

Discrimination of Radix *Angelica sinensis* from Different Producing Areas by Using Electronic Tongue and UPLC Methods

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Abstract: *Angelica sinensis* is a famous medicinal plant and dietary supplement, whose quality is different in different producing areas. However, limited research focused on the discrimination of producing areas for Radix *Angelica sinensis* (DG) based on electrochemical techniques. In this study, an array of seven taste sensors were employed to detect the DG from Gansu, Yunnan, Sichuan and Hubei province in China. The Ultra Performance Liquid Chromatography (UPLC) technology was used to determine the main chemical composition content and verify the analysis of electronic tongue. The results demonstrated that electronic tongue could discriminate the DG samples from different producing areas, and the UPLC determination verified the analysis of electronic tongue. This work was conducive to clarify the diversity and geoherbalism of herb drugs, which laid foundation for better and rational utilization of traditional Chinese medicine.

Keywords: Electronic Tongue, Chemometrics, *Angelica sinensis*, Sensory Evaluation, PCA

1. Introduction

Angelica sinensis (Oliv.) Diels is commonly medicinal plant, which is mainly distributed in Europe, North America and western of Asia, including China. Gansu, Yunnan, Sichuan and Hubei province are the main producing areas for *A. sinensis* in China [1]. Volatile oil, phthalides, organic acids, polysaccharides and flavonoid are main chemical components of Radix *Angelica sinensis* (DG) [2]. DG has been used for the treatment of gynecological diseases such as dysmenorrhea and amenorrhea for thousands of years in traditional Chinese Medicinal prescriptions [3]. The herb drugs derived from the root of *A. sinensis* are mainly beneficial for hematopoietic system, circulatory system, nervous system, immune system [4]. In addition, DG is largely served as the dietary supplement in Europe and American market [5, 6], and as health food for women care in Asia and European [7, 8].

Previous study showed that DG from different producing

areas possessed different contents of ferulic acid, volatile components, phthalides and nucleosides and had different quality for clinical application [9-13]. Traditional methods, such as characteristic and morphological identification, microscopic identification, are mainly relied on experience and subjective discrimination, which limited by high cost, a lack of taxonomic expertise and the considerable amount of time to sample and identify plants accurately. The physical and chemical method, such as ultraviolet spectrum (UV), high performance liquid chromatography (HPLC) and ultra performance liquid chromatography (UPLC) required various experiment conditions and complex operational procedure.

Electronic tongue which is equipped with an array of non-selective and broad-spectrum chemical sensors, emerged as a useful tool to provides an odor fingerprint of samples and to classify the samples according to their quality [14-21]. As a new technology and an supplement for traditional methodologies, e-tongue has been widely applied for

authentic assessment and quantitative analysis of food, water, wine, oil, crops and herbal medicines [22-25]. Moreover, studies also showed that the electrochemical device can be used as an effective and practical tool to distinguish materials according to their botanical origin [26-28]. In this study, electronic tongue was employed to discriminate the DG samples from Gansu, Yunnan, Sichuan and Hubei province in China. UPLC technique was performed to determinate the chemical content of main components, and verify the analysis of electronic tongue. This work aimed to explain the diversity and geoherbalsim of DG, which was conducive for safe and reasonable application of traditional Chinese medicine.

2. Materials and Methods

2.1. Reagents and Materials

The two-year old samples of DG were collected from Gansu, Yunnan, Sichuan and Hubei province of China in October to November of 2014 (Table 1). The samples were identified to *Angelica sinensis* (Oliv.) Diels by the Prof. Yulin Lin, and the voucher specimens were deposited in the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing, China.

Table 1. The samples for herb drugs of *Angelica sinensis* from China.

