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Clustering analysis of *Ampelopsis grossedentata* based the contents of dihydromyricetin and myricetin

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Abstract
Ampelopsis grossedentata (Vine tea) has been developed as a functional tea in China. Dihydromyricetin and myricetin are the main active ingredients. However, there have been no reports about the difference of active ingredient content in vine tea produced by different manufacturers. 21 samples were collected from different enterprises. The HPLC was used to determine the contents of dihydromyricetin and myricetin in vine tea, and the Euclidean distance and group-average method were used for cluster analysis. The results showed that the content interval of dihydromyricetin and myricetin in vine tea were 168.7-349.9 mg/g and 1.3-4.9 mg/g, respectively. And clustering analysis shows that samples 3, 4, 7, 8, 10, 13, 14, 17, 20 and 21 can be grouped into one class, 12 and 6 fall into one category respectively, and the other 9 samples into a class. The main factor determining the clustering attribution is the content ratio of dihydromyricetin to myricetin in vine tea.
Practical Applications: The contents of dihydromyricetin and myricetin in samples of ampelopsis grossedentata (vine tea) from 21 large-scale enterprises were determined simultaneously. The fluctuation range of dihydromyricetin and myricetin contents in vine tea was revealed. Ten vine tea samples are grouped into one class, corresponding to the first-class products reviewed by 14 experts, nine vine tea samples are grouped into another class, corresponding to the third class, and the other two samples are grouped into two categories, corresponding to the second-class products and fourth-class products. The main factor determining the clustering attribution is the content ratio of dihydromyricetin to myricetin in vine tea. These results lay the foundation for the development and application of the active ingredients based on vine tea.

Keywords: ampelopsis grossedentata; dihydromyricetin; myricetin; clustering analysis

Introduction
Ampelopsis grossedentata (Vine tea) is an ancient medicinal plant and has been developed as a functional tea in recent years in China. Dihydromyricetin and myricetin, the main active ingredients in vine tea (Chen *et al*, 2015) [1], have been reported to exert various physiological activities. Dihydromyricetin has the functions of scavenging free radicals, anti-oxidation (Zheng *et al*., 2014; Wang *et al*, 2017) [2, 3] anti-tumor (Zhou *et al*., 2012; Huang *et al*, 2016) [4, 5] reducing blood lipid and blood glucose level (Chen *et al*, 2016) [6], increasing superoxide dismutase activity (Song *et al*, 2017) [7] and liver protection effects (Gao *et al*., 2011) [8]. Myricetin has anti-inflammatory, anti-oxidation, anti-allergic, hypoglycemic and liver-preserving effects (Lin *et al*, 2012) [9].
Laifeng County is located in Enshi tujia and miao autonomous prefecture, Hubei province, China. Its longitude is 109.40, latitude is 29.49 degrees north. Laifeng vine tea is a national geographical indication certified agricultural product. The determination of dihydromyricetin and myricetin in Laifeng vine tea is mainly found in the product propaganda reports of a few enterprises, but no academic reports were found. Moreover, here have been no reports about the difference of active ingredient content in vine tea produced by different manufacturers.
In this study, 21 samples of vine tea produced by 21 scale enterprises (annual production of 500 kg) from Laifeng County were used as research materials. The contents of dihydromyricetin and myricetin were determined by HPLC method. The samples of the same quality were classified together by clustering analysis. In order to provide scientific basis for the establishment of quality standard and the development of high quality.

Materials and Methods
Chemicals and apparatus
Standard of dihydromyricetin and myricetin were purchased from Solarbio science and technology Co., Ltd, Beijing, China. Ultrasonic cell disruptor (Fuzhou golden speed equipment Co., Ltd, Fuzhou, China, 50Hz, 100 W) was employed for extraction of active ingredient. A microporous plate detector (Infinite ® M200 PRO, Switzerland) was used for scanning of extracts and standard. Methanol was of HPLC grade, and which was purchased from Aladdin Biotech Co., Ltd (Shanghai, China). HPLC system for active ingredient separation and quantification consisted of Waters e2695 separations module, Waters 2998 photodiode array detector and ODS-C18 column (250×4.6×5 mm).

