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ORIGINAL ARTICLE

Phytochemical Profiling of Different Processed Products from *Atractyloidis* Rhizome using UHPLC/Q-TOF-MS

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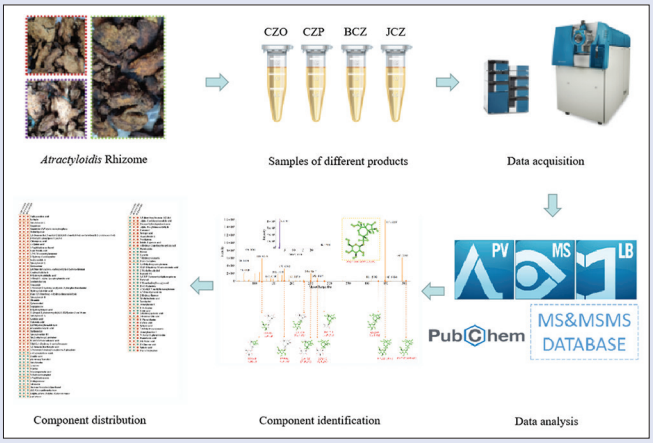
ABSTRACT

Background: *Atractyloidis* rhizome (cangzhu) was an important herb widely used in clinical application with different products, such as crude cangzhu (CZO), cangzhu pieces (CZP), bran frying cangzhu (BCZ), and Jiao cangzhu (JCZ). **Objectives:** To unscramble the phytochemical profiling of CZO, CZP, BCZ, and JCZ. **Materials and Methods:** In this paper, ultra-high performance liquid chromatography combined with quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF-MS/MS) technology and multivariate data analysis methods such as principal component analysis (PCA) and orthogonal partial least squares discrimination analysis (OPLS-DA) were employed to screen the different components among CZO, CZP, BCZ, and JCZ. **Results:** As a result, CZO, CZP, BCZ, and JCZ were separated obviously in PCA plot, and a total of 101 components were identified successfully. Among them, 54 chemical markers, including isoleucine, citric acid, L-phenylalanine, atractyloside A, etc., discriminating among CZO, CZP, BCZ, and JCZ, were screened out. **Conclusion:** UHPLC-Q-TOF-MS/MS technology combined with PCA and OPLS-DA could be successfully applied in clarifying the different ingredients between crude drug and its processed products of traditional Chinese medicine (TCM). Importantly, the established method revealed the reasonability of processing theory in TCM.

Key words: *Atractyloidis* Rhizome, chemical markers, identification, TCM, UHPLC-Q-TOF-MS/MS

SUMMARY

- The established method rapidly identified the chemical ingredients of different products from *Atractyloidis* Rhizome and revealed the reasonability of processing theory in TCM. Especially, UHPLC-Q-TOF-MS/MS based on multivariate data anaysis maybe a powerful approach for understanding phytochemical ingredients from other processed products in TCM.



Abbreviations used:
CZO: Original crude Cangzhu; CZP: Cangzhu pieces; BCZ: bran frying Cangzhu; JCZ: Jiao Cangzhu; TCM: Traditional Chinese medicine; UHPLC-Q-TOF-MS/MS: Ultra-high-performance liquid chromatography combined with quadrupole time-of-flight mass spectrometry; CE: collision energy; PCA: principal component analysis; OPLS-DA: orthogonal partial least squares discrimination analysis; VIP: variable importance in the projection

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INTRODUCTION

Metabolomics was a comprehensive means that could qualitatively or quantitatively analyze small molecular metabolites (<1000 Da), such as lipids, nucleic acids, organic acids, vitamins, *etc.*, based on high-throughput analysis technology and multivariate data processing methods. In recent years, metabolomics has been widely applied in the chemical analysis of traditional Chinese medicine (TCM), including *Astragalus*,[1] Pollen Typhae,[2] and Kaixinsan.[3] Up to now, metabolomics was further used for understanding the constituent, effect, and mechanism of TCM.

Nowadays, ultra-high-performance liquid chromatography combined with quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF-MS/MS) was widely applied in various multi-component qualitative and quantitative analysis research of TCM, such as the chemical identification of *Astragalus*,[1] corn silk,[4,5] Shizao decoction.[6] UHPLC-Q-TOF-MS/MS provided a powerful means for the identification of ingredients *in vitro* and *in vivo*.