Sample number	Collection location	Collection Time
Gansu 1	Zhifang village, Sigou township, Min county, Gansu	2014.10.21
Gansu 2	Lvye village, Mazi township, Min county, Gansu	2014.10.22
Gansu 3	Wendou village, Meichuan township, Min county, Gansu	2014.10.23
Yunnan 1-3	Machang village, Caohai township, Heqing county, Yunnan	2014.11.10
Sichuan 1-3	Shimeishan village, Shiquan township, Zhongjiang county, Sichuan	2014.11.17
Hubei 1-3	Hongta village, Chengguan township, Fang county, Hubei	2014.11.20

The acetonitrile of UPLC grade were purchased from ThermoFisher, USA. The formic acid of analytical grade were purchased from Fisher, USA. The deionized water was obtained from Milli-Q water system (Millipore, Bedford, MA, USA). The reference substance of femlic acid (110773-201012) were purchased from National Institutes for Food and Drug Control. The reference substance of senkyunolide A (Batch number: MUST-10102309), Ligustilide L (Batch number: MUST-11072416), n-butylphthalide (Batch number: MUST- 12020706), Butylidenephthalide (Batch number: 12071103) were purchased from Chengdu Mansite, Biological Technology Co., Ltd. (purities > 98%).

2.2. Instrumentation

The α -ASTREE electronic tongue sensor array were consist of the second set of sensor array (seven taste sensors: ZZ, AB, GA, BB, CA, DA, JE, and one reference electrode of silver chloride), sixteen-automatic sampler, data acquisition system and Alphasoftware V12 workstation software. The Acquity UPLC-DAD system (Waters, USA) was consist of quaternary high pressure gradient pump, vacuum degasser with solvent rack, automatic sampler, Empower 2 chromatographic workstation. The pure water were produced by ELGA PURELAB Classic-UVF pure water equipment (ELGA, UK).

2.3. Electronic Tongue Analysis

The decoction pieces of DG were put into the beaker for 10 g, and soaked for 30 min with 500 mL pure water, and then decocted for 30 min. The decocted solution were filtered immediately, and the residue were decocted for 30 min with 500 mL pure water. The decocted solution were filtered immediately, and combined this two filtrate with pure water to 1000 mL. The 10 mL filtrate were precision measured, and diluted with pure water to 100 mL. The obtained solution of

herb drugs were with the concentration of 1 mg·mL⁻¹, and then filtered with 0.45 μ m microfiltration membrane for electronic tongue analysis. The sample solution of 80 mL were put into the special beaker of electronic tongue, and detected the samples in the room temperature. The every sensor collected the data from every sample for 120 s, and then cleaned for 10 s. Every second collected one datum, and the data were recorded by data acquisition system. Every sample were detected for three times. Before the data collection, the electronic tongue system were processed of self-checking, diagnosis and straighten for ensuring the accuracy and reliability of collected data.

2.4. UPLC Determination

The five reference substance were weighed 1 mg critically and respectively. The reference substance were put into volumetric flask, and then diluted with 70% methyl alcohol to 10 mL. The solution were blended into 0.1 mg·mL⁻¹ for subsequent analysis. The herb drug of DG samples were crushed into powder, and filtered by three-sieve. The 0.2 g powder of DG were weighed precisely, and put into conical flask with cover. The conical flask was added into 20 mL methyl alcohol critically, and measured the weight after closing the cover. The solution was heated reflux for 30 min, and supplemented to 20 mL with 70% methyl alcohol after cooling completely. The obtained solution were filtered with 0.22 μ m microfiltration membrane before injecting into UPLC analysis.

The chromatographic column of BEH C₁₈ (2.1 mm×100 mm, 1.7 μ m) was employed in this study. The mobile phase were 0.1% methanoic acid (A): acetonitrile (B). The flow rate was 0.3 mL·min⁻¹. The column and autosampler were maintained at 35 and 5°C respectively. The injection volume was 2 μ L. The conditions of gradient elution were optimized as follows: 95% A: 5% B (0-4 min), 95% A: 5% B - 76% A:

24% B (4-7 min), 76% A: 24% B - 72% A: 28% B (7-8 min), 72% A: 28% B - 50: A: 50% B (8-10 min), 50: A to 50% B - 30% A: 70%B (10-12 min), 30% A: 70%B - 0% A: 100% B (12-15 min). The detection wavelength of femlic acid, senkyunolide A, Ligustilide L, n-butylphthalide was 281 nm, and the detection wavelength of butylidenephthalide was 261 nm. All experiments were performed in triplicate.

