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Original Research Article

Study on the Extraction Technology of Flavonoid from Dandelion

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Abstract

The extraction technology of total flavonoid from dandelion was optimized by single factor screening and orthogonal test. The method of solvent extraction was used, and ethyl acetate was used as solvent, solid-liquid ratio, extraction temperature and extraction time were invested to study the effects of the extraction. On the basis of single factor, three-factor and three-level orthogonal test were carried out to optimize the extraction process of flavonoid from dandelion. The results showed that the factors of the extraction effect of flavonoid were influenced by the time of extraction, the ratio of material to liquid, and the temperature of extraction in turn, it was found that the time of extraction had the most significant effect on the extraction of flavonoid from dandelion. The results showed that the optimal conditions of extraction were as following: the ratio of solid-liquid was 1:70, the temperature of extraction was at 70 ℃, and the time of extraction was 1.5 h. Flavonoid was extracted from dandelion at the best conditions, flavonoid was obtained, and the content of flavonoid was 7.18%. The process is simple, reproducible, economical and feasible.

Keywords: dandelion; flavonoid; orthogonal test; extraction.

INTRODUCTION

Dandelion is a perennial herbaceous plant of compositae, it is called dandelion, cao hua di ding, po po ding *et al*. it is various, rich in resources and widely distributed. There are about 2,000 species in the world and more than 100 species in China. 27 species of the genus are at least used in medicine. There are many common dandelions, such as Taraxacum of mongolicum Hand Mazz, Taraxacum of platyperidum Diers, Taraxacum of Sinicum Kitag, Taraxacum of ohwianum Kitam, Taraxacum of grypodon dahlst, and Taraxacum of falcilobum Kitag. Dandelion contains a variety of chemical components: carotenoids, triterpenoids, sterols, flavonoid, sesquiterpenoid lactones, caffeic acid, chlorogenic acid, volatile oil, coumarins, phenolic acids, fatty acids, choline, fructose, vitamins, proteins, minerals, *et al*. [1-4]. Flavonoid is the active ingredients in dandelion; natural flavonoid is mostly in the form of glycosides. In general, flavonoid is soluble in highly polar solvents such as water, ethanol, methanol, etc., it is insoluble in organic solvents, such as benzene, chlorine imitation and so on. The studies of modern pharmacological have shown[5] that dandelion has the functions of two clearing heat, detoxification, detumescence, diuresis, toning, it has larger efficacy on broad-spectrum bacteriostasis, anti-inflammatory, anti-tumor, liver and gallbladder protection, and anti-stomach injury, and these pharmacological activities are related to the flavonoid of dandelion[6]. Along with flavonoid from dandelion was widely used in pharmaceuticals, health products and other fields, how to extract effectively flavonoid from dandelions is important.

The flavonoid of dandelion is usually extracted by solvent extraction, ultrasonic assisted extraction, microwave assisted extraction [7-9], and methanol and ethanol are usually used as solvent. In this paper, ethyl acetate was used as solvent; the single factor experiment and orthogonal experiment were designed to determine the optimal extraction process, which provides the theoretical foundation for developing and using the flavonoid of dandelion.

MATERIALS

Experimental sample

Dandelion (which harvested in daqing)

Reagents

Rutin (chromatographical pure, ≥ 98%), which was purchased from hefei bomei biotechnology co. LTD; ethyl acetate, methanol, acetone, aluminum nitrate, anhydrous ethanol, sodium hydroxide and sodium nitrite, which were all analytically pure and

purchased from Damao chemical reagent factory in Tianjin.

Intruments

Electronic analytical balance (ALC-310.3), which purchased from Shanghai precision scientific instrument co., LTD. Concentrated type constant temperature heating magnetic stirrer (DF-10S), which purchased from gongyi yuhua instrument co., LTD. Uv-visible spectrophotometer (T6), which purchased from Beijing general instrument co., LTD. Circulating water type multipurpose vacuum pump (SHB-ⅢA), which purchased from zhengzhou Great Wall industry &trade co., LTD. Acidity meter (LE438), which purchased mettler-Toledo instrument LTD.