Extraction and preparation of dihydromyricetin and myricetin in vine tea
21 new vine tea samples were collected from 21 different enterprises producing vine tea on a large scale in Laifeng. The annual output of each enterprise is above 500 kg. And all vine tea samples were processed by young stems and leaves in May 2020. Take vine tea powder (less than 0.425

mm) 0.5 g (accurate to 0.001 g), add 20 ml absolute methanol. Then the sample was extracted by ultrasonic for 10 minutes (start 3 seconds after 6 seconds). Pour out the extract, repeat the operation once, the residue is washed with 4 ml methanol, the combined extract is placed in 50 ml capacity bottle, and the volume is fixed to the scale with methanol. Shake it well, filter, Discard the primary filtrate 10 ml, measure continuous filtrate 10 ml, put in 100 ml capacity bottle, dilute to scale with methanol. Finally, shake well and filter with 0.45μm microporous membrane to obtain the sample solution.

Contents analysis of dihydromyricetin and myricetin in vine tea

Two standards were dissolved with absolute methanol to different concentrations. The standard solution were scanned within 200-600 nm range to detect absorbance peak. Based on the scanning analysis by microporous plate detector, HPLC was used for further quantitative determination. The HPLC method was 0.1% phosphoric acid as solvent A (methanol as solvent B) 0-13 min, 70-40%, 13-16 min, 40-20%, 16-18 min, 20%, 18-20 min, 20-70%, 20-23 min, 70%, column temperature of 25 ℃, sample injection volume of 10 μl, flow rate of 1.0 ml/min. The detection wavelengths of dihydromyricetin and myricetin were 291 and 373 nm, respectively. When dihydromyricetin was detected, the sample solution was diluted 10 times with methanol, while the sample solution was not diluted when myricetin was detected.

Clustering analysis

Using SPSS 20.0 statistical software, based on the content of dihydromyricetin and myricetin, the Q-type systematic cluster analysis (Liu *et al*, 2014) [10] was carried out on 21 samples of Laifeng vine tea by the method of between-groups linkage, and the metric standard was Euclidean distance.

Results and discussion

Absorbance peak and retention time of dihydromyricetin and myricetin

The main absorbance peak of dihydromyricetin and myricetin standard solution were 291 nm and 373 nm, respectively (Fig. 1, Fig. 2).

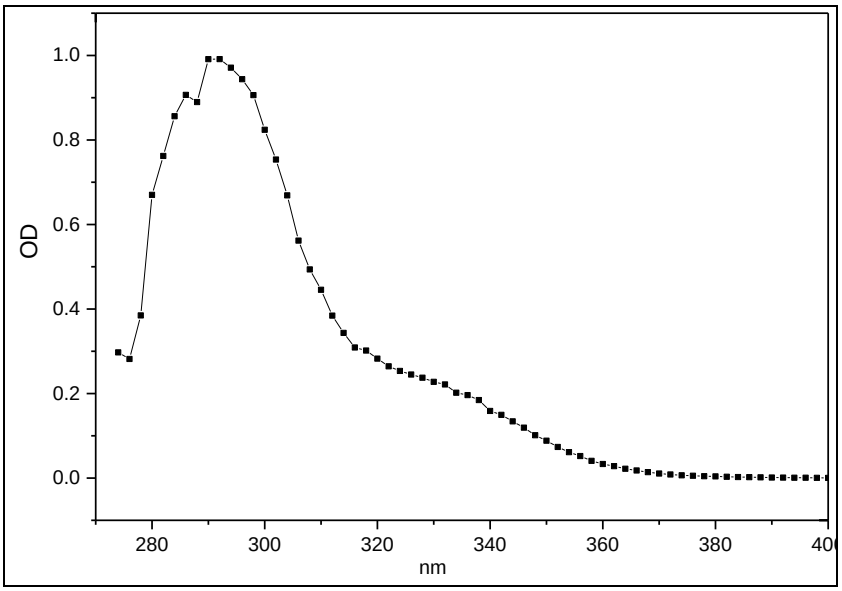

Fig 1: Scanning spectrum of dihydromyricetin standard solution (43.2 mg/L)