Atractyloidis Rhizome, derived from the root of *Atractylodes lancea* Thunb. (DC.) and *Atractylodes chinensis* (DC.) Koidz. in *Chinese*

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Pharmacopoeia (2020 Edition), was widely used for abdominal fullness, diarrhea, and edema.[7] *Atractyloidis* Rhizome expressed activities in anti-cancer, anti-bacterial, anti-inflammatory, and immunoregulation.[8] However, crude drug exhibit enormous dryness and may result in intense stimulation for the stomach and intestine. Generally, processed products with low dryness of *Atractyloidis* Rhizome were applied in clinical application, including cangzhu (CZO), cangzhu pieces (CZP), bran frying cangzhu (BCZ), and Jiao cangzhu (JCZ). Different preparation owned different medicinal efficacy. CZO could relieve rheumatic arthralgia and wet fever; however, CZP was applied in nausea and vomiting, typhoid fever, incoordination between spleen and stomach, and downward flow of damp heat.Compared to CZP, BCZ reduced the pungent in taste, weakens the dryness in nature, and strengthens the spleen and stomach. JCZ was used for eliminating dampness and arrest leucorrhea and spleen-deficiency diarrhea. In the current study, UHPLC-Q-TOF-MS/MS combined with multiplex data processing method was employed to explore the discrepancy of chemical ingredients among CZO, CZP, BCZ, and JCZ and to identify the main discrepant constituents. The obtained results supplied a reference for quality control of different processed products from *Atractyloidis* Rhizome; besides, the present paper also offered a scientific explanation of pharmaceutical preparation methods of TCM. The experimental process of the current study was shown in Figure 1.

MATERIALS AND METHODS

Chemical reagents

Cangzhu was collected from a medicinal material base in Qiqihar Medical University and identified by Doctor Qi Liu. The voucher specimen numbers were CZ-20190825-001, CZ-20190825-002, CZ-20190825-003, CZ-20190825-004, CZ-20190825-005, and CZ-20190825-006. We have obtained the approval from the ethics committee, the number was QMU-AECC-2021-134, the date was May 7, 2021.

The original crude cangzhu was dried and named CZO. Besides, CZP, BCZ, and JCZ were prepared independently as quintessential methods. CZO was soaked with water thoroughly, cut into thick pieces, and dried, then, the product was named CZP. The wheat bran was sprinkled into a heating wok and heated over medium, then CZO was put into the wok until smoke, stir fry until deep yellow, sieve the bran and cool, the product was named BCZ. CZO was put in a frying container and fried until brown over medium heat, then sprayed with a little water and fried over low heat until dry, the product was named JCZ. Methanol and acetonitrile of HPLC grade were bought from Thermo Fisher Scientific (Waltham, Massachusetts, America). Ultra-pure water was self-produced using a pure-ultra pure water system (Millipore, Bedford, MA, USA). Formic acids were purchased from Aladdin Reagent Co. Ltd. (Shanghai, China). Other reagents were of analytical grade.

Sample preparation

CZO, CZP, BCZ, and JCZ were smashed into powder and passed the No. 3 sieve. Then, 2.0 g of each group was added to 40 mL ultra-pure water, respectively, and extracted via hyperacoustic system for 30 min under 30 kHz frequency in 35°C water. After cooling, the extracted solution was centrifuged at 16,000 r/min for 10 min and further filtered through 0.22 μm membrane. Then, 1 mL of supernatant was diluted with 9 mL ultra-pure water and vortexed for 1 min. Finally, samples of CZO, CZP, BCZ, and JCZ were filtered through a 0.22 μm membrane and injected into the UHPLC-Q-TOF-MS/MS system.

Chromatography and massspectrum conditions

High-resolution AB SCIEX Triple TOF 5600 + coupled with ultra-high performance liquid chromatography was used in the analysis of different prepared products. A rapid ACQUITY™ UPLC HSS T_3 column (100mm × 2.1mm i.d., 1.8 μm, Waters Corporation, Milford, MA, USA) was used and the temperature was set at 40°C. The gradient elution containing 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) was set as follows: 0–1.5 min, 2–16% B; 1.5–2.0 min, 16–20% B; 2.0–4.0 min, 20–60% B; 4.0–4.5 min, 60–65% B; 4.5–8.0 min, 65–70% B; and 8.0–10.0 min, 70–100% B. The flow rate was maintained at 0.3 mL/min. The injection volume was set at 2 μL.

Electrospray ion source was employed in the massspectrum and the temperature was maintained at 600°C, the voltage of ionspray voltage floating was set at 5500 V in positive ion mode and 4500 V in negative ion mode; the pressure of nebulizer gas, auxiliary gas, and curtain gas were, respectively, set at 55, 55, and 30 psi. The voltage of declustering potential and collision energy were maintained at 100 V and 10 V, respectively. The scan range of *m/z* was set at 50–1000 Da and the accumulation time of TOFMS was 0.15 s. MS/MS fragments were acquired via an information-dependent acquisition mode under collision energy (CE) 40 ± 20 V. During the analysis period, a continuous calibration was conducted every 2 h.

