Research Article

Identification and Characterization of Key Chemical Constituents in Processed Gastrodia elata Using UHPLC-MS/MS and Chemometric Methods

Xide Ye\(^1,2\), Yanhong Wang\(^2\), Jianping Zhao\(^2\), Mei Wang\(^2\), Bharathi Avula\(^2\), Qiaozhen Peng\(^3\), Hui Ouyang\(^1\), Zhong Lingyun\(^1\), Jinlian Zhang\(^1\), and Ikhlas A. Khan\(^2,4\)

\(^1\)School of Pharmacy, Jiangxi University of Traditional Chinese Medicine, Nanchang, Jiangxi 344000, China
\(^2\)National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, Jackson, MS 38677, USA
\(^3\)School of Computer Information Engineering, Nanchang Hongkong University, Nanchang, Jiangxi 330063, China
\(^4\)Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, Jackson, MS 38677, USA

Correspondence should be addressed to Ikhlas A. Khan; ikhan@olemiss.edu

Received 4 July 2019; Revised 6 September 2019; Accepted 17 September 2019; Published 23 October 2019

Copyright © 2019 Xide Ye et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Gastrodia elata Blume belongs to the Orchidaceae family. G. elata is often processed when used in traditional Chinese medicine (TCM). In the current study, a traditional processing method, known as “Jianchang Bang,” was applied. Steamed and dried (S&D) G. elata was processed with ginger juice for up to 5 days (GEP5D). An UHPLC-MS/MS combined with a chemometric method was developed for the analysis of processed G. elata along with the raw material as well as steamed and dried G. elata. As a result, the primary marker compounds were identified with the aid of TOF-MS and MS/MS analyses. Compared with the raw material of G. elata with GEP5D, three new parishin-type compounds were identified according to their retention time, accurate mass, and fragmentation patterns. L’he chromatographic peak areas for marker compounds, including S-(gastrodin)-glutathione, S-(4-hydroxybenzylamine)-glutathione, and parishin-type compounds, changed significantly. L’his result indicated that by applying the “Jianchang Bang” method, changes in chemical composition in G. elata contents were observed. L’his study also demonstrated that chemometric analysis is helpful in understanding the processing mechanism and will provide scientific support for the clinical application of G. elata.

1. Introduction

The dry tuber of G. elata, commonly called as Tianma, Chi Jian, or Ming Tianma, has been officially listed in the Chinese Pharmacopeia [1] to treat symptoms of hyperactive liver, arrest endogenous wind, and stop tetany according to the theory of traditional Chinese medicine (TCM). G. elata is used for the prevention and treatment of neuralgic and nervous diseases, such as headaches, migraine, dizziness, rheumatism, convulsion, and epilepsy, for centuries in TCM [2–5]. Natural products have been widely accepted as important sources for nutritional supplements, dietary products, and drug discoveries due to their remarkable and multiple medical benefits for human health. On this account, herbal plants and their bioactive ingredients, especially those with new compounds, have been studied to highlight some of their potential therapeutic values and subjected to extensive research.

G. elata contains a variety of components that have been previously reported [6–8]. These structurally identified compounds were derived from organic solvent extracts of the rhizome which were produced from either fresh raw materials or from steamed and dried materials using different analytical techniques. In recent years, pharmaceutical studies suggested that phenolic glucoside gastrodin, gastrodigenin (p-hydroxybenzylalcohol), and parishins were
identified as secondary metabolites responsible for biological activities [9, 10]. Other compounds with relatively low concentrations, such as nucleosides, polysaccharides, and phospholipids, have also been reported in the literature [11, 12]. The bioactive compounds in G. elata have increasingly become the focus of this research for important potential sources in developing new drug products [13].

