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Study on extraction conditions and content comparison of total flavonoids from bitter bamboo shoots and sinocalamus affinis shoots

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Abstract. To optimize the extraction process of total flavonoids from bitter bamboo shoots and sinocalamus affinis shoots, and compare their contents, to provide technical reference for the value by two kinds of bamboo shoots. Based on the three factors of ethanol concentration, extraction time and extraction temperature, orthogonal test was designed to determine the content of total flavonoids in bamboo shoots and the optimum extraction conditions were selected. The results showed that the total flavonoids contents of bitter bamboo shoots reached to 10.655 mg/g when 60% ethanol concentration, 70℃ extraction temperature and 1.5 h extraction time; the total flavonoids contents of sinocalamus affinis shoots reached to 32.24 mg/g when ethanol concentration, extraction temperature and extraction time were 60%, 80 C and 1.5 h. The total flavonoids content of the sinocalamus affinis shoots was significantly higher than that of the bitter bamboo shoots, and the medical value was higher than that of the bitter bamboo shoots.

1. Introduction

China is the richest country of bamboo resources in the world. The bamboo planting area and bamboo industry output value are the first in China [1]. Bitter bamboo and sinocalamus affinis are widely distributed, and are mainly used in bamboo weaving and papermaking raw materials. However, they are still lacking in the utilization of medicinal value as common table vegetables.

Flavonoids are a natural organic compound, which can enhance the immune function of human body and play an important role in the treatment of cardiovascular diseases. [2-3]. The research on the extraction technology of Flavonoids is mainly concentrated on some medicinal plants and common vegetables. However, there are relatively few studies on bamboo flavonoids. Yang YF and Huang CL studied the flavonoids of bitter bamboo shoots by spectrophotometry. The effects of climate and soil conditions on the content of flavonoids in bamboo shoot were clarified [4].

Some other scholars have also carried out the extraction and determination of flavonoids in the leaves of sinocalamus affinis. For example, Jiang JB et al. used different solvents to extract total flavonoids from Xiangxi bamboo leaves. The results showed that the extraction effect was acetone > ethanol > ether [5]. So far, no extraction technology has been selected for total flavonoids from bitter bamboo shoots, and no comparison of total flavonoids contents from bitter bamboo shoots and

sinocalamus affinis shoots. Therefore, it is of great practical significance to optimize the extraction technology and content comparison of flavonoids from bitter bamboo shoots and sinocalamus affinis shoots.

2. Materials and Methods

2.1. Experimental Materials

The bitter bamboo shoots and sinocalamus affinis shoots were collected from "Bamboo Diseases and Pests Control and Resources Development Key Laboratory of Sichuan Province" in May and August of 2017.

2.2. Experimental Methods

2.2.1. Bamboo shoots sample preparation. The sinocalamus affinis shoots and the bitter bamboo shoots are cleaned and leachate, and then placed in the electrothermal constant temperature dryness box (to 50 degrees centigrade) and dried to the constant weight, and then mashed by the tissue mashed homogenizer. Select 60 target sieves to get very fine powder samples and store them in refrigerator.

2.2.2. Drawing of standard curve. Referring to Yin P et al. [6], the regression equation and the standard curve are drawn as shown in Figure 1.

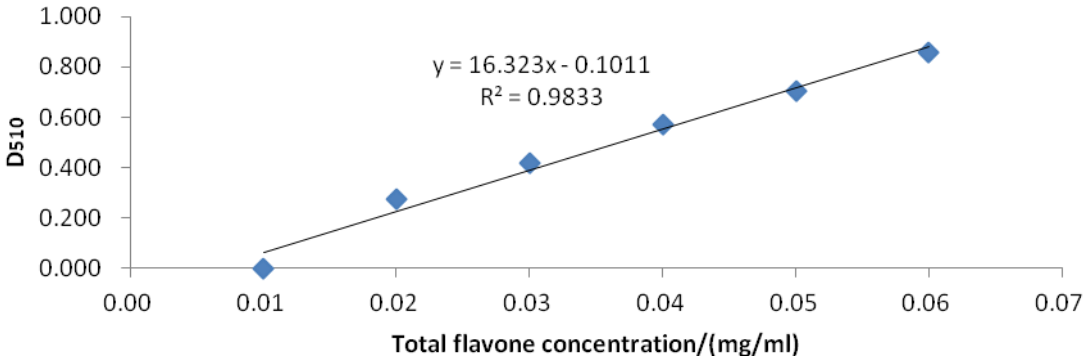

Fig. 1 Rutin standard curve

2.2.3. Determination of total flavonoids in bamboo shoots. The bitter bamboo shoots and the sinocalamus affinis shoots of 1g were accurately measured, and the absorbable value was measured under different ethanol concentration, different extraction temperature and different extraction time. And the total flavonoids content was calculated by Yin P et al. [4].