Data collection and processing

Data of CZO, CZP, BCZ, and JCZ samples were obtained via the UHPLC-Q-TOF-MS/MS technology under positive ion mode and negative ion mode. All the data were imported into EZinfo 2.0 software, and principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA) proceeded to figure out the differential compounds. AB SCIEX Qjet™ working station was used to screen the possible elemental compositions and the possible chemical structures based on public databases such as Chemspider. Then, MS/MS fragments were analyzed based on chromatographic peaks and chemical structures according to the existing reports or standard spectra comparison.

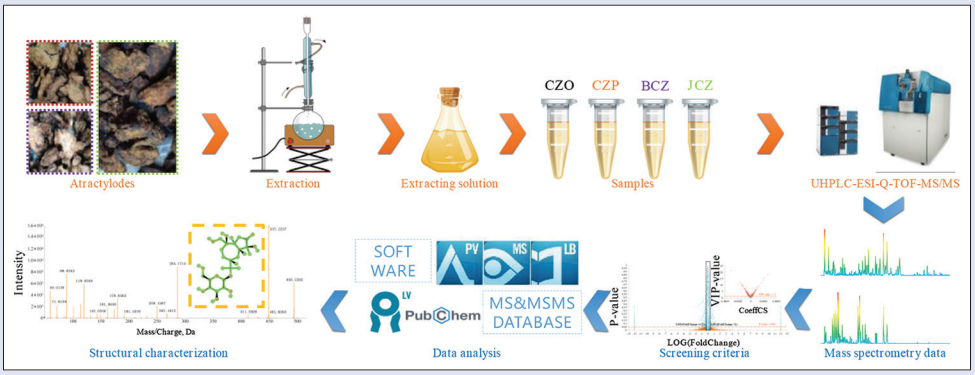

Figure 1: Experimental procedures for composition analysis of different processed products from *Atractyloidis* Rhizome based on UHPLC-Q-TOF-MS/MS

Table 1: Detailed information of identified components in *Atractyloidis* Rhizome by UHPLC-ESI-Q-TOF-MS/MS approach