METHODS

The pretreatment of sample

Dandelion, which harvested in daqing, was washed firstly, dried and crushed secondly, and it was sealed and refrigerated for using at last.

Determination of the maximum absorption wavelength

The power of dandelion (2.00 g), which dried in advance, was soaked in the solution of ethyl acetate, the flavonoid of dandelion was extracted under a certain ratio of material to liquid, temperature and time, the coarse solution of flavonoid was obtained, the coarse solution of flavonoid, which acquired in the previous step, was filtered then, at this stage, the solid impurities were removed, The solution of flavonoid was purified. The solution of flavonoid(2.00 mL), which purified, was put to a volumetric flask(25.00 mL) by a pipette, sodium nitrite(1 mL, 5 %) was added to volumetric flask then, the solution of flavonoid was shaken well, the volumetric flask was placed for 6 min, the solution of aluminum nitrate(10%, 1 mL) was added to volumetric flask, then the solution was shaken well once again, the volumetric flask was placed for 6 min in the same way, let the reaction finish well, and then ethanol solution(60%) was put until the volume of the solution was 25.00 mL, so the concentration of the solution was determined. An appropriate amount of the prepared solution was put into the colorimetric dish, and the absorbance of the solution was measured to determine the maximum absorption wavelength at 400~700 nm.

The establishment of standard curve

The standard sample of rutin(0.0147 g) was weighed, it was dissolved in the solution of methanol, the solution of rutin was transfered into the volumetric bottle(25.00 mL), then the solution of methanol was put in the volume until the volume was 25.00 mL, so the concentration of the solution was determined, the solution was shaken well, and the standard solution of rutin(0.5880 $mg \cdot mL^{-1}$) was obtained. The process was as follows:the standard solution of rutin(0.00, 1.00, 2.00, 3.00, 4.00, 5.00 and 6.00 mL) was put in volumetric flask(25.00 mL), respectively, in which the solution of sodium nitrite(1.00 mL, 5%) was put then, and the solution of rutin was shaken subsequently, the solution was set for 6 min, the solution of aluminum nitrate(1 mL, 10%) was added to the volumetric flask, and the solution was shaken well, let the solution stand for 6 min once again, let the reaction finish well, and then ethanol solution of methanol(60%) was put in the volume until the volume was 25.00 mL, so the concentration of the solution was determined. An appropriate amount of the prepared solution was put into the colorimetric dish, and the absorbance of the solution was measured to determine the maximum absorption wavelength.

The test of single factor

The flavonoid of dandelion was extracted by ethyl acetate which was used as solvent. the powder of dandelion(2.00 g), which weighed in advance, was put in a beaker, then the solution of acetate was put subsequently. The ratio of the dandelion to ethyl acetate was 1:30, 1:40, 1:50, 1:60, 1:60, and 1:70 respectively, the temperature of extraction was at 40, 50, 60, 70, and 80 ℃respectively, the time of extraction was 0.5, 1, 1.5, 2, and 2.5 h respectively, the test of single factor was invested on the basis of the content of the dandelion. Then the solution of extraction was purified by filtration, and then the purified solution of flavonoid was obtained. The solution(2.00 ml), which obtained in the above step, was put into a volumetric flask(25.00 ml), sodium nitrite(1 mL, 5 %) was added to volumetric flask then, the solution of flavonoid was shaken well, the volumetric flask was placed for 6 min, the solution of aluminum nitrate(10%, 1 mL) was added to volumetric flask, then the solution was shaken well once again, the volumetric flask was placed for 6 min in the same way, let the reaction finish well, and then ethanol solution(60%) was put until the volume of the solution was 25.00 mL, so the concentration of the solution was determined. An appropriate amount of the prepared solution was put into the colorimetric dish; the absorbance of the solution was measured at the maximum absorption wavelength. According to the standard curve which obtained according the above contents, so the content of flavonoid was calculated and the optimal extraction conditions were also determined.

