Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.130422

Quantitive analysis of flavonoids in ainaxiang tablets by high-performance liquid chromatography

Daopeng TAN[1], Guopan WANG[1], Jianmei WANG[1], Yidong FENG[2], Yuqi HE[1,3]*

Abstract:

Blumea balsamifera is a very famous medicinal plant and functional tea in many countries of Asia. Ainaxiang tablets are a preparation consisting of flavonoids from *B. balsamifera*. Today, a high performance liquid chromatography method was developed for simultaneous quantification of 15 flavonoids in Ainaxiang Tablets. The analysis was performed on a Kromasil C18 column (250 × 4.6 mm, 5 μm) with a binary gradient mobile phase of acetonitrile and 0.2% aqueous acetic acid. The method was validated in terms of linearity, sensitivity, stability, precision and accuracy. It was found that this method had linearity with R^2 at 0.9992- 0.9999 in the test range of 0.15-276.40 μg/mL. The limit of detection (LOD) and limit of quantification (LOQ) for 15 tested reference compounds were 0.01-0.28μg/mL and 0.02 - 0.55μg/mL, respectively. The relative standard deviations (RSD%) for intra-day and inter-day repeatability were not more than 3.18%. The analyzed samples were stable for at least 18 h. The spike recoveries for 15 analyzed samples were 106.17 - 95.02%. The results suggested that this newly developed HPLC method could be used for quantitative analysis of flavonoids in Ainaxiang Tablets and its raw materials.

Keywords: *Blumea balsamifera*; Ainaxiang tablets; HPLC quantification; flavonoids.

Practical Application: The investigation provides the important information for the quality control of Ainaxiang Tablets and *Blumea balsamifera*.

1 Introduction

In recent years, the research and development of functional products made from herbs has attracted increasing attention (Wang et al., 2022a, b, 2023). *Blumea balsamifera* DC. (Chinese name, Ainaxiang) is a very famous medicinal plant and functional tea in China and many countries of Asia, such as Malaysia, Philippines, Vietnam, and Thailand for the treatment of many diseases (Huang et al., 2022; Tan et al., 2020). Previous phytochemical investigations on this plant revealed the presence of flavonoids, monoterpenes, and sesquiterpenes (Tan & Yan, 2019; Tan et al., 2013; Widhiantara & Jawi, 2021). Ainaxiang tablets is made from the total flavonoids extracts of the leaves of *B. balsamifera*. Activating blood circulation to dissipate blood stasis with pulse-invigorating and heart-nourishing effects, Ainaxiang tablets is one of the commonly used traditional Chinese medicine preparation for the treatment of coronary heart disease and angina pectoris in China for many years. Flavonoids is an ubiquitous group of polyphenolic substances which are present in most plants. The increasing interest in flavonoids from *B. balsamifera* is due to the appreciation of their broad pharmacological activities such as radical scavenging (Ginting et al., 2022; Zhang et al., 2021), anti-cancer (Jiang et al., 2014), plasmin-inhibitory (Guan et al., 2022), liver-protective (Jirakitticharoen et al., 2022; Montealegre & De Leon, 2017) and xanthine oxidase inhibitory effects (Kubota et al., 2009; Ma et al., 2018; Nguyen & Nguyen, 2012; Wang & Zhang, 2020).

In recent years, many serious complications of traditional Chinese medicine preparations (TCMPs) were reported, the cause for which due largely to the lack of a practicable and reliable quality standard to monitor the properties changed in the procedure from preparation, transportation and storage to clinic usage. However, chemical ingredients in traditional Chinese medicines (TCMs) vary greatly with the geographical origin of the species, cultivation practice, time of harvest, storage condition, and methods of processing, which bring about the complexity and difficulty to the quality assessment of TCMPs and their raw materials. To the best of our knowledge, there is few previous reports that designed to analyze chemical constituents in the leaves of *B. balsamifera* (Liu et al., 2014; Tan et al., 2020).