Rt (min)	*m/z*	Name	Adducts	Formula
0.60	195.0512	D-gluconic acid	M-H	$C_6H_{12}O_7$
0.62	180.0635	D-(+)-galactose	M-H_2O-H, M-H, M+FA-H	$C_6H_{12}O_6$
0.62	387.1141	D-(+)-trehalose	M+FA-H	$C_{12}H_{22}O_{11}$
0.63	189.1342	L-NG-monomethylarginine	M+H	$C_7H_{16}N_4O_2$
0.63	365.1045	Leucrose	M+Na	$C_{12}H_{22}O_{11}$
0.65	116.0702	Proline	M+H	$C_5H_9NO_2$
0.65	133.0141	DL-malic acid	M-H	$C_4H_6O_5$
0.67	689.2069	3α,4β,3α-galactotetraose	M+Na	$C_{24}H_{42}O_{21}$
0.71	851.2603	Maltopentaose	M+Na	$C_{30}H_{52}O_{26}$
0.74	146.0917	4-guanidinobutyric acid	M+H	$C_5H_{11}N_3O_2$
0.82	192.0273	Citric acid	M-H_2O-H, M-H	$C_6H_8O_7$
0.82	344.0399	guanosine 3',5'-cyclic monophosphate	M-H	$C_{10}H_{12}N_5O_7P$
0.87	268.1048	Adenosine	M+H	$C_{10}H_{13}N_5O_4$
0.89	152.0565	2-hydroxy-6-aminopurine	M+H	$C_5H_5N_5O$
0.89	284.0996	Isoguanosine	M+H	$C_{10}H_{13}N_5O_5$
0.91	282.0842	Guanosine	M-H	$C_{10}H_{13}N_5O_5$
0.95	117.0193	Methylmalonic acid	M-H	$C_4H_6O_4$
1.05	132.1015	L-isoleucine	M+H	$C_6H_{13}NO_2$
1.35	178.0864	5-hydroxytryptophol	M+H	$C_{10}H_{11}NO_2$
1.44	249.1958	Matrine	M+H	$C_{15}H_{24}N_2O$
1.54	164.0719	L-phenylalanine	M-H	$C_9H_{11}NO_2$
1.62	221.0919	Indole-3-pyruvic acid	M+NH4	$C_{11}H_9NO_3$
1.67	218.1036	Pantothenic acid	M-H	$C_9H_{17}NO_5$
1.69	167.0353	Vanillic acid	M-H	$C_8H_8O_4$
1.78	109.0294	Pyrocatechol	M-H	$C_6H_6O_2$
1.78	153.0194	Gentisic acid	M-H	$C_7H_6O_4$
1.89	353.0875	Chlorogenic acid	M-H	$C_{16}H_{18}O_9$
1.92	473.2340	Atractyloside B	M+Na	$C_{21}H_{38}O_{10}$
2.03	187.0631	3-Indoleacrylic acid	M+H-H_2O, M+H, M+NH4	$C_{11}H_9NO_2$
2.05	204.0902	D-(+)-Tryptophan	M-H, M+FA-H	$C_{11}H_{12}N_2O_2$
2.08	471.2188	Atractyloside A	M+Na	$C_{21}H_{36}O_{10}$
2.25	137.0243	3,4-dihydroxybenzaldehyde	M-H	$C_7H_6O_3$
2.25	181.0145	4-hydroxyisophthalic acid	M-H	$C_8H_6O_5$
2.27	191.0562	(-)-quinic acid	M-H	$C_7H_{12}O_6$
2.33	417.1393	Syringin	M+FA-H	$C_{17}H_{24}O_9$
2.38	175.0613	2-isopropylmalic acid	M-H	$C_7H_{12}O_5$
2.51	137.0593	4-hydroxy-3-methoxybenzyl alcohol	M+H-H_2O	$C_8H_{10}O_3$
2.51	197.0456	Syringic acid	M-H	$C_9H_{10}O_5$
2.53	177.0195	7,8-dihydroxycoumarin	M-H	$C_9H_6O_4$
2.55	144.0803	1-naphthalenamine	M+H	$C_{10}H_9N$
2.55	181.0493	Caffeic acid	M+H	$C_9H_8O_4$
2.58	151.0405	3,4-dihydroxyacetophenone	M-H	$C_8H_8O_3$
2.60	196.0733	Ethyl 2,4-dihydroxy-6-methylbenzoate	M+H-H_2O, M+H	$C_{10}H_{12}O_4$
2.67	402.1518	Icariside F2	M-H, M+FA-H	$C_{18}H_{26}O_{10}$
2.71	515.1199	Cynarin	M-H	$C_{25}H_{24}O_{12}$
2.74	177.0542	trans-Ferulic acid	M+H-H_2O	$C_{10}H_{10}O_4$
3.06	599.2652	Atractyloside I	M+Na	$C_{27}H_{44}O_{13}$
3.20	206.0822	N-acetyl-L-phenylalanine	M-H	$C_{11}H_{13}NO_3$
3.25	173.0822	Suberic acid	M-H	$C_8H_{14}O_4$
3.29	161.0244	7-hydroxycoumarin	M-H	$C_9H_6O_3$
3.81	179.0699	Coniferyl aldehyde	M+H	$C_{10}H_{10}O_3$
3.81	209.0807	trans-3,5-dimethoxy-4-hydroxycinnamaldehyde	M+H	$C_{11}H_{12}O_4$
3.90	152.1197	(-)-carveol	M+H-H_2O, M+H	$C_{10}H_{16}O$
4.01	292.0946	Picrotoxinin	M+H, M+Na	$C_{15}H_{16}O_6$
4.07	137.0240	4-Hydroxybenzoic acid	M-H	$C_7H_6O_3$
4.08	206.0577	6,7-Dimethylesculetin	M+H, M+Na	$C_{11}H_{10}O_4$
4.08	250.1207	2,8-dimethyl-2-(.beta.-carboxyethyl)-6-hydroxychroman	M+H-H_2O, M+H	$C_{14}H_{18}O_4$
4.49	168.1149	(1R)-chrysanthemolactone	M+H-H_2O, M+H	$C_{10}H_{16}O_2$
4.51	423.2341	Atractyloside C	M+Na	$C_{21}H_{36}O_7$
4.59	202.1201	Sebacic acid	M-H_2O-H, M-H	$C_{10}H_{18}O_4$
4.79	416.2407	Atractyloside G	M-H, M+FA-H	$C_{21}H_{36}O_8$
5.11	261.1132	Osthol hydrate	M-H	$C_{15}H_{18}O_4$
5.17	236.1772	Curcumol	M+H-H_2O, M+H, M+NH4	$C_{15}H_{24}O_2$
5.42	215.1290	Undecanedioic acid	M-H	$C_{11}H_{20}O_4$
6.10	216.1511	α-hexylcinnamaldehyde	M+H-H_2O, M+H	$C_{15}H_{20}O$
6.25	220.1823	Farnesal	M+H-H_2O, M+H	$C_{15}H_{24}O$

Contd...

Table 1: Contd...