2.5. Data Processing

The data obtained from the electronic tongue were analyzed by Alphasoftware V12 workstation software, including the data processing and principal component analysis (PCA). The data analysis of UPLC, chemometrics and the correlation analysis between electronic tongue and UPLC were processed by SPSS 22.0 (SPSS Institute, Cary, NC, USA).

3. Results and Discussion

3.1. The Sensors Array Analysis

Seven sensors named ZZ, AB, GA, BB, CA, DA, JE were applied to detect the samples of DG from different producing areas. Because of various absorbed molecules, the different sensors were adsorbed diverse ionized molecules in the solution selectively, which made up of different response signals (Figure 1). The results showed that different sensors possessed different responses. Sensor BB was more sensitive to DG samples. The signals of DG samples from Hubei province were higher than other three producing areas, while signals of Sichuan province were lower than other three producing areas.

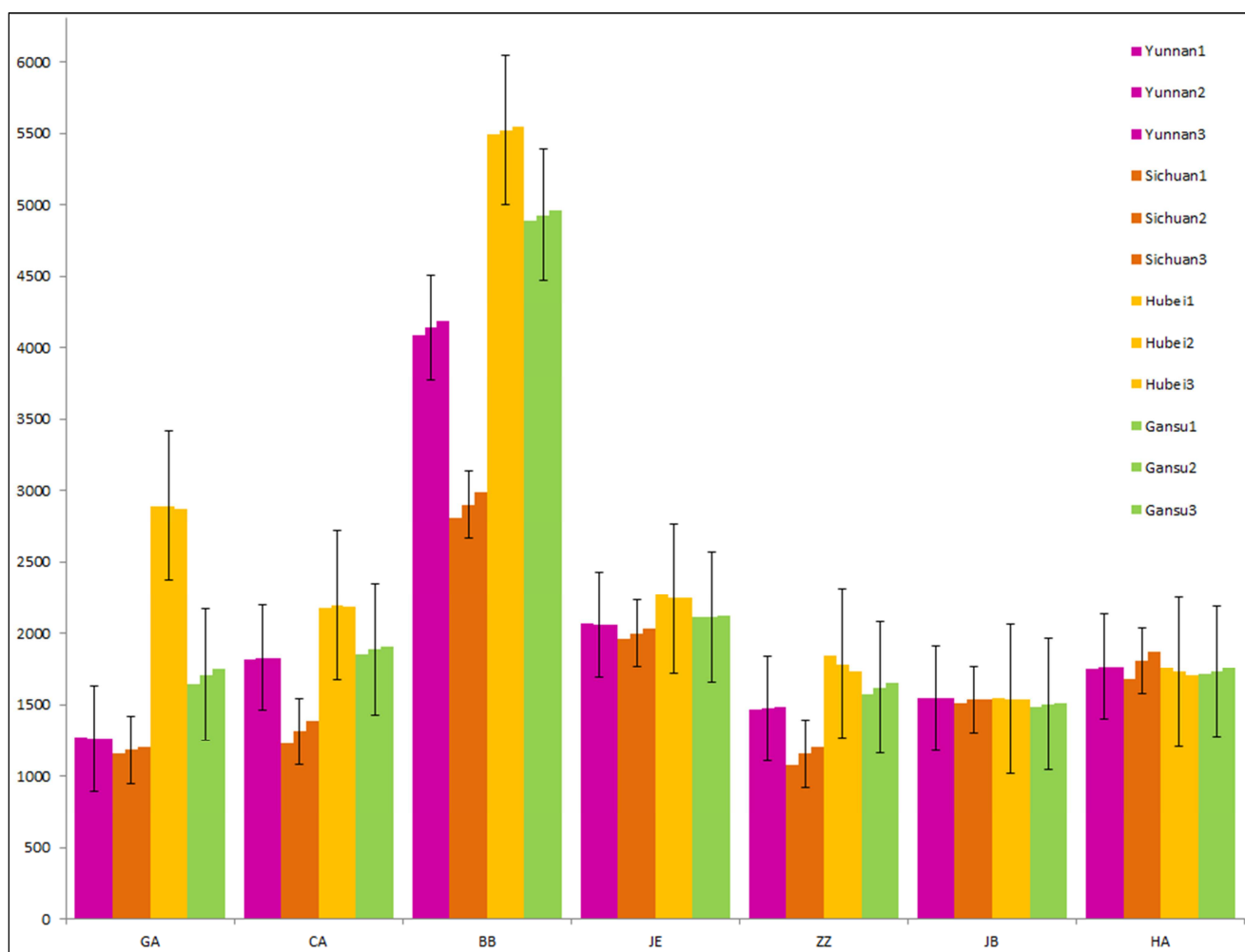


Figure 1. The response signal of seven sensors for DG from different producing areas.

The standard deviation for each producing area was presented in the histogram respectively.

3.2. The radar Map Analysis

The radar map showed the response values of every electrode for every sample visually, which was convenient for comparing the response values between different samples (Figure 2). From Figure 2a, response values of samples from the same producing areas were consistent, while the samples from different producing areas were discrepant. Figure 2b showed the differentiation between different producing areas clearly. DG samples from Sichuan province possessed significantly different response values compared to other samples.

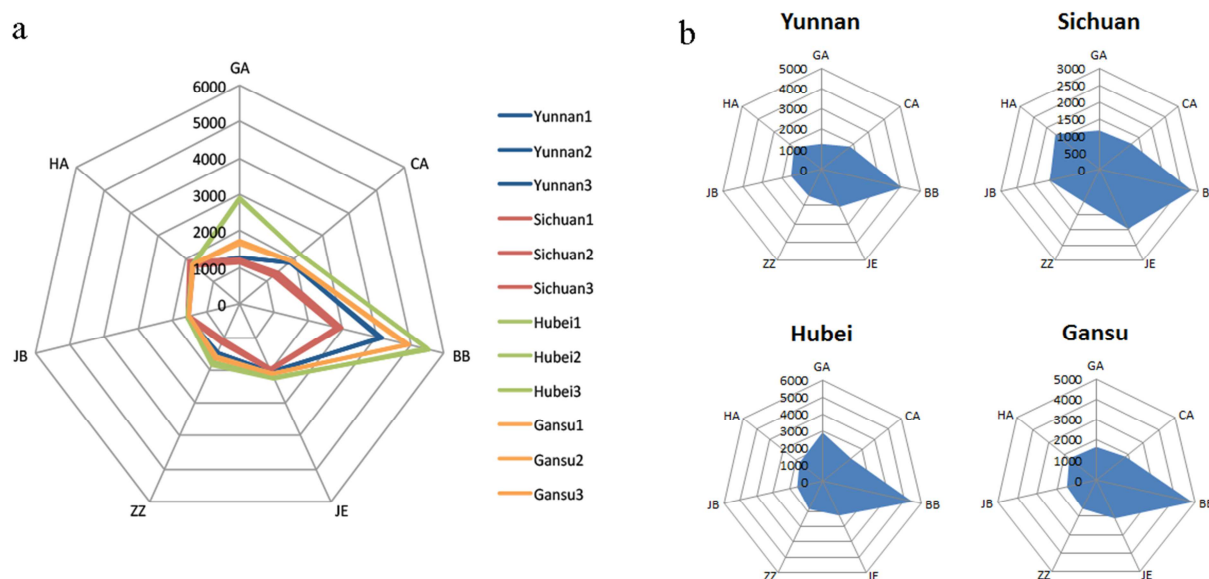


Figure 2. The radar map for DG from different producing areas.

