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Exploring the Quality of *lophatherum gracil* based on Taste and Quantitative Multi-component Determination

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Abstract: *Lophatherum gracile* is both edible and medicinal, and quality evaluation of *lophatherum gracil* is necessary to ensure the clinical efficacy of *lophatherum gracil*. In this study, an HPLC method was established to determine the contents of eight components in *lophatherum gracil*, namely orientin, isoorientin, vitexin, isovitexin, kaempferol, luteolin, caffeic acid and chlorogenic acid, and it was found that chlorogenic acid, kaempferol, isovitexin, luteolin, and isoorientin were the characteristic components for the quality difference of *lophatherum gracil* in different origin. At the same time, the taste of *lophatherum gracil* was determined, and it was found that the taste was differentiated by the content of the active components. The correlation study between the taste information and the content of active components found that the taste reacted to the high or low content of active components in the herbs, and the pattern presented could be used for rapid identification of the quality of herbs. In conclusion, the present study combines the taste of herbs with the analysis of the active components content to provide new ideas and means for the evaluation of the quality of herbs and to provide reference for their clinical application.

Keywords: *L. gracile*; Flavour; Constituent Content; Quality.

1. Introduction

'Quality evaluation through morphological identification' refers to the essence of the experience of discrimination which determine the authenticity, pros and cons of traditional Chinese medicine (TCM) based on their appearance manifest characteristics, such as shape, color, odor and taste. The taste of TCM is an important characteristic of TCM, and tasting TCM is the main way to identify TCM[1]. Therefore, the correct evaluation and quantification of the taste of TCM is of great significance to the quality control of TCM and to guide the rational use of medication in clinical practice.

Lophatherum gracile (*L. gracile*) is the dried stems and leaves of *lophatherum gracile* Brongn. of the family Gramineae, and have anti-inflammatory, antioxidant, antiviral, hepatoprotective and anticancer activities[2–6]. Orientin, isoorientin and isovitexin have been identified in the leaves of *L. gracile*. Orientin has anti-inflammatory, cardioprotective, hepatoprotective and other bioactivities[7–9], isoorientin has slowed down the development of arthritis, protects the nerves, and improves the diabetic nephropathy and other bioactivities [10–12], isovitexin has the regulation of ulcerative colitis, slowing down the aging process, and antidiabetic and other bioactivities[13–15]. L. *gracile* is both medicinal and edible[4]. *L. gracile* is mainly used in TCM for clearing heat, diuretic and regulating the liver[16], in order to ensure the clinical efficacy of *L. gracile*, the quality control of *L. gracile* is particularly important. At present, there is basically no research on the sensory evaluation of *L. gracile* using modern scientific methods, and it is necessary to apply the electronic tongue technology to quantify the taste of *L. gracile*, and to analyze the correlation with the content of the components.

Therefore, in this study, an HPLC method was established to determine the content of eight constituents, orientin, isoorientin, vitexin, isovitexin, chlorogenic acid, caffeic acid, luteolin, and kaempferol, in 18 batches of *L. gracile*. The taste of *L. gracile* were determined using electronic tongue techniques. The content of different components in *L. gracile* affects the taste. The use of intelligent sensory techniques to quantify the taste of *L. gracile* provides new ideas and means for the quality evaluation of *L. gracile* and provides reference for its clinical application.

2. Materials and Methods

2.1. Chemicals and Materials

Table 1. Information on 18 batches of L. gracile

Sample No.	Place of Origin	Sample No.	Place of Origin
S1	Zhejiang, China	S10	Jiangsu, China
S2	Zhejiang, China	S11	Hubei, China
S3	Fujian, China	S12	Hubei, China
S4	Jiangxi, China	S13	Hubei, China
S5	Guangxi, China	S14	Hubei, China
S6	Guangdong, China	S15	Hubei, China
S7	Anhui, China	S16	Hubei, China
S8	Sichuan, China	S17	Guizhou, China
S9	Yunnan, China	S18	Guizhou, China