The design of orthogonal experiment

In order to determine the optimal experimental conditions, on the basis of single factor experiment, three-factor and three-level orthogonal experiment was further designed to optimize the extraction conditions of flavonoid from dandelion.

Table-1: The table of orthogonal test

umber	A （The ratio of liquid and material）	B （The temperature of extraction/℃）	C （The time of extraction/h）
	1 : 50	60	1.5
	1 : 60	70	2.0
	1 : 70	80	2.5

RESULTS AND ANALYSIS

The determination of maximum absorption wavelength

As is shown in Figure 1, the absorption spectrum of the solution of flavonoid, which extracted from dandelion, is shown in the wavelength range of 400~700 nm. It can be seen that the absorbance of flavonoid to light is different in this range of 400~700 nm, and the strongest peak of absorbance is at 510 nm, it showed that the maximum absorbance wavelength of flavonoid to light is at 510 nm, Therefore, the absorbance of flavonoid was determined at 510 nm in the following experiment, it is as the maximum absorption wavelength in this study, so the content of flavonoid can be determined according to the Beer-Lambert Law.

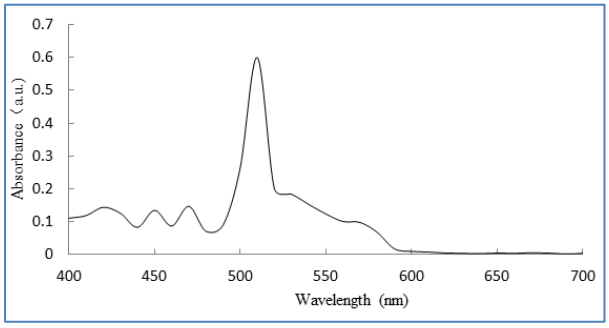

Fig-1: Ultraviolet-visible spectrum of the solution of flavonoid

The establishment of standard curve

The Rutin was used as the standard product, the standard solutions of different concentrations were prepared, and the absorbance of different standard solutions were measured at 510 nm, and the absorbance was obtained. According to the absorbance, the standard curve was drawn as shown in figure 2. To inspect the reliablity of the absorbance, through the linear fitting, the equation of standard curve was obtained as formula (1).

$$A=0.0110+3.7278C, \quad R^2=0.9921 \ (1)$$

It is shown that the content of flavonoid and the absorbance have a good linear relationship. Combining with the equation of the standard curve, the content of flavonoid can be calculated as formula (2).

$$Y=[\ (C\times D\times V)\ \times 10^{-3}/G\ ]\times 100\% \ (2)$$

In the formula, Y is the content of flavonoid in dandelion (%), C is the concentration calculated by the standard curve (mg· ml^{-1}), D is the multiple of dilution, V is the volume of the extraction (mL), G is the mass of the dandelion sample (g).

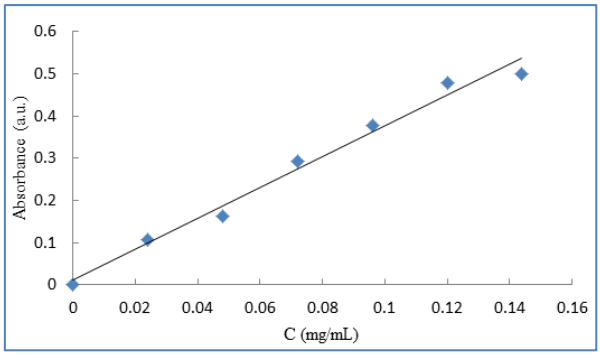

Fig-2: The standard curve of standard product

The test of single factor

The effect of ratio of material-liquid on content of flavonoid

In order to investigate the effect of the ratio of material to liquid on the content of flavonoid, we changed the amount of solvent which added in the system, the result was shown in figure 3.