In our previous studies (Tan & Yan, 2019; Tan et al., 2013), multiple flavonoids were isolated from *B. balsamifera* and structurally defined by MS and NMR. During the course of our continuing search for the quality control of *B. balsamifera* and its preparations, 15 flavonoids were used as bioactive markers and simultaneously determined by the established HPLC/PDA approach. This study represents the first detailed investigation of

Received 12 Nov., 2022
Accepted 27 Dec., 2022
[1] *Guizhou Engineering Research Center of Industrial Key-technology for Dendrobium Nobile, School of Pharmacy, Zunyi Medical University, Zunyi, China*
[2] *Shenzhen Neptunus Pharmaceutical Technology Research Institute Co., Ltd., Shenzhen, China*
[3] *Key Laboratory of Basic Pharmacology of Ministry of Education and Joint International Research Laboratory of Ethnomedicine of Ministry of Education, School of Pharmacy, Zunyi Medical University, Zunyi, Guizhou, China*
*Corresponding author: 1474755772@qq.com

the components of Ainaxiang tablets and could improve quality evaluation of Ainaxiang tablets and its raw materials.

2 Experimental

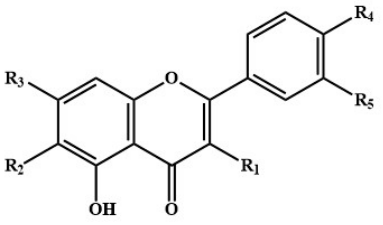
2.1 Materials and reagents

Four batches of the Ainaxiang tablets were manufactured by Shenzhen Neptunus pharmaceutical company. A total of 15 reference flavonoids (Figure 1) were isolated previously from *B. balsamifera* in our laboratory and elucidated by NMR, MS. The purities of these reference standards were determined to be higher than 96% by the HPLC peak area method.

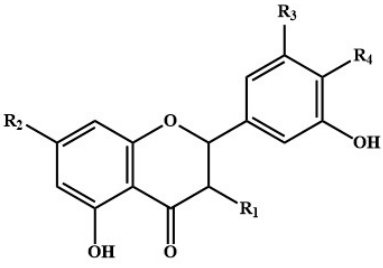
Acetonitrile (Merck, Germany) were HPLC grade and used with further purification. All other reagents were of at least analytical grade from Jinhuada Chemical Factory (Guangzhou, China). Water was purified using a Milli-Q water purification system (Millipore, USA).

2.2 Sample preparation

Each of the ground Ainaxiang tablets (0.15 g) was extracted with 80% methanol (25 mL) by reflux for 30 min. The sample solution was passed through a 0.45 μm membrane filter prior to injection.



No.	Name	R_1	R_2	R_3	R_4	R_5
1	Rutin	ORutinose	H	OH	OH	OH
2	Hyperoside	OGal	H	OH	OH	OH
3	Isoquercitrin	OGlc	H	OH	OH	OH
6	Quercetin	OH	H	OH	OH	OH
8	Chrysosplenol C	OCH_3	OH	OCH_3	OH	OCH_3
9	Diosmetin	H	H	OH	OCH_3	OH
10	Tamarixetin	OH	H	OH	OCH_3	OH
11	3,5,7-Trihydroxy-3',4'-dimethoxyflavone	OH	H	OH	OCH_3	OCH_3
14	Rhamnetin	OH	H	OCH_3	OH	OH
15	Ombuin	OH	H	OCH_3	OCH_3	OH



No.	Name	R_1	R_2	R_3	R_4
4	3,3',5,7-Tetrahydroxy-4'-methoxyflavanone	OH	OH	H	OCH_3
5	3',5,5',7-Tetrahydroxyflavanone	H	OH	OH	H
7	3,3',4',5-Tetrahydroxy-7-methoxyflavanone	OH	OCH_3	H	OH
12	3,3',5-Trihydroxy-4',7-dimethoxyflavanone	OH	OCH_3	H	OCH_3
13	Blumeatin	H	OCH_3	OH	H

Figure 1. The structures of 15 reference standards of Ainaxiang tablets.

The reference standards were accurately weighted and dissolved in methanol to prepare solutions with a concentration of approximately 2.9-276.4 μg/mL. A 2-100 fold dilution of the each solution was made to prepare the working solutions before injection.