Orientin (Lot: P24D1223J, >=98%, medlife), Isoorientin (Lot: P24JZ729G, >=98%, medlife), Vitexin (Lot: P24JZ729H, >=98%, medlife), Isovitexin (Lot: P24JZ726E, >=98%, PERFEMIKER), Luteolin (Lot:

P24JZ729I, >=98%, PERFEMIKER), Caffeic acid (Lot: P24D1223J, >=98%, medlife), Chlorogenic acid (Lot: P24JZ729F, >=98%, medlife), Kaempferol (Lot: J1110A5, >=98%, midlife), Formic acid (Lot: 20190328, AR, Damao Chemical Reagent Factory), Acetonitrile (HPLC, Concord Chemical Reagent Co., Ltd.), Methanol (HPLC, Concord Chemical Reagent Co., Ltd.), Water for Wahaha water.

Information on the 18 batches of herbs is shown in Table 1.

2.2. HPLC Sample Solution Preparation

Eighteen batches of *L. gracile* sample solution were prepared. An aliquot of 1.0 g fine powder of each sample was properly weighed and ultrasonically extracted with 20 ml of methanol/water (70/30, v/v) for 30 min at room temperature. The mixture was filtered through a 0.22-μm membrane filter. The filtrate was transferred into a sample vial for HPLC and UHPLC-Q-Orbitrap/MS analysis.

2.3. Sample Preparation for Taste Determination

Take 2.0g of *L. gracile* sample powder (through 65 mesh sieve), add 50mL of distilled water at 60°C, sonicate for 30min, let stand for 1h, filtered by filter paper and used for taste analysis.

2.4. HPLC Analysis

The HPLC analyses were performed on a Shimadzu high liquid chromatography system, and column (Luna C18, 4.6*250 mm, 5 μm; Phenomenex) with a mobile phase of 0.2% formic acid aqueous solution (A)-acetonitrile (B), and eluent B (acetonitrile) at a flow rate of 1.0 ml/min with the following gradient program: 0-40 min, 5%-20% (B); 40-45 min, 20%-23% (B); 45-65 min. 23%-40% (B); 65-66min, 40%-5% (B); 66-70min, 5%-5% (B). The injection volume was 10 μL. The column temperature was 30°C.

2.5. Taste Measurement Method

The electronic tongue is a new type of taste analysis and detection instrument composed of an array of interaction-sensitive sensors, signal adjustment circuits and its own pattern recognition algorithm software, which converts the taste characteristics of food liquids into electrical response values by simulating the five basic taste sensations of the human body (sour, sweet, bitter, salty and fresh) and quantifies the taste characteristics after analysis. The sensor system analyzed in this study consists of seven sensors whose molecular membranes can selectively adsorb different taste substances and convert taste characteristics into electrical signal data.

Before testing the samples, the sensors were activated, calibrated and diagnosed to ensure that they were in good condition. The sample collection time was 120s, and each analysis time was 180s. After the electronic tongue was activated and calibrated, 25 mL of the aqueous extract of *L. gracile* was added into the detection cup in sequence. The single sampling time was 120s, and the samples were washed with ultrapure water for 120s, the stirring rate was 3r/s, the data were collected at room temperature, and each sample of the aqueous extract of *L. gracile* was measured three times.

2.6. Data Processing and Multivariate Statistical Analysis

Pearson's correlation analysis was performed using SPSS (version 26.0, online application software), and bar charts and radar plots using Origen 2021 software.