Rt (min)	*m/z*	Name	Adducts	Formula
6.43	203.1790	(-)-caryophyllene oxide	M+H-H_2O	$C_{15}H_{24}O$
6.63	249.1482	Parthenolide	M+H	$C_{15}H_{20}O_3$
6.70	234.1616	Valerenic acid	M+H, M+Na	$C_{15}H_{22}O_2$
6.83	174.1052	3-methyl-1-phenylpent-1-yn-3-ol	M+H-H_2O, M+H	$C_{12}H_{14}O$
6.86	233.1186	α-Cyclohexylmandelic acid	M-H	$C_{14}H_{18}O_3$
6.92	247.1339	11-propan-2-ylidenetricyclo[4.3.1.12,5]undec-3-en-10-one	M+FA-H	$C_{14}H_{18}O$
7.16	275.1289	Atractylenolide I	M+FA-H	$C_{15}H_{18}O_2$
7.26	233.1533	Hydroxyvalerenic acid	M+H-H_2O	$C_{15}H_{22}O_3$
7.41	248.1407	Atractylenolide III	M+H-H_2O, M+H, M+NH4, M+Na	$C_{15}H_{20}O_3$
7.42	203.1442	3,5-ditert-butylbenzene-1,2-diol	M-H_2O-H	$C_{14}H_{22}O_2$
7.65	193.1220	Senkyunolide A	M+H	$C_{12}H_{16}O_2$
7.67	694.3395	Atractyloside E	M+NH_4, M+Na	$C_{32}H_{54}O_{16}$
7.96	182.0729	Atractylodin	M+H-H_2O, M+H	$C_{13}H_{10}O$
8.12	218.1667	Nootkatone	M+H-H_2O, M+H	$C_{15}H_{22}O$
8.22	313.2381	12,13-dihydroxy-9Z-octadecenoic acid	M-H	$C_{18}H_{34}O_4$
8.67	165.0696	2-hydroxyfluorene	M+H-H_2O	$C_{13}H_{10}O$
9.17	232.1461	Isoalantolactone	M+H-H_2O, M+H, M+Na	$C_{15}H_{20}O_2$
10.62	181.0643	Xanthydrol	M+H-H_2O	$C_{13}H_{10}O_2$
11.03	227.1064	Bis (4-methoxyphenyl) methanol	M+H-H_2O	$C_{15}H_{16}O_3$
11.66	141.0693	1-naphthalenemethanol	M+H-H_2O	$C_{11}H_{10}O$
11.66	181.1007	2-methylbenzhydrol	M+H-H_2O	$C_{14}H_{14}O$
11.66	240.1146	3,3',5,5'-tetramethyldiphenoquinone	M+H, M+Na	$C_{16}H_{16}O_2$
11.77	205.1946	(cis+trans)-nerodilol	M+H-H_2O	$C_{15}H_{26}O$
11.94	218.1667	Germacrone	M+H-H_2O, M+H, M+Na	$C_{15}H_{22}O$
12.24	452.2775	1-palmitoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine	M-H	$C_{21}H_{44}NO_7P$
14.83	258.1616	1,3-benzenediol, 5-methyl-2-[(1R,6R)-3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]	M+H, M+Na	$C_{17}H_{22}O_2$
15.05	435.2511	1-oleoyl-L-α-lysophosphatidic acid	M-H	$C_{21}H_{41}O_7P$
15.87	671.4672	1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphate	M-H	$C_{37}H_{69}O_8P$
15.89	271.2277	2-hydroxypalmitic acid	M-H	$C_{16}H_{32}O_3$
15.91	354.2760	1-monolinoleoyl-rac-glycerol	M+H-H_2O, M+H, M+NH_4, M+Na	$C_{21}H_{38}O_4$
16.50	304.2608	Oleamide	M+Na	$C_{18}H_{35}NO$
16.75	280.2399	9E,11E-octadecadienoic acid	M+H-H_2O, M+H, M+Na	$C_{18}H_{32}O_2$
18.50	149.0226	1,2-benzenedicarboxylic acid	M+H-H_2O	$C_8H_6O_4$
18.50	390.2761	Bis (2-ethylhexyl) phthalate	M+H, M+Na	$C_{24}H_{38}O_4$
18.68	360.3235	Erucamide	M+Na	$C_{22}H_{43}NO$

UHPLC-Q-TOF-MS/MS=ultra-high performance liquid chromatography combined with quadrupole time-of-flight mass spectrometry

RESULTS AND DISCUSSION

Identification of components

The collected MS and MS/MS fragments data were preliminarily processed by PeakView software. Finally, the components from *Atractyloidis* Rhizome were successively identified in view of the fragmentation rule, reported literatures, and MassBank database. The identified components in *Atractyloidis* Rhizome were shown in Table 1. As shown in Table 1, totally 101 ingredients under positive ion mode and negative ion mode were successfully identified, including representative constituents such as several kinds of atractyloside and other componnets.