2.3 HPLC analysis

The HPLC system consists of Waters 2995 controller and 2998 Photodiode Array detector. The separation of all the analytes was performed by using an Elite Kromasil C18 column (250 mm × 4.6 mm, 5 μm particle size) on the conditions of flow rate at 1.0 mL/min, column temperature at 25 °C, and detective wavelength at 254 and 289 nm. The acetonitrile and water containing 0.2% acetic acid were employed as mobile phases A and B, respectively. The binary gradient program was set as follows: eluent B maintained at 80% in the first 10 min, decreased from 80 to 75% in the next 5 min, and then maintained at 75% for 10min, decreased from 75 to 35% from 25 to 50 min and equilibrated for 10 min before the next injection. The injection volume was 10 μL with needle wash. Data collection and integration were performed by Waters Empower Chemstation Software.

2.4 Linear regression equation and calibration curve

Linear regression analysis for each of the 15 compounds was performed by external standard method. The linear regression equation ($y = ax + b$) and calibration curve were investigated between peak area (y) and the concentrations (x, μg/mL) of each reference standard.

2.5 Sensitivity and stability

The sensitivity study was done by analyzing the limit of detection (LOD) and the limit of quantification (LOQ). The LOD and LOQ for the 15 reference standards were determined at a signal/noise (S/N) ratio of about 2.3-3.6:1 and 8.1-10.3:1, respectively.

The same sample was stored at room temperature, and analyzed at 0, 2, 4, 8, 12, and 18 h, respectively. The RSD value of peak area was taken as a measurement of stability. The results showed that the sample solution was found to be stable within 18 h.

2.6 Precision and accuracy of the HPLC analysis

Intra- and inter-day variations were used to evaluate the precision of the development method. The relative standard deviation (RSD%) was used as a measure of precision. Intra- and inter-day repeatability was determined on six replicates of the same sample within one day and three consecutive days, respectively. RSD% was calculated by the following formula: RSD% = 100 × SD/mean, where SD represented standard deviation of six replicates and mean was the average content of six replicates.

Spike recovery was chosen to evaluate the accuracy of the HPLC assay. It was determined by adding the standards

with three different levels (high, middle and low) to the Ainaxiang tablets (batch no. 20100505). The mixture was extracted using the same procedure as sample preparation described in Section 2.2. and then filtered through a 0.45 μm membrane filter prior to HPLC analysis. The recovery of each spiked reference standards was calculated by the formula: Recovery% = [(found amount - original amount) /spiked amount] × 100%.

3 Results and discussion

3.1 Optimization of HPLC conditions

To develop a good separation, some HPLC analytical parameters including column, different mobile phases with and without acid and gradient programs were tested for good resolution. Finally, all analyzed ingredients were separated on an Elite Kromasil C18 column (250 mm × 4.6 mm, 5 μm) with the HPLC conditions as described in Section 2.3. Blumea flavonoids have two different UV λmax values at 254 nm for **1**-**3**, **6**, **8**-**11**, **14**, **15** and 289 nm for **4**, **5**, **7**, **12**, **13** (Figure 1). In order to simultaneously detect all the analyzed ingredients and obtain a better baseline separation, the detection wavelength was set at 254 and 289 nm. Typical HPLC chromatograms are shown in Figure 2.

3.2 Validation of the developed method

The established method was validated in terms of linearity, sensitivity, stability, precision, and accuracy. Linear regression equations (y = ax +b) were constructed by plotting peak areas (y) of each analyte against analyte concentrations (x, μg/mL). Table 1 summarized the linearity, test range, limit of detection (LOD) and limit of quantification (LOQ). The linearity is expressed in terms of the correlation coefficient (R^2). It was found that all analytes showed linearity with R^2 at 0.9992- 0.9999 in the test range. The LOD and LOQ for the 15 tested reference standards were 0.01 - 0.28 μg/mL and 0.02 - 0.55 μg/mL, respectively. The variations (RSD%) of peak areas of the analyzed compounds in the batch no. 20190505 were ≤ 2.25%, indicating the analyzed compounds in the samples was stable for 18 h. The overall intra- and inter-day variations were not more than 3.18%, respectively. The spike recoveries for 15 analyzed samples were 106.17- 95.02% (Table 2). The RSD% for intra- and inter-day repeatability was shown in Table 3. Above data demonstrated that the developed HPLC method was precise and accurate.