The radar map for samples of all and each producing areas were displayed in Figure 2a and 2b, which was able to compare the response values for DG from different regions obviously.

3.3. The PCA Analysis of Electronic Tongue

The response values were collected by the data acquisition system, and then analyzed in the Alphasoft V12 workstation software for principal component analysis (PCA). The PCA was calculated by dimension reduction analysis to obtain discrimination index, distance and relative standard deviation (RSD) between different data groups determined by electrodes. The groups of data were mutual independence, which demonstrated that the discrimination index was positive. The larger the discrimination index, the better between the samples. The larger the distance, the more obvious the difference of

taste between samples.

The results showed that the contribution rate of first two principal components was 98.997% and 1.003% respectively, and the accumulative contribution rate was 100.00% (Figure 3). That indicated the two dimensional subspaces decided by the feature vector corresponding with this two principal components could fully conserve the original data information. Samples from same producing areas possessed smaller dispersion compared with samples from different producing areas. The distance between different producing areas showed that the quality of samples from Gansu and Yunnan province were close, while samples from Hubei province possessed great difference with other producing areas. The results indicated that the electronic tongue could discriminate the samples of DG from different producing areas.

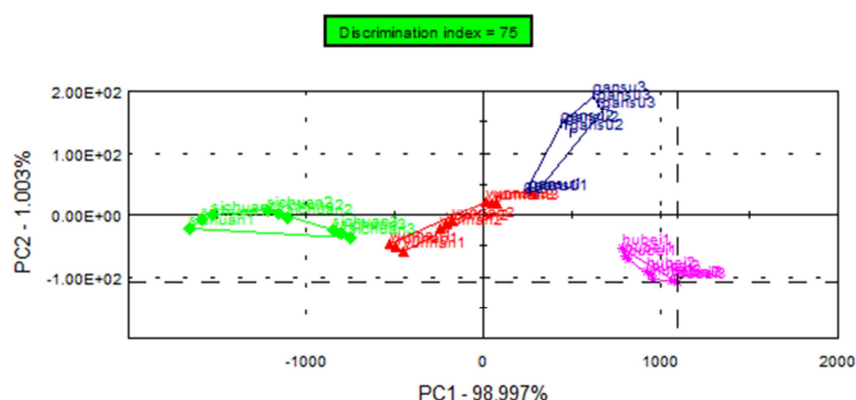


Figure 3. The PCA of electronic tongue for DG from different producing areas.

This analysis demonstrated that the samples from different producing areas separated for each other distinctly, and the samples from same producing areas got together, which indicated that electronic tongue could discriminate DG from different producing areas.

3.4. Determination of Chemical Content

The determination of the content for ferulic acid and four phthalide compositions were performed by UPLC system (Figure 4). The validation, precision, accuracy, and stability test for the method were according to the previous study of our

team [29]. The chemical contents, especially ligustilide L, ferulic acid and butylidenephthalide of DG from Gansu and Yunnan province were all higher than Sichuan and Hubei province. Ferulic acid is the measuring standard of DG in Chinese pharmacopoeia (CP) ($\geq 0.5\%$). Results showed that samples from Yunnan province possessed higher mass fraction of ferulic acid than other three province, and Hubei province possessed the lowest mass fraction of ferulic acid.

The content of ferulic acid in the samples from Sichuan (1/5 of the requirement of CP) and Hubei (only 1/50 of the requirement of CP) were less than the requirement of Chinese pharmacopoeia. The rank of the content for the phthalide compositions of four producing areas was as the following: Gansu > Yunnan > Sichuan > Hubei province, which was in consistent with the results of ferulic acid.