3. Results and Analysis

3.1. Multi-component Quantitative Results

Orientin, isoorientin, vitexin, isovitexin, luteolin, kaempferol, chlorogenic acid, and caffeic acid were mainly contained in *L. gracile* [17,18], and an HPLC method was established for the determination of these eight components. The methodology was validated by the established HPLC method, and good correlation coefficients (r > 0.990) were obtained for the curves of the eight analytes, repeatability and precision of each common peak relative retention time and relative peak area RSD values were less than 5.0%, the stability of the sample solution was good within 25h, and the method was feasible. The results of the determination of the eight components in 18 batches of *L. gracile* are shown in Table 2 and Figure 1. Among the 18 batches of the herbs, except for S5, which did not contain chlorogenic acid, the other batches contained eight compounds, and the content of each compound had variability in the samples of different batches, orientin, and isoorientin content was higher in all batches. The content of chemical compounds in the *L. gracile* is related to climatic factors, and the frost-free period and rainfall affect the content of chemical compounds in the leaves of *L. gracile* [19].

Peak area data of the 8 components from 18 batches of *L. gracile* were imported into SIMCA14.1 software for PCA analysis. The model fit and predictive ability of Ctr was better (R2X=0.897), with some differences between the 18 batches of samples. From the PCA plots (Figure 2 B), 18 batches of samples were classified into 2 categories, with S3, 6 and S7 as one category; the rest were classified as one category, consistent with the HCA (Figure 2 A) classification results. A permutation test (n=200) was performed to assess the potential overfitting of the discriminant model, as shown in Figure 2 C, with Q2 intercept < 0.05. Combined with the VIP values, as shown in Figure 2 D, the main characteristic components that could cause differences in the composition of *L. gracile* from different batches were screened out, and VIP>1 was selected as the component with a larger contribution. From the VIP plot, it can be seen that there are 5 fractions with VIP>1, and chlorogenic acid, kaempferol, isovitexin, luteolin, and isoorientin can be used as a characteristic component to distinguish the quality difference of *L. gracile* from different origins.

3.2. Taste Measurement Results