3. Single factor test and result analysis

3.1. The effect of ethanol concentration on the extraction of total flavonoids

The bitter bamboo shoots and the sinocalamus affinis shoots powder of 1g were accurately identified in the centrifuge tube of 50ml, 30%, 40%, 50%, 60%, 70%, 80% ethanol extractant 40ml were added to the centrifuge tube, and placed in the constant temperature water bath at 50 C during 2.0 h. Then, the centrifuge 10min was taken under 4000 r/min. Finally, the 1ml supernatant was taken to determine the absorption value.

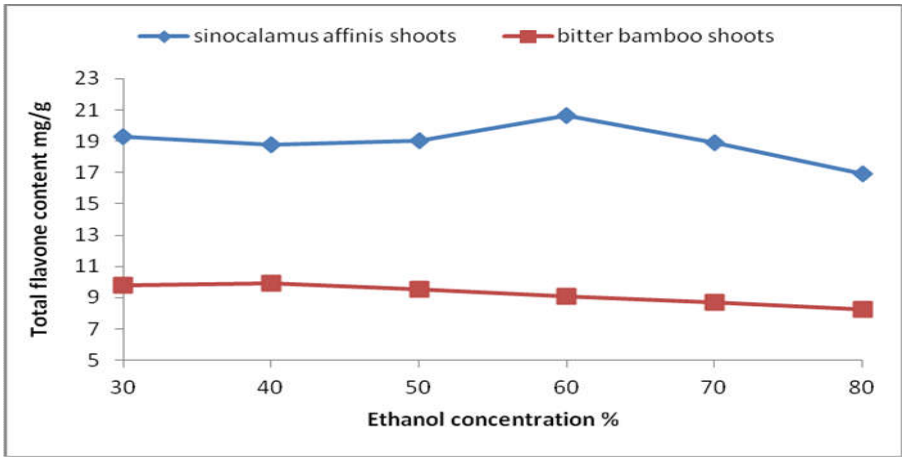

Fig. 2 Effect of ethanol concentration on extraction of total flavonoids from shoots of bitter bamboo and sinocalamus affinis

According to Fig. 2, the ethanol concentration had no obvious effect on the content of total flavonoids in bitter bamboo shoots. When the concentration of ethanol was 40%, the content of total flavonoids in bitter bamboo shoots was the highest, and then decreased slightly. Therefore, the four levels of 30%, 40%, 50% and 60% of bitter bamboo shoots were selected for orthogonal experiment. In terms of bamboo shoots, bamboo shoots generally rose first and then decreased. When the concentration of ethanol was 60%, the total flavonoids content of sinocalamus affinis shoots was the highest. Therefore, the orthogonal test of sinocalamus affinis shoots was conducted at four levels of 50%, 60%, 70% and 80%.

3.2. The effect of extraction temperature on the extraction of total flavonoids

1 g bamboo shoots powder and 30% ethanol extractant (40 ml) were added to the 50ml centrifuge tubes. And then they were placed in a constant temperature water bath at 30℃、40℃、50℃、60℃、70℃、80℃ during 2.0 h. Finally, the contents of total flavonoids were measured.

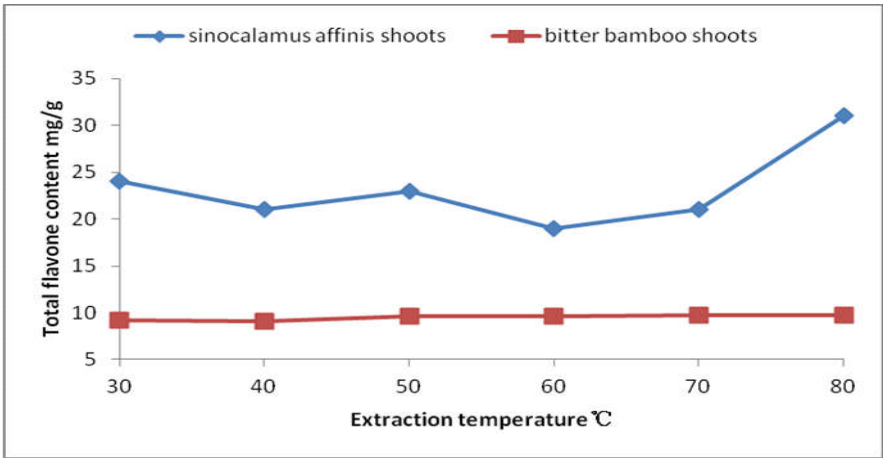

Fig. 3 Effect of extraction temperature on extraction of total flavonoids from shoots of bitter bamboo and sinocalamus affinis