3.3 Sample determination

Due to the co-existing multiple bioactive components in the TCMPs and their raw materials, it is far from enough to just monitor one component for the quality control of its raw material and products. Therefore, current study developed a simple and accurate assay method for simultaneous determination of 15 major bioactive compounds in the Ainaxiang tablets (Table 4). The content of compound **4** under our extraction and determination method existed in the most abundant amount comparing with the other 14 bioactive compounds.

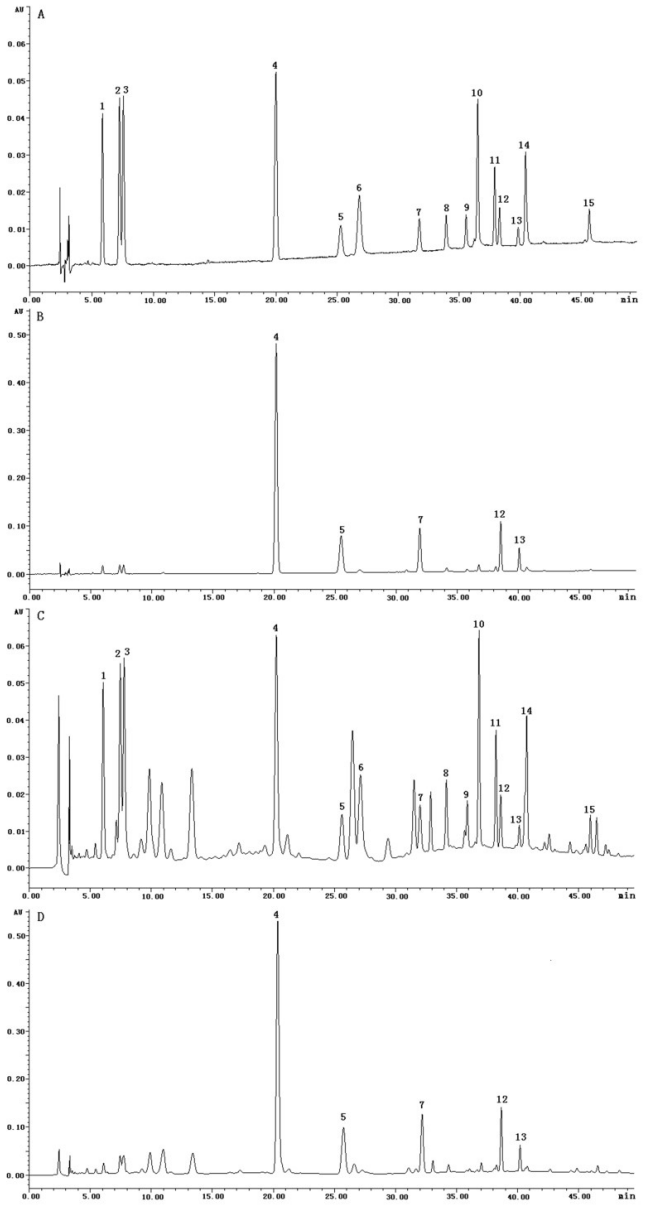


Figure 2. HPLC chromatographs (A: reference standards at 254 nm; B: reference standards at 289 nm; C: the sample of batch no. 20190505 at 254 nm; D: the sample of batch no. 20190505 at 289 nm; **1**: Rutin; **2**: Hyperoside; **3**: Isoquercitrin; **4**: 3,3',5,7-Tetrahydroxy-4'-methoxyflavanone; **5**: 3',5,5',7-Tetrahydroxyflavanone; **6**: Quercetin; **7**: 3,3',4',5-Tetrahydroxy-7-methoxyflavanone; **8**: Chrysosplenol C; **9**: Diosmetin; **10**: Tamarixetin; **11**: 3,5,7-Trihydroxy-3',4'-dimethoxyflavone; **12**: 3,3',5-Trihydroxy-4',7-dimethoxyflavanone; **13**: Blumeatin; **14**: Rhamnetin; 15: Ombuin).

Generally, therapeutic effects of TCMPs are integrative results of multiple bioactive components. Currently, multiple TCMPs products are made by independent manufacturers using raw herbs from different areas, and their chemical ingredients and clinical efficacy vary greatly. Therefore, there is a significant importance to establish the good agriculture practice (GAP) standard to grow medicinal plants with stable and consistent chemical ingredients.