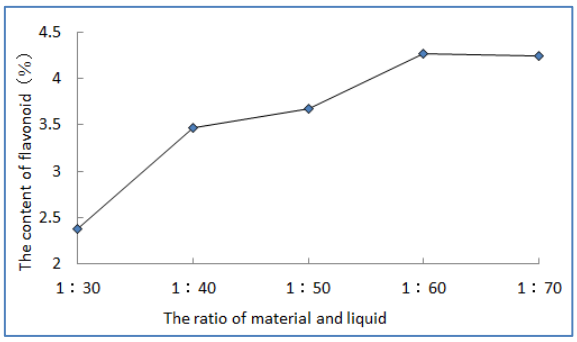

Fig-3: The effect of the ratio solid-liquid

As is shown in Figure 3, the content of flavonoid from dandelion was changed with the ratio of material-liquid. It can be seen that the content of flavonoid increases with the increase of the ratio of material-liquid, when the ratio of material to liquid reached 1:60, the content of flavonoid reached the highest level, when the ratio of material to liquid continued to increase, the content of flavonoid did not change much, this is mainly because the dissolution of flavonoid requires a certain amount of solvent, when the ratio of material to liquid is relatively lower, the amount of solvent is less, the flavonoid in dandelion cannot be completely extracted by the solvent, and when the ratio of material to liquid is relatively higher, the amount of solvent is too much, the flavonoid in

dandelion was completely extracted by the solvent, the excessive solvent is also a waste meaningless, so the optimal ratio of material to liquid is 1:60.

The influence of extraction temperature on the content of flavonoid

In order to investigate the effect of the difference of temperature on the content of flavonoid, we changed the amount of solvent which added in the system, the result was shown in figure 4.

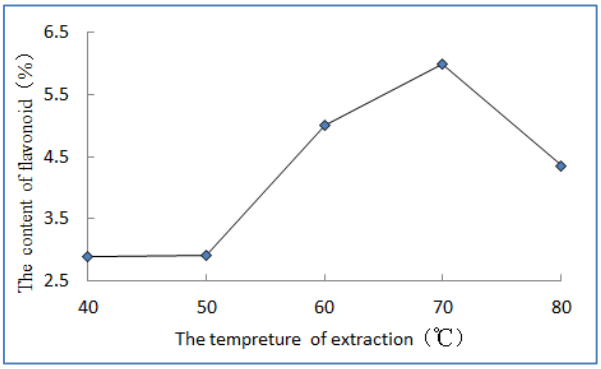

Fig-4: The effect of different temperature

As is shown in Figure 4, the content of flavonoid is changed with the temperature of extraction. The change is not obvious at the low temperature, with the increase of temperature, the content of flavonoid increases gradually, when the temperature is at 70 □, and the content of flavonoid reaches the highest level. This may be due to the fact that high temperature can facilitate the leaching of flavonoid, and high temperature may affect the stability of flavonoid. Therefore, the optimal temperature of extraction is at 70 □.

The influence of extraction time on the content of flavonoid

In order to investigate the effect of the difference of time on the content of flavonoid, we changed the amount of solvent which added in the system, the result was shown in figure 5.