G. elata has to be processed before clinical application according to the Chinese pharmacopoeia. The processing of G. elata can cause changes in the content, potentially in the chemical property of the ingredients, or both of them, and influence its clinical efficacy. Processing methods, including mixing G. elata with wine, honey, Tribulus terrestris, wheat bran, or serofluid, have been recorded in some ancient books of TCM [14–17], but the most commonly used processing method is to steam and dry the tuber and then cut it into slices [18]. Among the main genres of TCM, “Jianchang Bang” is a genre located in the Nanxiong county of Jiangxi Province in China. In this, steamed and sliced G. elata is mixed with ginger juice and then dried at room temperature. The processing method has been believed to be a special traditional processing means because of local clinical application of the theory of “toxicity reducing and efficacy enhancing.” After processing with ginger juice, the plain property of G. elata would be turned to warm, according to the theory of TCM. Compared with the pharmacopoeia processing method, the product processed with ginger juice by the “Jianchang Bang” method might have more power to relieve headache and reduce side effects of vomiting caused by the smell of G. elata. Although the processed product of G. elata with ginger has shown good efficacy practically, there is limitation for its clinical application owing to the lack of scientific evidences. Therefore, finding out the differences in the chemical composition of G. elata obtained before and after processing by the “Jianchang Bang” method is the key to revealing the concoction mechanism that can be used for providing clinical application.

Many studies have been carried out and focused on the differences in composition changes in processed G. elata products [19–22]. Li et al. used the UHPLC/Q-TOF-MS/MS method for the identification of 64 chemical components in G. elata by comparing the retention time, accurate mass measurements, and characteristic fragmentation patterns [23]. Yet, these studies were only based on the conventional processing methods, such as steaming and drying or sulfur fumigation, rather than processing G. elata with accessories including ginger or wine. It is hard to determine and characterize the key chemical constituents and markers in processed G. elata using the “Jianchang Bang” method.

In order to understand the chemical composition of G. elata processed with ginger juice, a UHPLC-MS/MS combined with multivariate statistical analysis method was developed. The method facilitated the elucidation of characteristic fragmentation pathways of targeted and nontargeted constituents, especially of key chemical ones, in processed G. elata using the “Jianchang Bang” method by offering accurate mass measurement and significant molecular ions with different ionization modes, and then confirmed their molecular structures using the MS/MS fragmentation pattern. This analysis can also offer an excellent resolution of chromatographic separation of main components from different processed products of G. elata. This is the first time that UHPLC-MS/MS and chemometric methods are being used for the identification of G. elata processed with ginger using the “Jianchang Bang” method.

2. Materials and Methods

2.1. Chemicals and Reagents. Methanol, acetonitrile, and formic acid were of HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ, USA). Water used as the UHPLC mobile phase was purified using a Millipore Synergy UV Water Purification System (Millipore SAS, Molsheim, France). Reference standards parishin A (No: wqk17110609), parishin B (No: wqk17052501), and parishin C (No: wqk17120605) were purchased from Sichuan Vikki Biotechnology Co., Ltd. (Xi’an, China), and their purities were ≥98%.

Dried rhizomes of G. elata were obtained from Zhaotong county, Yunnan Province, China (Jiangxi Wuzhou Pharmaceutical Co., Ltd.) and identified by Dr. Lingyun Zhong (Jiangxi University of Traditional Chinese Medicine). Fresh ginger was purchased from a local supermarket in Mississippi, USA.

2.2. Sample Preparations

2.2.1. Raw and Processed Plant Samples. G. elata (400 g) was steamed, cut into slices, and dried. Ginger juice (50 mL) was obtained by grinding fresh ginger using a mortar and pestle. Dried slices of G. elata (30 g) were mixed with the ginger juice (3.75 mL), which is the “Jianchang Bang” method. Three different materials, such as the raw material, dried slices of G. elata after steaming, and the one processed by the “Jianchang Bang” method (steamed and dried slices of G. elata processed in ginger juice for 5 days) were frozen at −20°C for a day and then taken out for freeze-drying (LABCONCO).