Table 1. Linearity and sensitivity of the HPLC analysis.

No.	Compound name	Calibration curve[a]	R^2	Linear range(μg/mL)	LOD[b] (μg/mL)	LOQ[c] (μg/mL)
1	Rutin	y=37.49x+5.05	0.9998	0.24~24.00	0.02	0.04
2	Hyperoside	y=48.77x-2.30	0.9999	0.25~25.26	0.03	0.05
3	Isoquercitrin	y=48.71x+2.49	0.9994	0.26~26.10	0.03	0.05
4	3,3',5,7-Tetrahydroxy-4'-methoxyflavanone	y=7.18x-5.97	0.9998	2.76~276.40	0.28	0.55
5	3',5,5',7-Tetrahydroxyflavanone	y=8.29x-9.56	0.9998	2.94~58.82	0.12	0.24
6	Quercetin	y=68.14x-23.02	0.9995	0.82~16.38	0.07	0.16
7	3,3',4',5-Tetrahydroxy-7-methoxyflavanone	y=5.82x-4.66	0.9994	3.04~60.80	0.24	0.61
8	Chrysosplenol C	y=44.92x-7.82	0.9992	0.38~38.23	0.03	0.08
9	Diosmetin	y=79.71x-4.94	0.9994	0.17~17.17	0.01	0.03
10	Tamarixetin	y=72.72x-13.44	0.9994	0.17~16.75	0.07	0.17
11	3,5,7-Trihydroxy-3',4'-dimethoxyflavone	y=70.14x-4.17	0.9996	0.16~40.20	0.01	0.02
12	3,3',5-Trihydroxy-4',7-dimethoxyflavanone	y=7.99x-1.24	0.9998	0.69~34.50	0.07	0.14
13	Blumeatin	y=8.26x-1.57	0.9997	0.81~16.29	0.06	0.16
14	Rhamnetin	y=57.54x-12.94	0.9994	0.30~29.79	0.01	0.02
15	Ombuin	y=108.25x-7.86	0.9994	0.15~14.55	0.01	0.02

[a]y is the peak area and x is the concentration of compound (μg/mL). [b]LOD refers to the limit of detection, S/N = 2.3-3.6:1. [c]LOQ refers to the limit of quantification, S/N = 8.1-10.3:1.

Table 2. Recovery of the targets (n=3).

No.	Original (mg)	Spiked (mg)	Found (mg)	Recovery (%)[a]	RSD (%)[b]
1	0.150	0.096	0.247	101.05 ± 0.70	0.69
	0.111	0.120	0.232	100.28 ± 1.83	1.82
	0.097	0.144	0.243	101.51 ± 2.10	2.07
2	0.155	0.101	0.257	100.71 ± 0.62	0.61
	0.115	0.126	0.242	100.61 ± 2.46	2.44
	0.100	0.152	0.254	101.66 ± 2.03	2.00
3	0.164	0.104	0.267	98.76 ± 0.37	0.37
	0.121	0.130	0.253	100.65 ± 2.28	2.27
	0.105	0.157	0.265	101.79 ± 1.93	1.90
4	1.686	1.106	2.797	100.47 ± 0.74	0.74
	1.251	1.382	2.663	102.22 ± 2.41	2.36
	1.086	1.658	2.786	102.50 ± 2.47	2.41
5	0.393	0.235	0.628	100.22 ± 1.48	1.48
	0.291	0.294	0.589	101.30 ± 3.51	3.46
	0.252	0.353	0.614	102.23 ± 2.00	1.95
6	0.093	0.066	0.158	99.72 ± 2.00	2.00
	0.069	0.082	0.150	98.56 ± 4.72	4.79
	0.060	0.098	0.156	97.54 ± 4.19	4.30
7	0.435	0.243	0.697	108.01 ± 4.76	4.42
	0.322	0.304	0.641	104.90 ± 4.92	4.69
	0.280	0.365	0.662	104.78 ± 2.41	2.30
8	0.057	0.031	0.088	99.93 ± 1.72	1.72
	0.042	0.038	0.082	102.87 ± 2.11	2.05
	0.038	0.046	0.084	102.26 ± 0.89	0.87
9	0.021	0.014	0.034	98.53 ± 1.49	1.51
	0.015	0.017	0.032	99.78 ± 2.15	2.15
	0.013	0.021	0.033	95.52 ± 4.30	4.50
10	0.099	0.067	0.166	100.35 ± 0.32	0.32
	0.073	0.084	0.159	101.95 ± 2.49	2.44
	0.064	0.101	0.167	103.11 ± 2.16	2.09
11	0.052	0.032	0.084	99.04 ± 1.20	1.21
	0.039	0.040	0.079	100.52 ± 3.57	3.55
	0.034	0.048	0.082	100.87 ± 2.18	2.16
12	0.211	0.138	0.352	101.72 ± 1.68	1.65
	0.157	0.172	0.332	101.69 ± 3.50	3.45
	0.136	0.207	0.347	101.96 ± 2.23	2.19
13	0.095	0.065	0.160	99.87 ± 4.86	4.87
	0.071	0.081	0.157	106.17 ± 0.37	0.35
	0.061	0.098	0.154	95.02 ± 1.43	1.50
14	0.102	0.060	0.159	96.21 ± 1.18	1.23
	0.076	0.074	0.148	96.91 ± 2.93	3.02
	0.066	0.089	0.154	98.43 ± 2.10	2.13
15	0.012	0.012	0.024	99.65 ± 1.35	1.35
	0.009	0.015	0.024	102.23 ± 1.95	1.91
	0.008	0.017	0.026	105.20 ± 1.97	1.88

