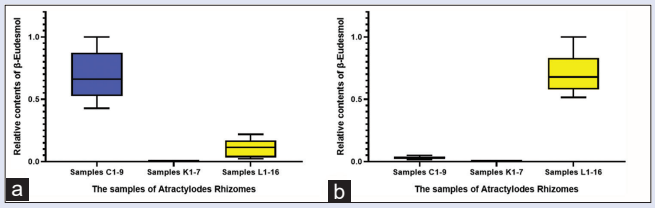

Figure 6: Boxplots of.Atractylodin (a) and β eudesmol (b). The relative content of the two components was calculated with the highest peak area in the sample as references. The relative content of Atractylodin in samples C1-10, K1-7 and L1-16 were 0.69, 0.00 and 0.11, respectively. And The relative content of β eudesmol in samples C1-10, K1-7 and L1-16 were 0.03, 0.00 and 0.71, respectively

of *Atractylodes* rhizome and the classification of species. The results of PCA provided a preliminary overview of the gathering and separation among the different species of *Atractylodes*. To further understand these differences, we employed a PLS-DA model.

Partial least-squares discriminant analysis

The PLS-DA score plot indicated an obvious distinction among the three species based on the 119 peaks obtained [Figure 4]. It indicated that the chemical components of samples had remarkable differences among the 119 selected peaks, characterizing these differences. In particular, the samples of *A. japonica* showed distinct components from that of the others. The scores t_1 which is fitted to be the first principal component and t_2 which is fitted to be the second principal component are new variables summarizing the *X*-variable, which are the values of the components. According to the cross-validation, the scores t_1 and t_2 depicted 39.4% of the variation in *X* (R^2X = 0.394) and 81.5% of the variation in *Y* (R^2Y = 0.815) and foresaw 67.8% (Q^2 [cum] = 0.678). In this study, the PLS-DA model effectively distinguished the three kinds of *Atractylodes* rhizome.

The VIP values of the primary compounds are ranked from high to low, revealing the differences in chemical components in sample identification. The VIP plot of PLS-DA [Table 3] showed that β-eudesmol and atractylodin may have greater effects than the others on the distinction of different kinds of *Atractylodes* rhizome. The PCA loading graph showed the degree of original variables in the different components. As shown in Figure 5, β-eudesmol had a negative contribution to P1, whereas atractylodin had a positive contribution to P1. Moreover, it implies that these two compounds lead to most of these variables. According to the VIP value [Table 3], the contribution of each variable from each compound was quantified for the classification, and we found that the greater the VIP value is, the more significant the variance is in the difference between the various species of *Atractylodes*. The VIP value of 53 physicochemical components was found to be higher than 1. Table 3 lists the top 10 components of the VIP value. Especially, the VIP values of β-eudesmol and Atractylodin were both >2. It indicated that these two components may have different contents in the samples. Among them, β-eudesmol and atractylodin were noted as the most important variables for the classification.

One-way analysis of variance and boxplots

One-way ANOVA was performed for comparison between the peak volumes of β-eudesmol and atractylodin among the three species of *Atractylodes*. The results showed that there were significant differences among the three species ($P < 0.01$).

Thus, β-eudesmol and atractylodin can be used to distinguish different species of *Atractylodes*. To further confirm the accuracy of the results, the boxplots were drawn for the first two components [Figure 6]. The remarkable differences in the contents of these two components were found among three kinds of *Atractylodes* rhizome. The two components showed different dispersions. There was a high level of the atractylodin in the samples of *A. chinensis*, a medium level in *A. lancea*, and a low level in *A. japonica*, whereas there was a high level of the β-eudesmol in *A. lancea*, a medium level in *A. chinensis*, and a low level in *A. japonica*. In brief, these two components have a significant influence on the classification of the samples.

CONCLUSION

In this study, GC × GC-MS was developed by integrating PCA and PLS-DA to investigate the volatile components of *Atractylodes* rhizome comprehensively. The superior separation efficiency of this method allowed us to identify some of the new components from the complex matrix. This method helped us to distinguish various *Atractylodes* rhizome samples according to their raw profiles. It can be used as a rapid and effective method to distinguish herbal medicines particularly those containing essential/volatile oils.

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Jie Lu and WenTing Chen contributed equally to this work.

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Conflicts of interest

There are no conflicts of interest.

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