2.2.2. Sample Preparation. After grinding to a fine powder using a pulverizing machine (Chart 5 Retsch. MM400), each sample (2.0 g), such as the raw material of G. elata, dried slices of G. elata after steaming, and samples processed by the “Jianchang Bang” method, was weighed in a centrifuge tube, then 3.5 mL of 50% methanol (methanol/water = 1:1, v/v) was added, and the mixture was sonicated in a water bath for 30 min. After that, the sample was centrifuged for 10 min at 959 × g. The supernatant was transferred to a 10-ml volumetric flask. The procedure was repeated for a total of three times and the respective supernatants were combined. The final volume was adjusted to 10 ml with 50% methanol and mixed thoroughly. Prior to injection, an adequate sample (ca. 2 ml) was passed through a 0.45-μm PTFE membrane filter and collected in an LC vial.

2.3. UHPLC Chromatographic Conditions. The UHPLC analyses were performed on a Waters Acquity UPLC™ system (Waters Corp., Milford, MA, USA) that included a
binary solvent manager, a sample manager, a heated column compartment, and a photodiode array (PDA) detector [24]. The separation was carried out on an Acquity UPLC™ HSS T3 column (100 mm × 2.1 mm i.d., 1.8 μm). The sample temperature and column temperature were maintained at 10°C and 45°C, respectively. The mobile phase consisted of water containing 0.05% formic acid (v/v) (A) and acetonitrile with 0.05% formic acid (B). The analysis was conducted at a flow rate of 0.5 mL/min and performed using the gradient elution as follows: 0–2.5 min, 1% B; 2.5–10 min, 1% B to 9% B; 10–14 min, 9% B to 20% B; 14–18 min, 20% B to 30% B; 18–21 min, 30% B to 60% B; 21–24 min, 60% B to 80% B; and 24–25 min, 80% B to 100% B. Each run was followed by a 3-min wash with 100% B and an equilibration period of 3.5 min with initial conditions. A Strong needle wash solution (90/10; acetonitrile/water, v/v) and a weak needle wash solution (10/90; acetonitrile/water, v/v) were used.

2.4. ESI-QTOF-MS. The high-resolution mass spectrometric analyses were performed using electrospray ionization (ESI) in the negative mode on a Waters Xevo G2-S QTOF mass spectrometer (Waters Corporation). The MS instrument was operated in the following conditions: mass scan range of 100–1500 Da, capillary voltage of 2.0 kV, cone voltage of 40 V, source temperature of 80°C, desolvation temperature of 450°C, desolvation gas flow of 900 L/hr, cone gas flow of 50 L/hr, and collision energy of 6 eV. Leucine-enkephalin was used for lock mass correction at a concentration of 5 μg/mL and a flow rate of 10 μL/min. Ions [M-H]− (m/z 554.2615 Da) and fragments at m/z 276.6268 Da of leucine-enkephalin were applied to ensure mass accuracy during the MS analysis. The lock spray interval was set at 30 s, and the data were averaged over three scans. The mass spectrometer was programmed to switch between two steps: between low (10 V) and elevated (30–50 V) collision energies on the gas cell, using a scan time of 0.1 s per function.

2.5. Statistical Analysis. PCA is one of the statistical analysis techniques used for finding the difference between different sample groups [25, 26]. In the study, data were achieved using Waters MassLynx™ Software v. 4.1 connected to MarkerLynx™ XS Application Manager (Waters Corporation, Milford, USA). A series of procedures in the software were used to handle a retention time of 0.1 min, the chromatogram from 0.2 to 25 min, the mass range of 100–1500 Da, the mass window of 0.02 Da, and the noise elimination level of 10.00%. All compounds identified in G. elata samples before and after processing using ginger juice were distinguished via PCA and Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) through the MarkerLynx XS Application Manager, as shown in Figure 1. The model has 3 score components for the samples: G. elata raw material, dried G. elata, and G. elata processed with GEP5D. These scores display a good separation for the three groups. The scores t[1] (x-axis) and t[2] (y-axis) are key variables in summarizing and separating the data. Each point in the plot corresponds to an observation. The groups are shown in different colors. This variation was assuredly caused by the use of the “Jiangchang Bang” method on G. elata in this study.