Take the identification of atractyloside A, for example, under negative ion mode, the molecular ion was 447.2227 in $[M-H]^-$ loading form and 493.2282 in $[M + FA-H]^-$ loading form. The primary MS/MS fragments include the following: *m/z* 285.1714, *m/z* 267.1612, *m/z* 249.1507, *m/z* 231.0874, *m/z* 179.0562, *m/z* 119.0349, and *m/z* 113.0608. *M/z* 285.1714 fragment may originate from α-cleavage between parent nucleus and glycoside bond. The concrete fragmentation information was shown in Figure 2.

Multivariate data analysis

The multivariate data results were shown in Figure 3. CZO and other three kinds of *Atractyloidis* Rhizome located in different spatial positions, displaying that the ingredients of CZO were obviously distinctive among the other three groups. Compared with PCA, OPLS-DA score plot made it easier to locate the chemical differences between groups. Obviously, the variable importance in the projection values greater than 0.05 by OPLS-DA were reasonable. Finally, the different chemical markers between every two groups were precisely located.

Content comparison of ingredients from different processed products

The variety and amount of identified ingredients in CZP, BCZ, and JCZ were compared with CZO group. Compared with CZO, the content of 73 constituents, such as atractyloside A, atractyloside B, atractyloside C, atractyloside G, atractylenolide I, atractylenolide III, etc., was decreased in CZP; on the contrary, the content of 28 ingredients, such as (-) caryophyllene oxide, atractyloside E, atractylodin, matrine, vanillic acid, (-)-carveol, etc., was markedly increased in CZP. Compared with CZO, the content of 55 constituents, including atractyloside A, atractyloside B, atractyloside C, atractyloside E, atractyloside G, atractylenolide III, syringic acid, DL-malic acid, etc., was signally reduced in BCZ; however, 56 ingredients containing (-) caryophyllene oxide, vanillic acid, atractylodin, atractylenolide I, farnesal, (-)-carveol, etc., were dramatically increased in BCZ. Compared with CZO, 62 constituents covering atractyloside A, atractyloside B, atractyloside C, atractyloside E, atractyloside G, atractylenolide I, etc., were visibly

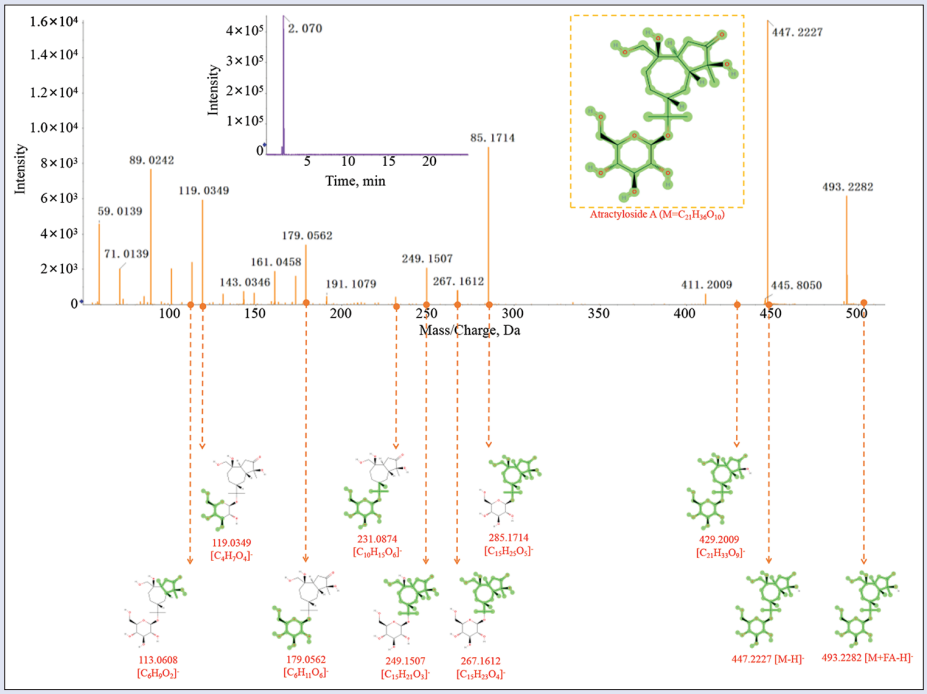

Figure 2: The MS and MS/MS information of atractyloside A detected in *Atractyloidis* Rhizome by UHPLC-ESI-Q-TOF-MS/MS