In the single factor test, the total flavonoids content of the bitter bamboo shoots tended to be stable with the increase of temperature. When the extraction temperature was 70℃, the content of total flavonoids in the bitter bamboo shoots was the highest. On the contrary, the total flavonoid content of

sinocalamus affinis shoots appeared irregular trend with the increase of temperature. The total flavonoid content increased most rapidly between 70℃ and 80℃ (Fig.3). At this time, 30% ethanol extract began to boil, resulting in the consumption of extractant. Considering that the experiment was a single variable, it did not raise the temperature again. So he orthogonal test of sinocalamus affinis shoots was conducted at four levels of 50℃, 60℃, 70℃ and 80℃.

3.3. The effect of extraction time on the extraction of total flavonoids

1 g bamboo shoots powder and 30% ethanol extractant (40 ml) were added to the 50ml centrifuge tubes. And then they were placed in a constant temperature water bath at 50℃ of different duration (0.5h、1.0h、1.5h、2.0h、2.5h、3.0h). Finally, the contents of total flavonoids were and measured. With the increase of extraction time, the total flavonoid content of bitter bamboo shoots firstly increased and then decreased. When the extraction time was 2h, the total flavonoid content of bitter bamboo shoots was highest. Therefore, four levels of 1.5h, 2.0h, 2.5h and 3.0h were selected for orthogonal experiment. The change trend of sinocalamus affinis shoots with extraction time was more significant than that of bitter bamboo shoots. When the extraction time was 1.0h, the total flavonoid content of bamboo shoots was highest. So the four levels of 0.5h, 1.0h, 1.5h and 2.0h were selected for orthogonal experiment (Fig.4).

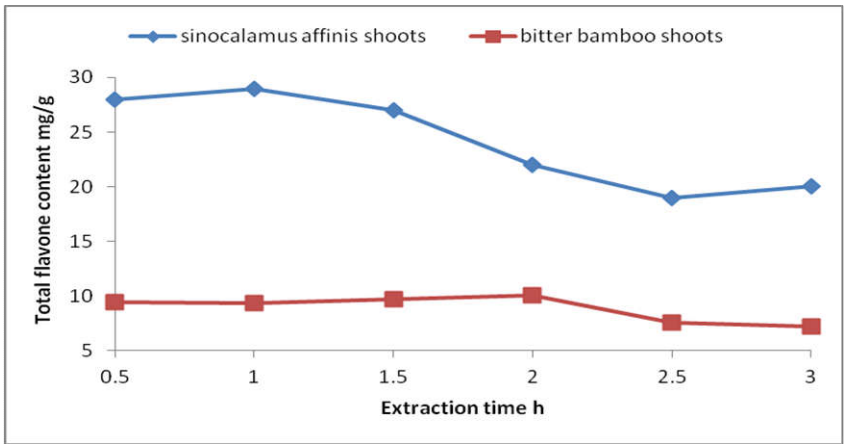

Fig. 4 Effect of extraction time on extraction of total flavonoids from shoots of bitter bamboo and sinocalamus affinis

4. Orthogonal experiment and result analysis

4.1. Setting of experimental conditions

According to the single factor experiment results, orthogonal test was conducted by selecting the most significant levels of total flavonoids extraction.

Tab. 1 Orthogonal test factors and levels of bitter bamboo shoots

	Factor		
Level	A. Ethanol concentration (%)	B. Extraction temperature (℃)	C. Extraction time(h)
1	30	50	1.0
2	40	60	1.5
3	50	70	2.0
4	60	80	2.5

Tab. 2 Orthogonal test factors and levels of sinocalamus affinis shoots

Level	Factor		
	A. Ethanol concentration (%)	B. Extraction temperature (℃)	C. Extraction time(h)
1	50	50	0.5
2	60	60	1.0
3	70	70	1.5
4	80	80	2.0

4.2. Orthogonal verification test

If the results are not consistent with the results obtained from the extreme difference analysis in the analysis of the orthogonal test results, it is necessary to carry out the verification test.