3. Results
Parishin A, B, and C (Figure 2) are major components in G. elata and eluted at 14.19, 12.64, and 13.01 min, respectively, under optimized conditions (Figure 3). Their structures in G. elata were unambiguously confirmed by comparing the retention time, accurate mass, and fragmentation pattern with that of reference standards. For instance, parishin A is a conjugate comprising three gastrodin units based on a citric acid unit by ester linkage. On the MS spectra of parishin A, ions at m/z 995.2997 Da are corresponding to the deprotonated molecular ion C_{45}H_{55}O_{25}− ([M-H]−, calc. 995.3038). When applying collision energy, it produces fragments at m/z 727.2067, 459.1053, 441.0986, 423.0921, 397.1124, 369.0175, and 161.0436 Da, corresponding to [M-C_{13}H_{16}O_{3}]−, [727-C_{13}H_{16}O_{2}]−, [459-H_{2}O]−, [441-H_{2}O]−, [423-C_{3}H_{4}]−, [397-CO]−, and [C_{8}H_{8}O_{2}]−, respectively, that were observed in the MS/MS spectra of parishin A (Figure 4). The fragmentation pathway is proposed in Figure 5.

Parishin B and C are isomers that the gastrodin unit connects at 2′ and 3′, respectively (Figure 2). They showed different retention times but had fragmentation pattern identical to that of parishin A.

Compound 9 is eluted at 12.92 min and found to be a deprotonated molecular ion at 889.2624 Da ([C_{38}H_{42}O_{37}]−, calc. 889.2614). When applying collision energy, it generates fragments at m/z 757.2218 Da [C_{35}H_{41}O_{20}]− corresponding to the loss of 132 Da. This indicates that compound 9 contains a pentose. Meanwhile, fragments at m/z 757 Da are 30 Da higher than key fragments at m/z 727 Da in parishin A, B, or C, which suggests that the fragment at m/z 757 Da exists as a methoxy group. In G. elata, reported compounds parishin L and H contain a methoxy unit on their molecules [23]. Therefore, the fragmentation pattern of compound 9 is proposed in Figure 6. This compound is identified as a pentose derivative of parishin H or its isomer, a new marker compound identified only in the processed sample by the “Jianchang Bang” method.

Compound 16 is a new mark in GEP5D that eluted at 13.9 min and appeared to be deprotonated molecular ion at 1319.4051 Da ([C_{57}H_{75}O_{35}]−, calc. 1319.4094) but does not show in the raw sample at the same retention time. When employing collision energy, fragment ions at m/z 1051.3173 Da and 889.2628 Da related to the losses of gastrodin residue (268 Da) and hexose (162 Da), respectively, are observed. In the following fragmentation pathway, fragment ions of compound 16 are the same as that of parishin A. Therefore, compound 16 is tentatively identified as a derivative of parishin A plus two units of hexoses, according to its fragmentation pattern (Figure 7).

Deprotonated molecular ions of compound 14 are at 1157.3589 Da ([C_{51}H_{65}O_{30}]−, calc. 1157.3566). The elution time of compound 14 (13.77 min) is different from that of parishin F (14.13 min) in G. elata samples, but compound 14 has a fragmentation pattern identical to that of parishin F [23]. Therefore, compound 14 is confirmed as an isomer of parishin H.
parishin F, a new marker compound found only in the GEP5D sample by the “Jianchang Bang” method, as shown in Table 1.