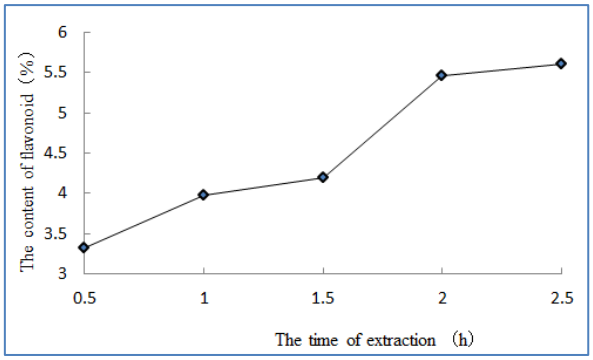

Fig-5: The effects of different time of extraction on the content of flavonoid

As is shown in Figure 5, the content of flavonoid was changed under the time of extraction. It can be seen that the content of flavonoid increases gradually with the extension of the extraction time, when the time of extraction is more than 2 hours, the content of flavonoid does not increase significantly, this is mainly because the dissolution of flavonoid requires a certain time, and the extraction time is relatively short, so the flavonoid in dandelion cannot be fully extracted, which results the low content of flavonoid in the extract. However, after 2 h, the content of flavonoid did not increase significantly, so the optimal time of extraction was 2 h.

The analysis of orthogonal test and variance

The single factor test was used to determine the level range of factors, and the three factors were the ratio of material to liquid (A), the temperature of extraction (B), and the time of extraction (C). Three factors and three levels was designed, the orthogonal test of three factors and three levels was used to optimize the extraction process of flavonoid from dandelion. The results of test were shown in table 2.

Table-2: The table of orthogonal design and results/$L_9(^3_3)$

	A	B	C	
Number	**The ratio of material to liquid**	**The temperature of extraction(□)**	**The time of extraction(h)**	**The content of flavonoid (%)**
1	1	1	1	3.83
2	1	2	2	3.75
3	1	3	3	2.02
4	2	1	2	2.34
5	2	2	3	2.93
6	2	3	1	4.63
7	3	1	3	3.63
8	3	2	1	7.26
9	3	3	2	4.98
K_1	9.60	9.80	15.72	
K_2	9.89	13.94	11.06	
K_3	15.87	11.63	8.58	
k_1	3.20	3.27	5.24	
k_2	3.30	4.65	3.69	
k_3	5.29	3.88	2.86	
R	2.09	1.38	2.38	

Table-3: The analysis of variance

Differences between the source	SS	df	MS	F	Significant difference
A	8.3382	2	4.1691	313.4662	**
B	2.8694	2	1.4347	107.8722	**
C	8.7558	2	4.3779	329.1654	**
Error	0.0266	2	0.0133		
Total	19.99	8			

As is shown in table 2, the influence of each factor on the effect of extraction is as follows: the time extraction > the ratio of solid-liquid > the temperature of extraction, that is to say, the time of extraction has the largest influence on the effect of extraction, followed by the ratio of solid-liquid and the least effect was the temperature of extraction. Within the scope of the experiment, the optimal process of extraction on total flavonoid from dandelion was $A_3B_2C_1$, that is, the ratio of solid-liquid was 1:70, the temperature of extraction was at 70 □, and the time of extraction was 1.5 h.

As is shown in table 3, according to the analysis of variance, three factors, such as the ratio of solid-liquid, the temperature of extraction and the time of extraction, have a significant influence on the extraction effect of total flavonoid.

The experiment of verification

The total flavonoid in dandelion was extracted under the optimal conditions of extraction, that is, the ratio of material to liquid was 1:70, the temperature of extraction was at 70 □, and the time of extraction was 1.5 h. The content of flavonoid was 7.18%, and the reproducibility was good.

CONCLUSION

Flavonoid, which has a variety of physiological effects, is more and more focused on in recent years. The results showed that the content of flavonoid in dandelion was rich. On the basis of the test of single factor, the experiment of three-factor and three-level was designed, then the test of orthogonal was carried out to determine the optimal extraction conditions for total flavonoid from dandelion, ethyl acetate was used as the agent of extraction: the ratio of solid-liquid was1:70, the temperature of extraction was at 70 □, the time of extraction was 1.5 h, Each factor has different influence on the effect of extraction, the order of influence is: the time of extraction > the ratio of solid-liquid > the temperature of extraction. The total flavonoid in dandelion was extracted under the best extraction technology, and the content of flavonoid was 7.18%. The process is simple, reproducible, economical and feasible.

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