[a]Recovery% = [(found amount - original amount)/spiked amount] × 100%; [b]RSD% = (SD/mean) × 100%.

Table 3. Intra-day and inter-day variability of the targets.

No.	Intra-day[a] (n=6)		Inter-day[b] (n=6)	
	Content (μg/mL)	RSD[c] (%)	Content (μg/mL)	RSD (%)
1	11.656 ± 0.284	2.44	11.853 ± 0.258	2.18
2	12.364 ± 0.108	0.87	12.392 ± 0.119	0.96
3	13.051 ± 0.098	0.75	13.055 ± 0.111	0.85
4	133.087 ± 0.904	0.68	133.910 ± 1.203	0.90
5	31.328 ± 0.227	0.73	31.549 ± 0.302	0.96
6	8.731 ± 0.040	0.48	8.309 ± 0.083	1.00
7	34.983 ± 1.113	3.18	34.701 ± 0.868	2.50
8	4.502 ± 0.089	1.97	4.619 ± 0.111	2.41
9	1.610 ± 0.016	0.98	1.627 ± 0.017	1.03
10	7.734 ± 0.038	0.49	7.835 ± 0.078	0.99
11	4.203 ± 0.028	0.67	4.197 ± 0.030	0.72
12	17.291 ± 0.339	1.96	17.032 ± 0.141	0.83
13	7.437 ± 0.113	1.52	7.519 ± 0.117	1.56
14	8.037 ± 0.044	0.54	8.067 ± 0.082	1.02
15	0.962 ± 0.017	1.75	0.979 ± 0.019	2.00

[a]Intra-day precision on one day for tested six times; [b]Inter-day precision on three consecutive days; [c]RSD% = (SD/mean) × 100%.

Table 4. The contents of the 15 targets in the Ainaxiang tablets (μg per tablet, mean ± SD, n=3)

No.	20190505	20191102	20191010	20191203
1	347.19 ± 2.36	329.77 ± 1.02	304.95 ± 5.27	366.30 ± 4.09
2	357.41 ± 2.80	362.76 ± 0.88	245.89 ± 3.63	427.98 ± 0.11
3	377.88 ± 2.70	381.35 ± 0.77	271.62 ± 3.58	388.98 ± 4.39
4	3869.18 ± 22.79	4054.43 ± 12.53	4196.32 ± 8.33	4055.99 ± 35.79
5	904.91 ± 12.08	779.94 ± 7.38	994.19 ± 4.09	950.75 ± 22.22
6	198.52 ± 5.57	195.27 ± 0.96	252.66 ± 0.51	253.83 ± 2.90
7	999.60 ± 3.99	455.00 ± 2.41	1037.79 ± 27.18	917.02 ± 4.50
8	130.65 ± 0.69	86.63 ± 0.49	133.13 ± 0.45	141.82 ± 0.50
9	46.00 ± 1.56	47.69 ± 0.17	64.70 ± 0.10	41.22 ± 0.40
10	225.52 ± 3.06	219.37 ± 0.88	221.80 ± 0.41	254.20 ± 1.75
11	120.64 ± 0.64	120.36 ± 0.27	123.52 ± 0.24	107.331 ± 1.35
12	488.41 ± 1.04	445.30 ± 1.07	560.90 ± 24.75	506.26 ± 6.24
13	220.91 ± 2.16	190.53 ± 0.48	233.76 ± 2.54	224.99 ± 2.10
14	235.26 ± 2.10	224.24 ± 0.88	242.34 ± 6.76	241.71 ± 3.60
15	28.50 ± 0.18	16.70 ± 0.35	29.47 ± 0.28	29.44 ± 0.44