Peak areas of many key marker compounds in Table 1 are different in raw, S&D, and GEP5D samples. These compounds are mainly parishin-type compounds, except for compounds 1 and 5 which are derivatives of glutathione. As seen in Table 1, the peak areas of maker compounds in S&D samples are usually decreased when compared with that of raw samples. Among them, the most obvious decline is found for compound 12: the peak area of this compound in the raw sample is more than sixfold that of the dried sample at the same optimized analysis conditions. The peak area of compound 3 also experiences a great decrease after
processing *G. elata* by the drying method. Yet, there are few marker compounds, such as compounds 5, 8, and 11, whose peak areas increase slightly when compared with raw samples and dried samples. However, the peak areas of some compounds, such as compounds 1, 2, 3, 12, 15, and 19, in GEP5D samples have been found to be lower than that of raw samples but higher than that of S&D samples. Moreover, the peak areas of compounds 6 and 11 in GEP5D samples are greater than that of raw samples. On the basis of MS and MS/MS data, it has been found that pentose or hexose derivatives of parishin-type compounds can be accumulated in *G. elata* samples processed by the “Jianchang Bang.” This observation has been proved by the identification of new marker compounds 9, 14, and 16.

In Table 1, parishin-type compounds are characterized following the fragmentation pattern of parishin A, B, and C according to MS and MS/MS data. Two derivatives of glutathione are determined as follows. Deprotonated
Figure 4: MS and MS/MS spectra of parishin A.

Figure 5: Proposed fragmentation pathway for parishin A.
molecular ions of compound 5 at 12.32 min are at 412.1171 Da ([C_{17}H_{22}N_{3}O_{7}S]^{-}, calc. 412.1184). On the MS/MS spectrum, the fragment ions at 306.0758 Da [C_{10}H_{16}N_{3}O_{6}S]^{-} correspond to the loss of the 4-hydroxy benzyl group. Therefore, compound 5 is tentatively identified as S-(4-hydroxybenzyl)-glutathione [25]. Compound 1 at 9.37 min (m/z 574.1700, [C_{23}H_{32}N_{3}O_{12}S]^{-}, calc. 574.1712) is 162 Da higher than compound 5, which suggests that compound 1 is a hexose derivative of compound 5. Considering gastrodin as one of the main components, compound 1 is characterized as S-gastrodin glutathione, and its fragmentation pathway is shown in Figure 8.
4. Discussion

For the UHPLC/Q-TOF-MS/MS analysis, different UHPLC columns, column temperatures, mobile phases, gradient elutions, and flow rates were evaluated to achieve the optimal separation. As a result, acetonitrile–water with 0.05% formic acid combined with the optimized gradient elution on an UPLC HSS T3 column at 45°C offered an appropriate chromatographic separation and MS responses in the negative ion scan mode.

The OPLS-DA was used for the identification and differentiation of potential markers based on their peak