Intelligent sensory technology, also known as sensory analysis technology, and sensory evaluation, mainly uses human senses as indicators to analyze products qualitatively and quantitatively [20,21]. Intelligent sensory technology is applied in TCM tablets to identify the quality of the tablets quickly and effectively, and to promote the innovation and development of the quality evaluation technology of TCM tablets [22,23]. Electronic tongue is a modern intelligent sensory analysis and testing instrument developed by imitating the mechanism of human taste perception, which is used to determine the taste of the samples, with high sensitivity, reliability, repeatability, and can effectively avoid the errors generated by human factors[24,25]. Yang[26] et al. used the electronic tongue to separate and identify a variety of bitter compounds in ginseng.

Table 2. Results of the content of 8 compounds (mg/g) (n=3)

NO.	Chlorogenic acid	Caffeic acid	Isoorientin	Orientin	Isovitexin	Vitexin	Luteolin	Kaempferol
S1	0.06±0.00	0.02±0.00	0.34±0.00	1.56±0.00	0.03±0.00	0.11±0.00	0.02±0.00	0.14±0.00
S2	0.23±0.00	0.02±0.00	0.61±0.00	0.72±0.00	0.03±0.00	0.16±0.00	0.04±0.00	0.15±0.00
S3	0.17±0.00	0.02±0.00	0.81±0.00	2.30±0.01	0.07±0.00	0.24±0.00	0.03±0.00	0.10±0.00
S4	0.01±0.00	0.01±0.00	0.07±0.00	0.47±0.00	0.01±0.00	0.04±0.00	0.01±0.00	0.14±0.00
S5	0.00±0.00	0.01±0.00	0.02±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.13±0.00
S6	0.07±0.00	0.02±0.00	0.66±0.00	2.16±0.00	0.05±0.00	0.22±0.00	0.02±0.00	0.09±0.00
S7	0.16±0.00	0.03±0.00	1.85±0.00	0.73±0.00	0.07±0.00	0.23±0.00	0.07±0.00	0.35±0.00
S8	0.05±0.00	0.02±0.00	0.34±0.00	1.03±0.00	0.03±0.00	0.09±0.00	0.02±0.00	0.10±0.00
S9	0.02±0.00	0.01±0.00	0.28±0.00	1.06±0.00	0.02±0.00	0.09±0.00	0.02±0.00	0.16±0.00
S10	0.03±0.00	0.01±0.00	0.38±0.00	1.28±0.00	0.03±0.00	0.10±0.00	0.02±0.00	0.11±0.00
S11	0.03±0.00	0.01±0.00	0.17±0.00	0.41±0.00	0.02±0.00	0.06±0.00	0.01±0.00	0.17±0.00
S12	0.21±0.00	0.02±0.00	0.65±0.00	0.77±0.00	0.07±0.00	0.19±0.00	0.04±0.00	0.15±0.00
S13	0.03±0.00	0.01±0.00	0.17±0.00	0.40±0.00	0.02±0.00	0.05±0.00	0.01±0.00	0.14±0.00
S14	0.02±0.00	0.01±0.00	0.15±0.00	0.37±0.00	0.01±0.00	0.05±0.00	0.01±0.00	0.12±0.00
S15	0.04±0.00	0.01±0.00	0.15±0.00	0.36±0.00	0.02±0.00	0.06±0.00	0.01±0.00	0.14±0.00
S16	0.03±0.00	0.01±0.00	0.17±0.00	0.40±0.00	0.02±0.00	0.06±0.00	0.01±0.00	0.14±0.00
S17	0.06±0.00	0.01±0.00	0.48±0.00	0.21±0.00	0.03±0.00	0.03±0.00	0.02±0.00	0.15±0.00
S18	0.07±0.00	0.01±0.00	0.53±0.00	0.22±0.00	0.05±0.00	0.04±0.00	0.03±0.00	0.15±0.00