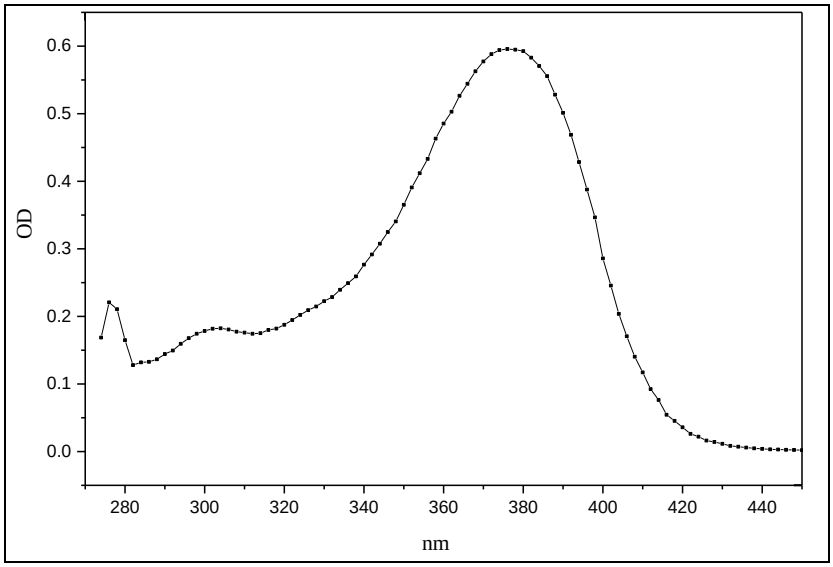

Fig 2: Scanning spectrum of myricetin standard solution (19.2 mg/L)