| Compound | Retention time (min) | Mass \( m/z \) | Peak area | Name | Fragmentation ions |
|----------|----------------------|----------------|-----------|------|-------------------|
| 1        | 9.37                 | 574.1700       | 13383     | S-(Gastrodin)-glutathione | 412.1168, 306.0758, 272.0877, 254.0773, 210.0867 |
| 2        | 11.80                | 727.2089       | 7734      | Isomer of parishin B      | 441.1028, 397.1129, 369.1189, 161.0447 |
| 3        | 12.02                | 727.2082       | 6542      | Isomer of parishin B      | 441.1017, 423.0917, 399.0924, 397.1118, 369.1176, 161.0438 |
| 4        | 12.20                | 889.2631       | 5478      | Isomer of parishin H      | 727.2081, 441.1037, 423.0923, 397.1158, 323.0960, 263.0748, 161.0435 |
| 5        | 12.32                | 412.1171       | 1055      | S-(4-Hydroxybenzyl)-glutathione | 306.0733, 272.0825, 254.0826, 210.0857 |
| 6        | 12.57                | 889.2625       | 765       | Isomer of parishin V      | 727.2065, 423.0901, 323.0976, 263.0471, 161.0442 |
| 7        | 12.64                | 727.2089       | 111564    | Parishin B                | 441.1028, 423.0928, 397.1134, 323.0970, 161.0440 |
| 8        | 12.78                | 889.2626       | 6887      | Parishin V                | 727.2051, 423.0912, 397.1130, 323.0970, 161.0440 |
| 9        | 12.92                | 889.2624       | 0         | Pentose derivative of isomer of parishin H | 757.2218, 423.0952, 397.1127, 323.0952, 161.0444 |
| 10       | 13.01                | 727.2086       | 54794     | Parishin C                | 441.1022, 423.0923, 397.1125, 369.1179, 161.0443 |
| 11       | 13.12                | 889.2621       | 1681      | Parishin I                | 727.2092, 423.0890, 323.0974, 161.0443 |
| 12       | 13.51                | 473.1298       | 1874      | Demethyl derivative of parishin N | 441.1042, 423.0969, 397.1045, 169.0110 |
| 13       | 13.69                | 1157.3590      | 5981      | Isomer of parishin F      | 889.2646, 727.2120, 423.0960, 323.1023, 161.0433 |
| 14       | 13.77                | 1157.3589      | 0         | Isomer of parishin F      | 889.2661, 727.2122, 423.0913, 323.0916, 161.0523 |
| 15       | 13.86                | 473.1290       | 6617      | Demethyl derivative of parishin N | 423.0891, 397.1096, 169.0130 |
| 16       | 13.90                | 1319.4051      | 0         | Derivative obtained by adding two glucose to parishin A | 1051.3173, 889.2628, 727.2076, 473.1293, 423.0913, 397.1132, 169.0135 |
| 17       | 14.13                | 1157.3595      | 10521     | Parishin F                | 889.2638, 727.2078, 423.0935, 323.0958, 161.0448 |
| 18       | 14.19                | 995.3057       | 117584    | Parishin A                | 727.2077, 441.1008, 423.0918, 397.1118, 369.1174, 161.0446 |
| 19       | 14.21                | 727.2092       | 25233     | Isomer of parishin B      | 441.1025, 423.0920, 397.1129, 369.1180, 161.0401 |
| 20       | 14.37                | 1025.3162      | 7106      | Parishin L                | 889.2578, 757.2204, 727.2094, 423.0911, 369.1133, 161.0443 |

**Figure 8:** Proposed fragmentation pathway for compound 1.
areas [27]. As the most important observation, three new markers, such as compounds 9, 14, and 16, along with at least 17 known compounds in G. elata were identified by the differences in compounds obtained from raw and GEP5D samples. In total, 20 key marker compounds were obtained from raw, S&D, and GEP5D samples based on differences identified by the comparison of their peak areas under the same optimized analysis conditions (Table 1).

An UHPLC-MS/MS method combined with multivariate statistical analysis was conducted to determine and characterize key marker compounds in G. elata raw material and processed samples by steaming or the “Jianchang Bang” method. Because of the significant evaluating means which reflected the changes in major compounds in different samples of G. elata processed by ginger, new marker compounds have been found and many important constituents experienced great changes between raw and GEP5D or raw and dried samples in peak areas.

5. Conclusions

In this study, unsupervised PCA can differentiate G. elata raw material, steamed samples, and samples processed by the “Jianchang Bang” method. OPLS-DA of raw material vs. GEP5D samples processed by the “Jianchang Bang” method provides a tool to select and determine marker compounds. A total of 20 marker compounds were characterized based on the accurate mass of MS and fragment ions of MS/MS data as well as by comparing with corresponding reference standards and literature. Compounds 9, 14, and 16 are new markers of parishin-type compounds in G. elata samples processed with ginger by the “Jianchang Bang” method. Except for two derivatives of glutathione (1 and 5), other marker compounds are parishin-type constituents. These results suggest that the developed UHPLC-MS/MS method combined with PCA and OPLS-DA can characterize key components in G. elata processed with ginger. This method will support the clinical application of G. elata processed by the “Jianchang Bang” method in a scientific way.