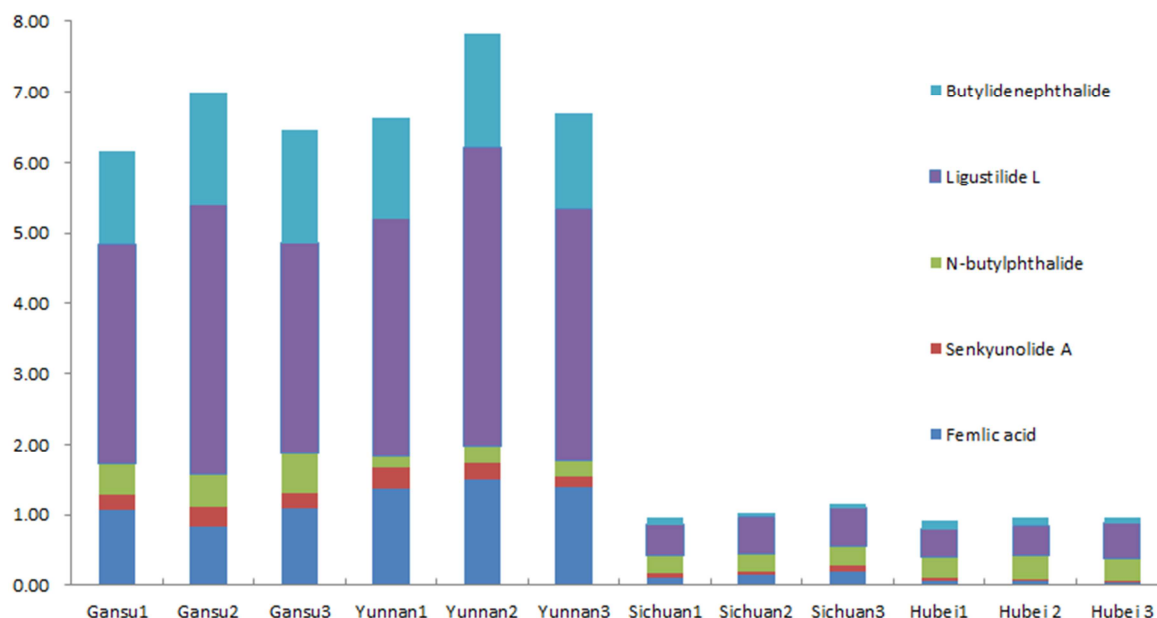


Figure 4. The content of five main chemical components for DG from different producing areas.

The results showed that the samples from Gansu and Yunnan province possessed higher content than Sichuan and Hubei province.

The content datas collected from UPLC were subsequently employed for PCA, aimed to verify the analysis of electronic tongue (Figure 5). Results showed that samples from four producing areas were distinguished obviously. The samples of Sichuan and Hubei province were closer than other two

provinces. The results were in consistent with the detection of electronic tongue, which can be a verification to the analysis of electronic tongue. The results were also in conformity with the previous research of Tan [30], who distinguished *Radix Angelica sinensis* from different regions in China by headspace solvent free microextraction (HS-SFME)/ gas chromatography-mass spectrometry (GC-MS).