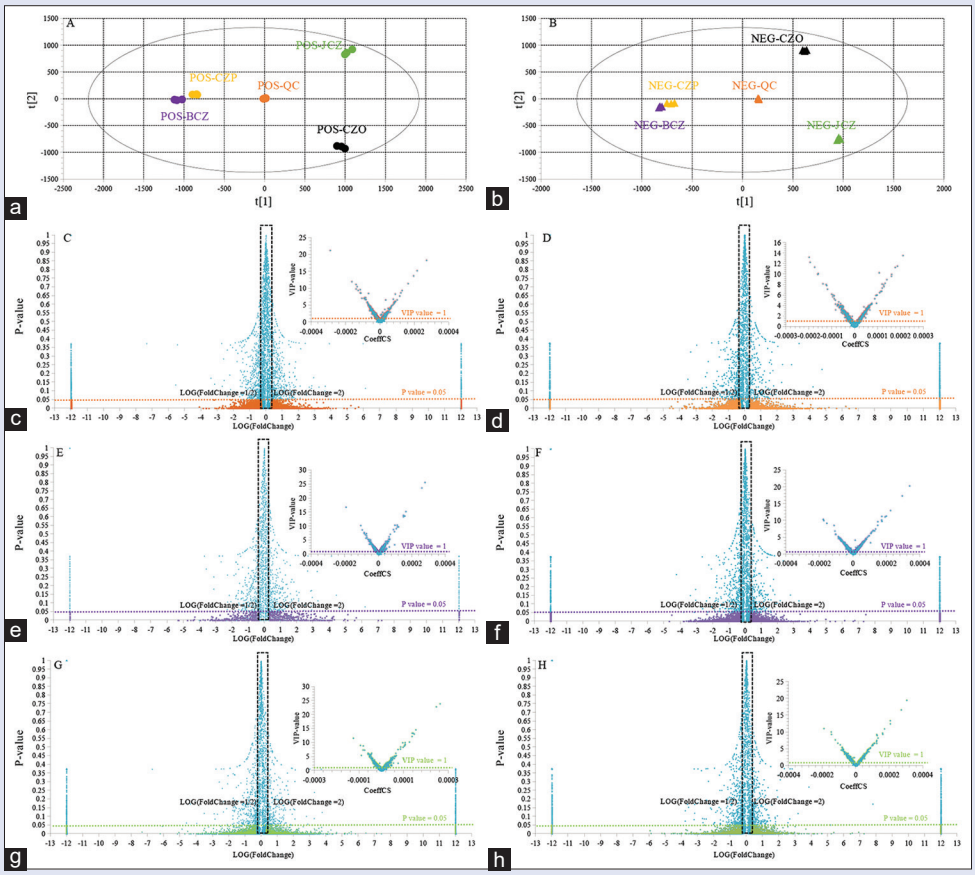

Figure 3: The principal component analysis (PCA) and Variable importance in the projection (VIP) analysis of different processed products of Atractyloidos Rhizome in positive ion mode and negative ion mode (a) PCA analysis in positive ion mode (b) PCA analysis in negative ion mode (c) VIP analysis (cangzhu pieces – CZP vs. cangzhu – CZO) in positive ion mode (d) VIP analysis (CZP vs. CZO) in negative ion mode (e) VIP analysis (bran frying cangzhu – BCZ vs. cangzhu CZO) in positive ion mode (f) VIP analysis (BCZ vs CZO) in negative ion mode (g) VIP analysis (Jiao cangzhu – JCZ vs. cangzhu – CZO) in positive ion mode (h) VIP analysis (JCZ vs CZO) in negative ion mode