Abbreviations

G. elata: Gastrodia elata
TCM: Traditional Chinese medicine
S&D: Steamed and dried
GEP5D: G. elata was processed with ginger juice for up to 5 days
UHPLC-MS/MS: Ultra-high-performance liquid chromatography-tandem mass spectrometry
PCA: Principal component analysis
OPLS-DA: Orthogonal partial least squares-discriminant analysis
TOF: Time of flight
ESI: Electrospray ionization
ESI-QTOF-MS: Electrospray ionization quadrupole time-of-flight mass spectrometry
UHPLC/Q-TOF-MS/MS: Ultra-high-performance liquid chromatography-quadrupole time-of-flight tandem mass spectrometry.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

No potential conflicts of interest were reported by the authors.

Acknowledgments

This research is supported by the National Natural Science Fund of China (No. 81760712), the Jiangxi Provincial Special Scientific Fund for First-Class Discipline of Traditional Chinese Medicine (JXSYLKX-ZHYAO040), and the Jiangxi Province Special Funds for Visiting Scholars in the Plan of Young and Mid-Aged Teachers’ Development. The research is also partially supported by the United States Department of Agriculture, Agricultural Research Service (Specific Cooperative Agreement no. 58-6408-1-603-07).

References

[1] Z.-W. Wang, Y. Li, D.-H. Liu et al., “Chemical constituents from the rhizomes of Gastrodia elata f. glauca and their potential neuroprotective effects,” Phytochemistry Letters, vol. 24, pp. 167–171, 2018.
[2] U. Ramachandran, A. Manavalan, H. Sundaramurthi et al., “Tianna modulates proteins with various neuro-regenerative modalities in differentiated human neuronal SH-SY5Y cells,” Neurochemistry International, vol. 60, no. 8, pp. 827–836, 2012.
[3] H. Ouyang, M. Zhou, Y. He et al., “Metabolites profiling of Pulsatilla saponin D in rat by ultra performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC/Q-TOF-MS/MS),” Fitoterapia, vol. 96, pp. 152–158, 2014.
[4] P.-J. Chen and L.-Y. Sheen, “Gastrodiae Rhizoma (tiân má): a review of biological activity and antidepressant mechanisms,” Journal of Traditional and Complementary Medicine, vol. 1, no. 1, pp. 31–40, 2011.
[5] N.-K. Huang, Y.-L. Lin, J.-J. Cheng, and W.-L. Lai, “Gastrodia elata prevents rat pheochromocytoma cells from serum-deprived apoptosis: the role of the MAPK family,” Life Sciences, vol. 75, no. 13, pp. 1649–1657, 2004.
[6] W.-C. Chen, Y.-S. Lai, K.-H. Lu et al., “Method development and validation for the high-performance liquid chromatography assay of gastrodin in water extracts from different sources of Gastrodia elata Blume,” Journal of Food and Drug Analysis, vol. 23, no. 4, pp. 803–810, 2015.
[7] W.-C. Chen, Y.-S. Lai, S.-H. Lin et al., “Anti-depressant effects of Gastrodia elata Blume and its compounds gastrodin and 4-hydroxybenzyl alcohol, via the monoaminergic system and neuronal cytoskeletal remodeling,” Journal of Ethnopharmacology, vol. 182, pp. 190–199, 2016.
[8] Q. Guo, Y. Wang, S. Lin et al., “4-Hydroxybenzyl-substituted amino acid derivatives from Gastrodia elata,” Acta Pharmaceutica Sinica B, vol. 5, no. 4, pp. 350–357, 2015.

[9] Z. Li, Q. Wang, H. Ouyang et al., “New compounds with neuroprotective activities from Gastrodia elata,” Phytochemistry Letters, vol. 15, pp. 94–97, 2016.