4 Conclusion

In the present study, a simple and accurate HPLC/PDA analytic method was developed and employed to quantify 15 bioactive flavonoids occurred as a very complex mixture in the Ainaxiang tablets. The established method was simple, reliable, high throughout, and environmentally friendly and would be potentially helpful to improve the quality control of the leaves of *B. balsamifera* and Ainaxiang tablets precisely.

Conflict of interest

The authors declare no conflict of interest.

Availability of data and material

The data generated and analyzed in this study are available from the corresponding author on request.

Funding

This work was financially supported by the Department of Science and Technology of Guizhou Province (Nos. QKHZC [2021] general 476, QKHZC [2023] general 426 and QKHPTRC [2018]5772-001), Guizhou Postgraduate Research Fund Project (YJSKYJJ [2021] 189), Guizhou Engineering Research Center of Industrial Key-technology for Dendrobium Nobile (QJJ [2022]048 and QJJ [2022]006).

References

Ginting, B., Maulana, I., Yahya, M., Saidi, N., Murniana, M., Hasballah, K., Maulidna, M., & Rawati, S. (2022). Antioxidant and antiproliferative activities of n-hexane extract and its fractions from *Blumea balsamifera* L. leaves. *Journal of Advanced Pharmaceutical Technology & Research*, 13(3), 216-220. PMid:35935701.

Guan, L., Yang, Y., Jiang, P., Mou, Q., Gou, Y., Zhu, X., Xu, Y. W., & Wang, R. (2022). Potential distribution of *Blumea balsamifera* in China using MaxEnt and the ex situ conservation based on its effective components and fresh leaf yield. *Environmental Science and Pollution Research International*, 29(29), 44003-44019. http://dx.doi.org/10.1007/s11356-022-18953-1. PMid:35122650.

Huang, X. L., Wang, D. W., Liu, Y. Q., & Cheng, Y. X. (2022). Diterpenoids from *Blumea balsamifera* and their anti-inflammatory activities. *Molecules*, 27(9), 2890. http://dx.doi.org/10.3390/molecules27092890. PMid:35566241.

Jiang, Z. L., Zhou, Y., Ge, W. C., & Yuan, K. (2014). Phytochemical compositions of volatile oil from *Blumea balsamifera* and their biological activities. *Pharmacognosy Magazine*, 10(39), 346-352. http://dx.doi.org/10.4103/0973-1296.137377. PMid:25210324.

Jirakitticharoen, S., Wisuitiprot, W., Jitareerat, P., & Wongs-Aree, C. (2022). Phenolics, antioxidant and antibacterial activities of immature and mature blumea balsamifera leaf extracts eluted with different solvents. *Journal of Tropical Medicine*, 2022, 7794227. http://dx.doi.org/10.1155/2022/7794227. PMid:36438181.

Kubota, H., Kojima-Yuasa, A., Morii, R., Huang, X., Norikura, T., Rho, S. N., & Matsui-Yuasa, I. (2009). Anti-obesity effect of *Blumea balsamifera* extract in 3T3-L1 preadipocytes and adipocytes. *The American Journal of Chinese Medicine*, 37(5), 843-854. http://dx.doi.org/10.1142/S0192415X09007326. PMid:19885945.