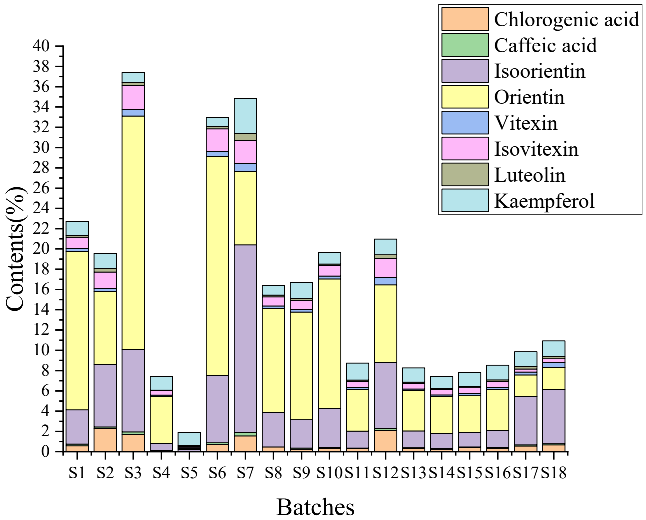

Figure 1. Results of the content of 8 compounds (%) (n=3)

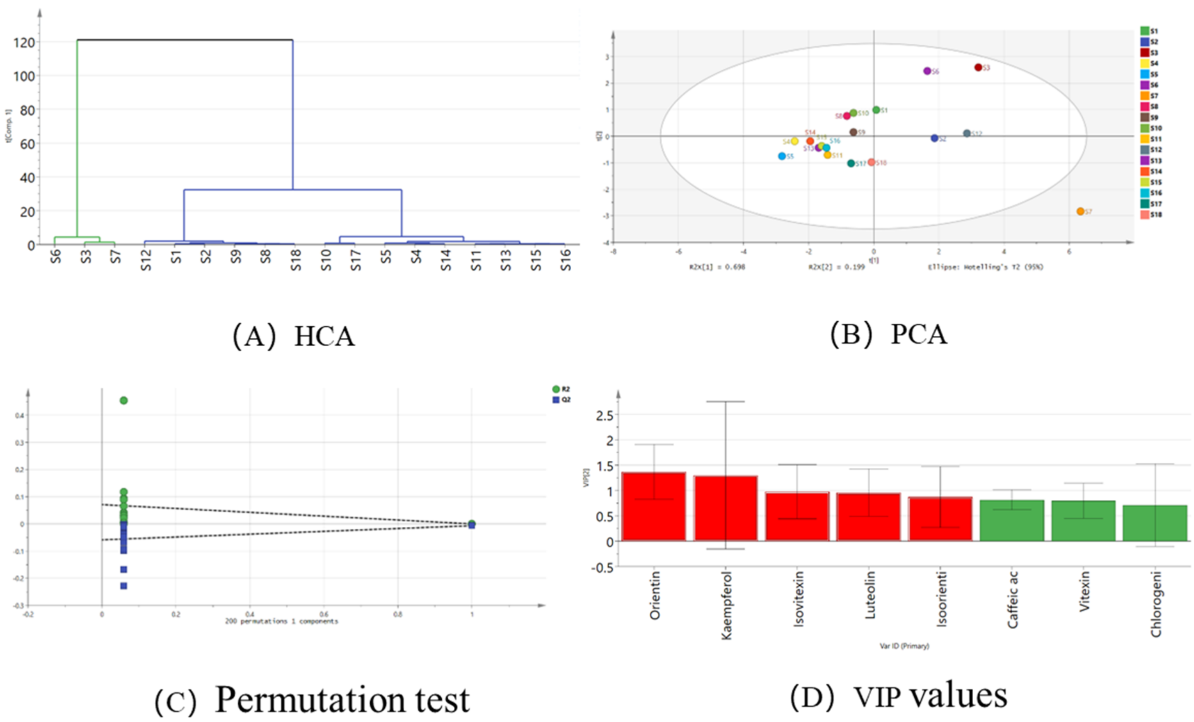

Figure 2. Statistical analysis of peak areas of eight components in 18 batches of samples

Table 3. Description of the performance of the electron tongue effect sensor

Sensor name	AHS	PKS	CTS	NMS	CPS	ANS	SCS
Performance description	Sensitive to acidity	General purpose sensors	Sensitivity to salty	Sensitive to fresh	General purpose sensors	Sensitivity to sweetness	Sensitivity to bitter

Table 3 lists sensor performance. Radar plots were made using Origin2021 software (Figure 3A). From the radar plot, it was observed that the 18 batches of aqueous extract of *L. gracile* had more significant differences in the ringing of AHS (sour), SCS (bitter), ANS (sweet) and NMS (fresh) sensors, while the CTS (salty) differences were not significant. The data obtained were analyzed using Alphasoft software to make PCA plots (Figure 3B). As can be seen from the PCA plot, S11 and S8 were similar in taste; S10 and S5 were more different.

Pearson's correlation analysis of taste and eight chemical compounds was performed using SPSS 26.0 software (Table 4). From the results, it can be seen that kaempferol was positively correlated with acidity (AHS); isoorientin, isovitexin, luteolin, and kaempferol were positively correlated with savory taste (CTS); kaempferol was positively correlated with fresh taste (NMS); isovitexin, luteolin, and kaempferol were positively correlated with sweetness (ANS); kaempferol was positively correlated with bitterness (SCS); kaempferol was positively correlated with sourness, saltiness, freshness, sweetness and bitterness; and chlorogenic acid, caffeic acid, and orientin were negatively correlated with sourness, saltiness, freshness, sweetness and bitterness. In other words, the heavier the flavor of *L. gracile* (the more sour, salty, fresh, sweet and bitter), the higher of the content of kaempferol, and the lower of the content of chlorogenic acid, caffeic acid and orientin.