[10] C.-J.-S. Lai, Y. Yuan, D.-H. Liu et al., “Untargeted metabolite analysis-based UHPLC-Q-TOF-MS reveals significant enrichment of p-hydroxybenzyl dimers of citric acids in fresh beige-scape Gastrodia elata (Wutianma),” Journal of Pharmaceutical and Biomedical Analysis, vol. 140, pp. 287–294, 2017.

[11] B.-W. Kim, S. Koppula, J.-W. Kim et al., “Modulation of LPS-stimulated neuroinflammation in BV-2 microglia by Gastrodia elata: 4-Hydroxybenzyl alcohol is the bioactive candidate,” Journal of Ethnopharmacology, vol. 139, no. 2, pp. 549–557, 2012.

[12] C. Tang, L. Wang, X. Liu, M. Cheng, Y. Qu, and H. Xiao, “Comparative pharmacokinetics of gastrodin in rats after intragastric administration of free gastrodin, parishin and Gastrodia elata extract,” Journal of Ethnopharmacology, vol. 176, pp. 49–54, 2015.

[13] M. Matias, S. Silvestre, A. Falcão, and G. Alves, “Gastrodia elata and epilepsy: rationale and therapeutic potential,” Phytomedicine, vol. 23, no. 12, pp. 1511–1526, 2016.

[14] Y. Liu, R. Ran, G. Huang et al., “Study on the best initial processing technology of Gastrodia elata,” Pharmaceutical Chemistry Journal, vol. 52, no. 3, pp. 224–230, 2018.

[15] Z. W. Ning, C. Q. Mao, T. L. Lu et al., “Effects of different processing methods on effective components and sulfur dioxide residue in Gastrodiae Rhizoma,” China Journal of Chinese Materia Medica, vol. 39, no. 15, pp. 2814–2818, 2014.

[16] J. Kwon, N. Kim, D. Lee et al., “Metabolomics approach for the discrimination of raw and steamed Gastrodia elata using liquid chromatography quadrupole time-of-flight mass spectrometry,” Journal of Pharmaceutical and Biomedical Analysis, vol. 94, pp. 132–138, 2014.

[17] A. Feng, B. Tian, J. Hu, and P. Zhou, “Recent applications of capillary electrophoresis in the analysis of traditional Chinese medicines,” Combinatorial Chemistry & High Throughput Screening, vol. 13, no. 10, pp. 954–965, 2010.

[18] J. H. Zhu, K. Yu, X. G. Chen et al., “Comparison of two sample preconcentration strategies for the sensitivity enhancement of flavonoids found in Chinese herbal medicine in micellar electrokinetic chromatography with UV detection,” Journal of Chromatography A, vol. 1166, no. 1-2, pp. 191–200, 2007.

[19] R.-t. Tian, P.-s. Xie, and H.-p. Liu, “Evaluation of traditional Chinese herbal medicine: chaihu (Bupleuri Radix) by both high-performance liquid chromatographic and high-performance thin-layer chromatographic fingerprint and chemometric analysis,” Journal of Chromatography A, vol. 1216, no. 11, pp. 2150–2155, 2009.

[20] Z. Cui, N. Ge, A. Zhang, Y. Liu, J. Zhang, and Y. Cao, “Comprehensive determination of polycyclic aromatic hydrocarbons in Chinese herbal medicines by solid phase extraction and gas chromatography coupled to tandem mass spectrometry,” Analytical and Bioanalytical Chemistry, vol. 407, no. 7, pp. 1989–1997, 2015.

[21] Z. Li, Y. H. Wang, H. Ouyang et al., “A novel dereplication strategy for the identification of two new trace compounds in the extract of Gastrodia elata using UHPLC/Q-TOF-MS/MS,” Journal of Chromatography B, vol. 988, pp. 45–52, 2015.

[22] P. Fasinu, N. P. Dhammika Nanayakkara, Y. H. Wang et al., “Formation primaquine-5,6-orthoquinone, the putative active and toxic metabolite of primaquine via direct oxidation in human erythrocytes,” Malaria Journal, vol. 18, no. 30, pp. 1–8, 2019.