The total flavonoids of bamboo shoots was obtained under the optimum extraction conditions. The content of the total flavonoids was compared with the content of visual analysis, and the optimum extraction conditions were determined. On the other hand, no verification test is needed.

4.3. Orthogonal test results

According to Tab. 3, the highest flavonoids content of bitter bamboo shoots is 10.66mg/g, and the best extraction conditions are 60% ethanol concentration, 70 ℃ extraction temperature and 1.5h extraction time. According to the extreme analysis, the factors affecting the extraction effect of the total flavonoids of bitter bamboo shoot were extraction time > extraction temperature > ethanol concentration, and the optimum extraction conditions were 30% ethanol concentration, 70 ℃ extraction temperature and 1.5h extraction time. As the two results are not consistent, verification test should be carried out.

Tab. 3 The results of orthogonal test of bitter bamboo shoots

Test number	Factor			Total Flavonoids content (mg/g)
	A. Ethanol concentration (%)	B. Extraction temperature (℃)	C. Extraction time (h)	
1	1	1	1	9.24
2	1	2	2	9.04
3	1	3	3	8.77
4	1	4	4	8.93
5	2	1	2	8.99
6	2	2	1	9.91
7	2	3	4	9.11
8	2	4	3	7.83
9	3	1	3	7.44
10	3	2	4	8.77
11	3	3	1	9.20
12	3	4	2	8.92
13	4	1	4	8.28
14	4	2	3	7.12
15	4	3	2	10.66
16	4	4	1	9.18
K1	35.98	33.96	37.54	
K2	35.84	34.84	37.60	
K3	34.33	37.73	31.16	
K4	35.23	34.85	35.09	
R	1.65	3.78	6.45	

When the ethanol concentration is 60%, the extraction temperature is 80 ℃, and the extraction time is 1.5h, the total flavonoid content of sinocalamus affinis shoots is 32.24 mg/g (Tab.4). The results of range analysis showed that the extraction temperature was the most important factor, and the extraction time was the least factor affecting the extraction of total flavonoids from sinocalamus affinis shoots. The results of visual analysis and range analysis are consistent and no need for further verification tests.

Tab. 4 The results of orthogonal test of sinocalamus affinis shoots

Test number	Factor			Total Flavonoids content (mg/g)
	A. Ethanol concentration (%)	B. Extraction temperature (℃)	C. Extraction time (h)	
1	1	1	1	17.36
2	1	2	2	23.16
3	1	3	3	21.28
4	1	4	4	27.84
5	2	1	2	24.08
6	2	2	1	29.72
7	2	3	4	32.08
8	2	4	3	32.24
9	3	1	3	25.00
10	3	2	4	23.36
11	3	3	1	29.92
12	3	4	2	29.16
13	4	1	4	21.48
14	4	2	3	28.48
15	4	3	2	29.60
16	4	4	1	27.44
K1	89.64	87.92	104.44	
K2	118.12	104.72	106.00	
K3	107.44	112.88	107.00	
K4	107.00	116.68	104.76	
R	28.48	28.76	2.56	

4.4. The results of verification test of bitter bamboo shoots

The content of total flavonoids in bitter bamboo shoots reached 10.655 mg/g, when ethanol concentration is 60%, extraction temperature is 70℃ and extraction time is 1.5h. This result is significantly lower than that of the total flavonoid content obtained when ethanol concentration is 60%, extraction temperature is 70℃, and extraction time is 1.5h. Therefore, the optimum extraction conditions of total flavonoids from bitter bamboo shoots were ethanol concentration of 60%, extraction temperature of 70℃, and extraction time of 1.5h.

Tab. 5 The results of verification test of bitter bamboo shoots

Ethanol concentration (%)	Extraction temperature (℃)	Extraction time (h)	Total Flavonoids content (mg/g)
30	70	1.5	9.56

5. Conclusion

When ethanol concentration, extraction temperature and time were 60%, 70℃, and 1.5h respectively, the content of total flavonoids in bitter bamboo shoots was up to 10.655 mg/g. When ethanol

concentration, extraction temperature and time were 60%, 80 ℃ and 1.5 h, the total flavonoids content of sinocalamus affinis shoots was 32.24 mg/g. In addition to a small difference in extraction temperature, the best extraction conditions are almost identical, and the results are believable. The total flavonoid content of sinocalamus affinis shoots was significantly higher than that of bitter bamboo shoots, and the value of medicinal development was higher.

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