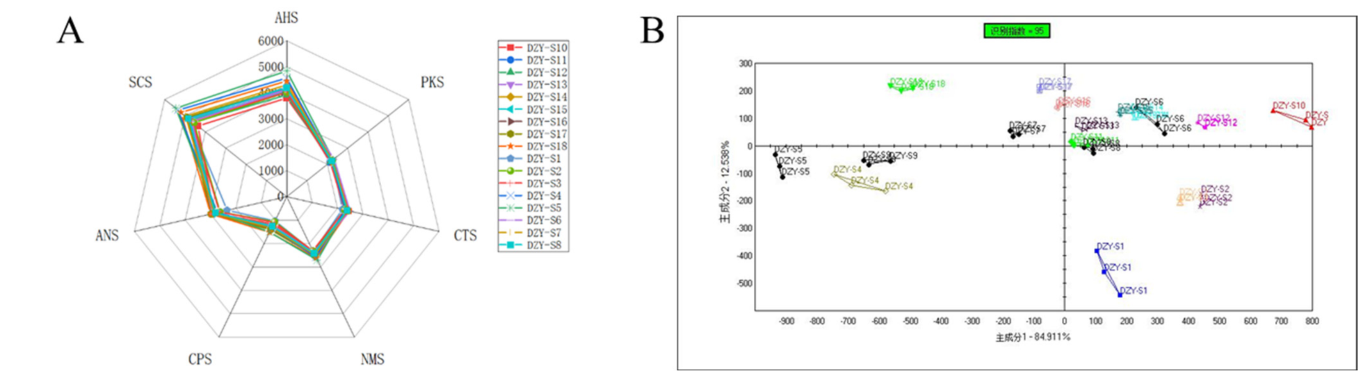

Figure 3. Results of the determination of the flavour of *L. gracile.* (A) Radar plot of the flavor of aqueous extracts of *L. gracile.* (B) PCA plot of the taste of aqueous extracts of *L. gracile.*

Table 4. Correlation between taste and eight chemical constituents of *L. gracile*

	Chlorogenic acid	Caffeic acid	Isoorientin	Orientin	Isovitexin	Vitexin	Luteolin	Kaempferol
AHS (Acid)	-.414**	-0.234	-0.170	-.386**	-.322*	-.424**	-0.211	0.204
PKS (General)	-0.163	-0.003	0.124	-0.131	-0.048	-0.105	0.069	0.127
CTS (Salty)	-0.193	-0.111	0.119	-0.246	0.139	-0.113	0.117	0.080
NMS (Fresh)	-.489**	-.373**	-0.214	-.577**	-.347*	-.577**	-0.230	0.198
CPS (General)	-.509**	-.406**	-0.197	-.656**	-0.268	-.593**	-0.176	0.204
ANS (Sweet)	-0.250	-0.259	0.013	-.497**	0.015	-.309*	0.048	0.132
SCS (Bitter)	-.464**	-.301*	-0.211	-.461**	-.340*	-.491**	-0.240	0.214

Note: ** at the 0.01 level (two-tailed), the correlation is significant; * at the 0.05 level (two-tailed), the correlation is significant

In this study, the taste of herbs determined by electronic tongue was combined with the content analysis, and the correlation between taste information and active ingredient content found that the taste reacted to the high or low content of active ingredients in herbs, and the presented pattern could be used for rapid identification of the quality of herbs.

4. Conclusion

Methodological validation of the HPLC method have good precision, stable and the method feasible. The results of multi-component quantitative determination of 18 batches of *L. gracile* showed that all batches contained 8 compounds except for S5, which did not contain chlorogenic acid, and the content of each compound had variability in the samples of different batches. Chlorogenic acid, kaempferol, isovitexin, luteolin, and isoorientin can be used as the characteristic components to distinguish the quality difference of *L. gracile* from different origins. The contents of the compounds were differentiated in different batches of the samples, among which the contents of orientin and isoorientin were higher in all batches. The electronic tongue measurement of the flavour of *L. gracile* shown that the heavier the flavour (more acidic, fresh, sweet and bitter), the content of kaempferol was higher and the content of chlorogenic acid, caffeic acid and orientin was lower. In this study, an HPLC method was established for the determination of eight compounds, at the same time, the taste of *L. gracile* were determined, and the taste was combined with the content of active ingredients to analyse the quality of *L. gracile*, which provides new ideas and means for the evaluation of the quality of *L. gracile*, and provides a reference for its clinical application.

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