Liu, X., Zhang, Q., Wu, Z., Shi, X., Zhao, N., & Qiao, Y. (2014). Rapid elemental analysis and provenance study of *Blumea balsamifera* DC using laser-induced breakdown spectroscopy. *Sensors*, 15(1), 642-655. http://dx.doi.org/10.3390/s150100642. PMid:25558999.

Ma, J., Ren, Q., Dong, B., Shi, Z., Zhang, J., Jin, D. Q., Xu, J., Ohizumi, Y., Lee, D., & Guo, Y. (2018). NO inhibitory constituents as potential anti-neuroinflammatory agents for AD from *Blumea balsamifera*. *Bioorganic Chemistry*, 76, 449-457. http://dx.doi.org/10.1016/j.bioorg.2017.12.008. PMid:29275263.

Montealegre, C. M., & De Leon, R. L. (2017). Effect of *Blumea balsamifera* extract on the phase and morphology of calcium oxalate crystals. *Asian Journal of Urology*, 4(4), 201-207. http://dx.doi.org/10.1016/j.ajur.2016.08.009. PMid:29387552.

Nguyen, M. T., & Nguyen, N. T. (2012). Xanthine oxidase inhibitors from Vietnamese *Blumea balsamifera* L. *Phytotherapy Research*, 26(8), 1178-1181. http://dx.doi.org/10.1002/ptr.3710. PMid:22821854.

Tan, D., & Yan, Q. (2019). Flavonoids from the aerial parts of *Blumea megacephala*. *Chemistry of Natural Compounds*, 55(5), 927-928. http://dx.doi.org/10.1007/s10600-019-02876-4.

Tan, D., Yan, Q., & Kang, H. (2013). Chemical constituents from *Blumea balsamifera*. *Chemistry of Natural Compounds*, 48(6), 1072-1073. http://dx.doi.org/10.1007/s10600-013-0468-5.

Tan, D., Yang, Z., Zhang, Q., Ling, H., Du, Y., Lu, Y., Xie, T., Zhou, X., Qin, L., & He, Y. (2020). Simultaneous quantitative determination of polyphenolic compounds in *Blumea balsamifera* (Ai-Na-Xiang, Sembung) by high-performance liquid chromatography with photodiode array detector. *International Journal of Analytical Chemistry*, 2020, 9731327. http://dx.doi.org/10.1155/2020/9731327. PMid:32256597.

Wang, G., Wang, J., Deng, Y., Qin, L., He, Y., & Tan, D. (2022a). Chemical constituents and nutritional health functions of Dendrobium nobile: a review. *Food Science and Technology*, 42, e84522. http://dx.doi.org/10.1590/fst.84522.

Wang, J., Wang, G., Wang, X., Qin, L., Xu, C., She, X., He, Y., & Tan, D. (2022b). Chemical constituents and bioactivities of *Rosa roxburghii*: a systematic review. *Food Science and Technology*, 42, e72722. http://dx.doi.org/10.1590/fst.72722.

Wang, G., Wang, J., Zhou, Z., Bai, L., Qin, L., He, Y., & Tan, D. (2023). Establishment of fingerprints and determination of various ingredients of Yanlishuang pills by GC-MS. *Food Science and Technology*, 43, e121322. http://dx.doi.org/10.1590/fst.121322.

Wang, Y. H., & Zhang, Y. R. (2020). Variations in compositions and antioxidant activities of essential oils from leaves of Luodian *Blumea balsamifera* from different harvest times in China. *PLoS One*, 15(6), e0234661. http://dx.doi.org/10.1371/journal.pone.0234661. PMid:32544201.

Widhiantara, I. G., & Jawi, I. M. (2021). Phytochemical composition and health properties of Sembung plant (*Blumea balsamifera*): a review. *Veterinary World*, 14(5), 1185-1196. http://dx.doi.org/10.14202/vetworld.2021.1185-1196. PMid:34220120.

Zhang, B., Tang, M., Zhang, W., Zhangb, C., Ai, Y., Liang, X., Shi, Y., Chen, Y., Zhang, L., & He, T. (2021). Chemical composition of *Blumea balsamifera* and *Magnolia sieboldii* essential oils and prevention of UV-B radiation-induced skin photoaging. *Natural Product Research*, 35(24), 5977-5980. http://dx.doi.org/10.1080/14786419.2020.1809401. PMid:32856487.