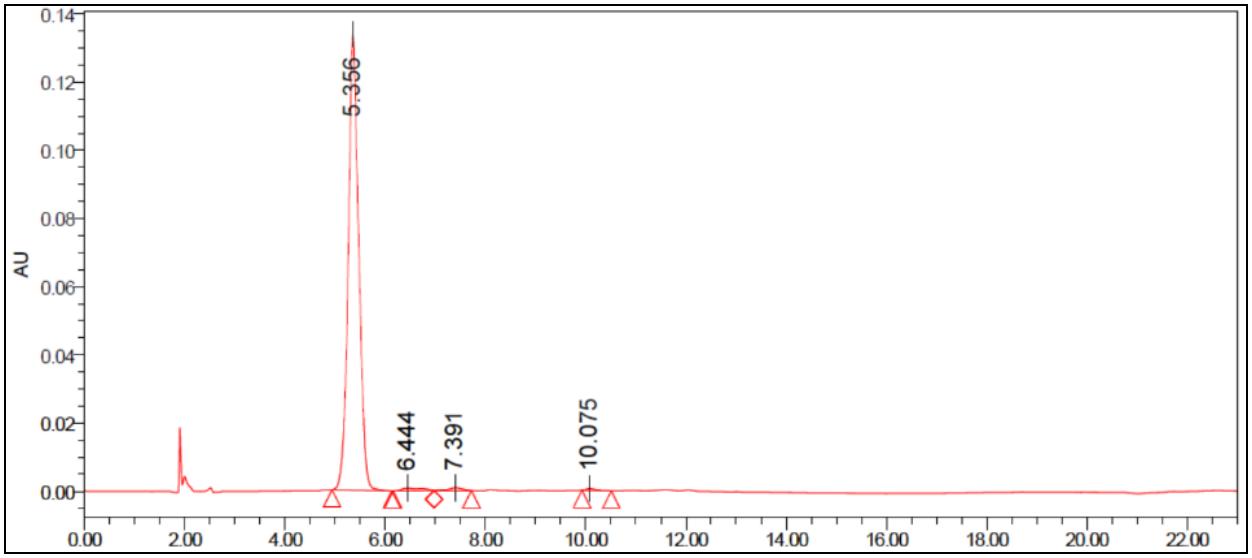

Fig 3: HPLC separation of dihydromyricetin standard solution

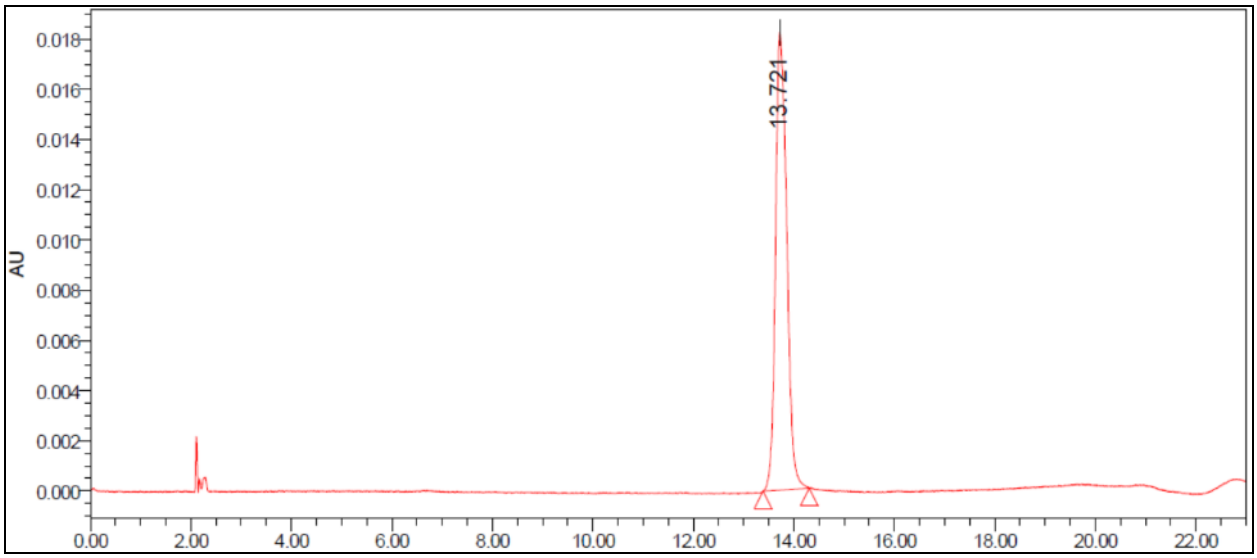

Fig 4: HPLC separation of myricetin standard solution

According to the standard solution dada, the retention time of the main peak of dihydromyricetin solution is 5.4 min (Fig. 3), while that of myricetin solution is 13.7 min (Fig. 4). These retention times are basically consistent with the data reported in the literature (Feng *et al*, 2018; Chen *et al*, 2015) [1].

Contents of dihydromyricetin and myricetn in vine tea

The content interval of dihydromyricetin in vine tea was 168.7-349.9 mg/g, while that of myricetin was 1.3-4.9 mg/g (Table 1). The content of dihydromyricetin in Laifeng vine tea is basically the same as that of 168.0-335.8 mg/g in 18 samples of vine tea throughout China (Fan *et al*, 2014) [12]. Different from the content reported by Chen *et al*. (2015) [1], Laifeng vine tea contained higher dihydromyricetin content and lower myricetin content. However, the content of dihydromyricetin in vine tea is 55-209 times that of myricetin, which was the first report.