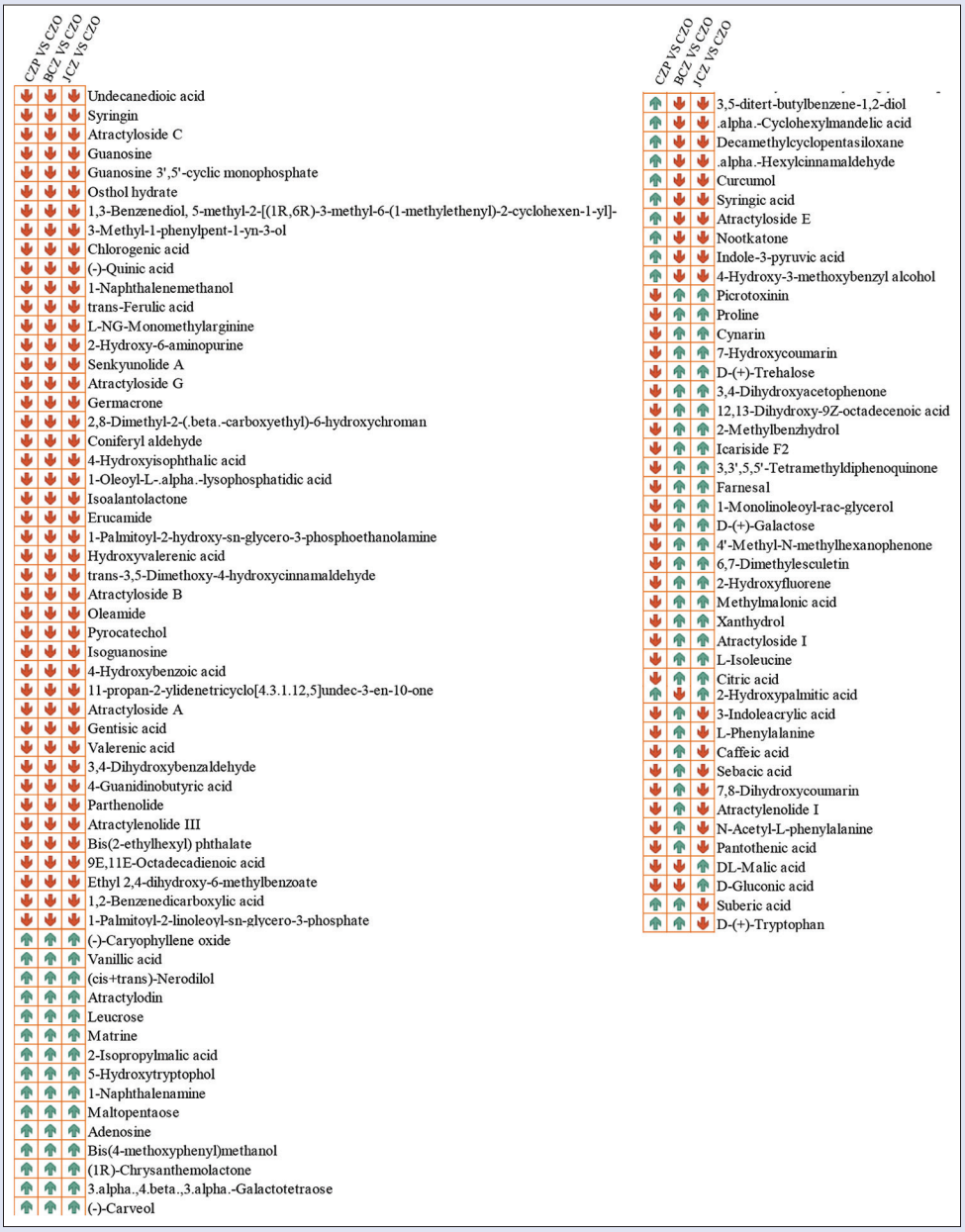

Figure 4: Detailed comparison of chemical ingredient among cangzhu pieces (CZP), bran frying cangzhu (BCZ), Jiao cangzhu (JCZ), and cangzhu (CZO). (Red: the content was decreased compared to CZO; Green: the content was increased compared to CZO)

decreased in JCZ; however, the content of other 39 constituents, such as (-) caryophyllene oxide, vanillic acid, (-)-carveol, etc., was overtly increased in JCZ. The detailed information was displayed in the Figure 4.

As shown in Figure 4, after processing procedure, most of the constituents especially primary active constituents, such as atractyloside A, atractyloside B, atractyloside C, atractyloside G, and atractylenolide III, were simultaneously reduced; these constituents maybe the internal reason of low dryness effect *in vivo*. Take atractyloside A and atractylenolide III, for example, the content of them were reduced seemingly, however, the blood concentration and total discharge rate were increased after their administration to rats. The result indicated that constituents of processed products were more easily absorbed into the blood and played a stronger therapeutic effect.[9,10] Atractyloside A and atractylenolide III maybe part of pharmacodynamic material basis for spleen heighten. Up to now, the pharmacokinetics study of other vital constituents such as atractyloside B, atractyloside C, and atractyloside G were not reported. The blood concentration and total discharge rate of atractyloside B, atractyloside C, and atractyloside G may as well increased *in vivo*. The deduction needs more experimental evidence in further study.

CONCLUSION

In this paper, the ingredients of different products from *Atractyloidis* Rhizome were successively analyzed based on an UHPLC-Q-TOF-MS/MS technology coupled with multivariate data processing method. As a result, a total of 101 constituents were successfully identified in *Atractyloidis* Rhizome. As a consequence, CZO, CZP, BCZ, and JCZ separated obviously in PCA loading plot, and 54 chemical markers discriminating among CZO, CZP, BCZ, and JCZ were screened out. The

current results clarified chemical discrimination in CZO, CZP, BCZ, and JCZ. The results guided chemical information for quality control among different processed products. The established method clarified the scientificity of TCM's preparation in clinical application.

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Conflicts of interest

There are no conflicts of interest.

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