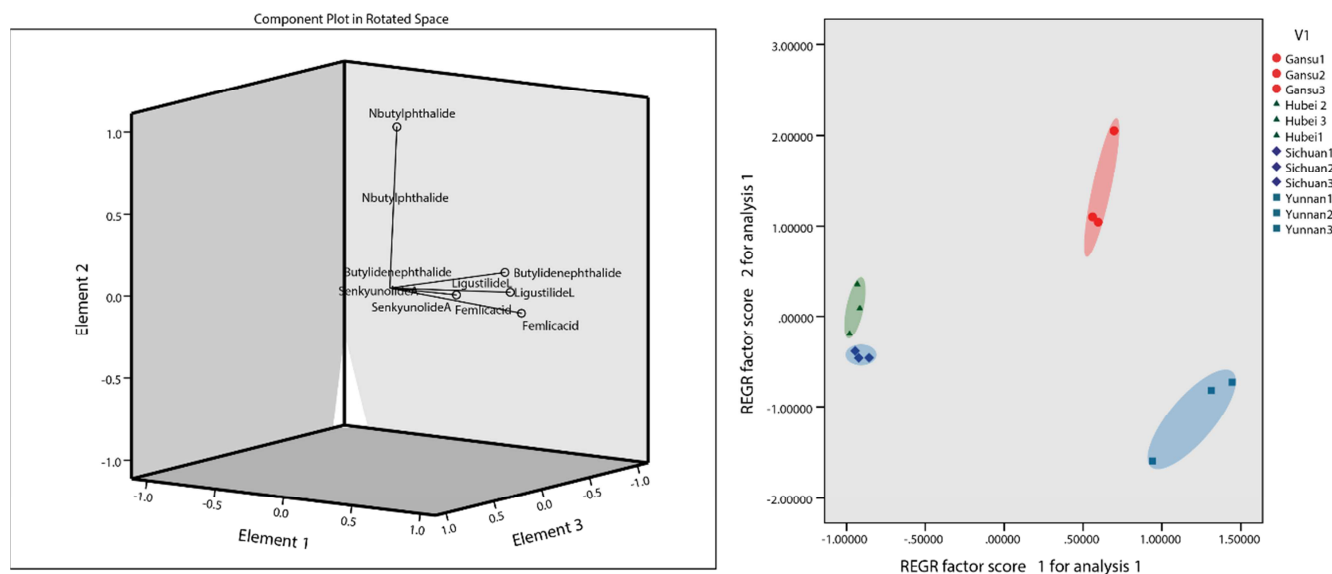


Figure 5. The PCA of the content of chemical components for DG from different producing areas.

The results indicated that DG from different regions possessed different chemical content, and the analysis was in accordance with the analysis of electronic tongue.

3.5. Correlation Analysis

Based on response values from electronic tongue and chemical content from UPLC, the correlation analysis was performed (Figure 6). The results of correlation analysis demonstrated that the response values of GA and JE were almost negatively correlative with all chemical components of DG samples except nbutylphthalide, while CA, BB and ZZ sensors were positively correlative with the chemical components strongly. We could deduce that the response values from GA, JE CA, BB and ZZ sensors might be consisted of the content of nbutylphthalide.

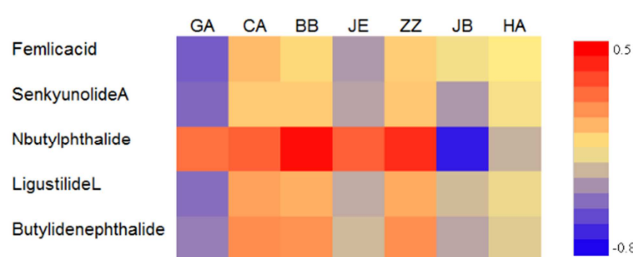


Figure 6. The correlation analysis between electronic tongue and chemical components for DG from different producing areas.

The results showed that the content of main chemical components were correlative with the response values of seven sensors.

4. Conclusions

In this work, electronic tongue and UPLC were employed to discriminate DG samples from four producing areas. The results showed that electronic tongue could distinguish DG samples from different producing areas, and the determination of chemical content by UPLC verified the accuracy of electronic tongue. This research was helpful to illustrate the diversity and geoherbalism of herb drugs and contributes to the development of industry of traditional Chinese medicine.

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Authors' Contributions

Linfang Huang, LinLi and Sihao Zheng contributed to conceive and design this study; Sihao Zheng, Yulin Lin and performed the experiments; LinLi, Jiaji Ding and Sihao Zheng

analyzed the data. Jiaji Ding wrote the manuscript. All the authors revised the manuscript carefully and approved the final manuscript.

Competing Interests

The authors declare no competing financial interests.

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