Table 1: Content of active ingredients in vine tea based HPLC analysis

Sample No.	Contents of dihydromyricetin (mg/g)		Contents of myricetin (mg/g)		Ratio
	Mean	S.D.	Mean	S.D.	
1	274.52	2.14	1.32	0.04	207.97
2	290.99	5.38	1.80	0.14	161.66
3	270.69	2.39	2.56	0.02	105.74
4	283.30	0.50	2.22	0.13	127.61
5	271.76	6.60	1.30	0.02	209.05
6	272.33	5.88	4.93	0.34	55.24
7	263.27	6.81	2.08	0.13	126.57
8	234.60	6.34	1.85	0.01	126.81
9	234.16	1.27	1.39	0.06	168.46
10	260.94	7.32	2.17	0.10	120.25
11	346.68	5.45	2.25	0.15	154.08

12	168.68	2.43	1.85	0.08	91.18
13	249.08	0.63	1.98	0.07	125.80
14	307.72	1.48	2.46	0.11	125.09
15	310.95	8.11	1.59	0.09	195.57
16	300.64	2.83	1.40	0.03	214.74
17	244.08	4.48	2.07	0.08	117.91
18	324.91	4.63	1.86	0.06	174.68
19	349.89	1.42	2.30	0.08	152.13
20	317.95	1.16	3.01	0.08	105.63
21	326.85	1.57	2.85	0.11	114.68

Clustering analysis of vine tea sample

The data of dihydromyricetin and myricetin in each sample are discrete, so it is not easy to compare the similarity degree intuitively. The classification results of cluster analysis are objective and scientific, and a large number of traits can be comprehensively investigated at the same time (Liu *et al*, 2019) [13]. Therefore, the samples were classified into different groups by systematic cluster analysis (Zhu *et al*, 2019) [14].

At a distance of 5.0 in Euclidean distance, 21 samples of Laifeng vine tea can be clustered into 4 classes. The samples 3, 4, 7, 8, 10, 13, 14, 17, 20 and 21 can be grouped into one class (Class 1), 12 and 6 fall into one category respectively (Class 2 and Class 4), and the other 9 samples (1, 2, 5, 9, 11, 15, 16, 18 and 19) into a class (Class 3, Fig. 5). Combined with the data of expert quality review (data not shown), class 1, 2, 3 and 4 basically correspond to the first, second, third and fourth classes of vine tea. In addition, it can be seen that the factors determining the clustering attribution are not the content of dihydromyricetin or myricetin, but mainly the ratio of the two. The range of fluctuations in the ratio of Class 1 is 105.6-127.6, the ratio of Class 2 is 91.2, the fluctuation range of Class 3 is 152.1-214.7, and the ratio of Class 4 is 55.2.

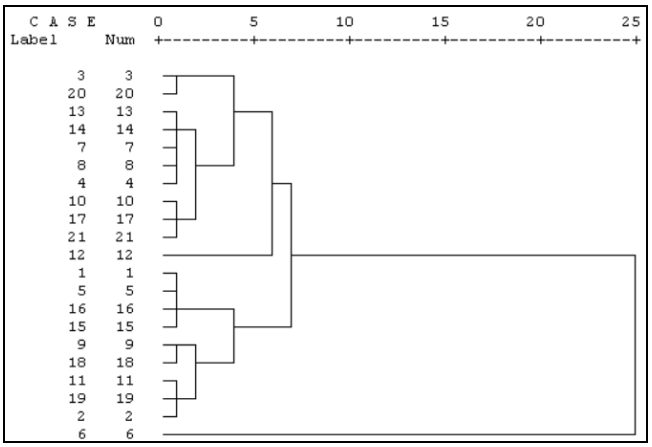

Fig 5: Dendrogram of clustering analysis of 21 samples of vine tea

Conclusions

The content range of dihydromyricetin and myricetin in 21 samples of Laifeng's original vine tea were 168.7-349.9 mg/g and 1.3-4.9 mg/g, and the ratio fluctuates between 55 and 215.

Clustering analysis divided 21 vine tea samples into 4 categories. The main factor determining the clustering attribution was the content ratio of dihydromyricetin to myricetin in vine tea. The quality of Class 1 is the best, Class 4 is the worst, and Class 2 and Class 3 ranked second and third, respectively.

Acknowledgments

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