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Research Article

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A comparative study between solvent and micro-wave assisted extraction technology of pigment from orange peel

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ABSTRACT

In order to conform the extraction condition of pigment from orange peel, methods of pigment extracted from orange peel by solvent of ethyl ethanol and micro-wave assisted extraction were compared. In the method extracted by solvent of ethyl ethanol, the optimized conditions were: the concentration of ethanol was 95%; the ratio of raw material to solvent was 1:20 (g/ml); extraction time was 1.5 h; extraction temperature was 55 ℃; extraction times was 3 times. In the method of micro-wave assisted extraction, the optimized conditions were: the concentration of ethanol was 95%; the ratio of raw material to solvent was 1:40 (g/ml); microwave irradiation time was 80s; extraction times were 3 times; microwave irradiation power was middle and high frequency.

Key words: pigment; orange peel**;** extraction; microwave assistant; optimization

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INTRODUCTION

Citrus reticu lata Banco, which belongs to *Rutaceae Citrus*, is widely distributed in North America, Latin America, Asia and the Mediterranean coast [1]. *Citrus* cultivation area in China ranks first in the world, while yield of *citrus* ranks third in the world, following the United States and Canada [2]. It is favored by the majority of consumers as it is sweet and juicy, fresh and delicious, mellow, rich in nutrition. In modern industrial production, orange juice production will reach 1.5 million tons in the year of 2015, while 25% to 40% of the *citrus* hide trimmings byproducts could be produced along with the production and processing of orange juice. Beneficial nutrient content in these hide trimmings includes gum, essential oils, natural pigment and other substances [3]. Currently, except a small amount of orange peel are used for extractions of essential oils and medicine (Tangerine peel), most enterprises treat orange peel as the landfill or process them into animal feed, while others directly use them as garbage disposal. The feed processing usually should go through drying and grinding so that energy consumption is too large [4, 5]. People tend to utilize byproducts of hide trimmings for turning waste into treasure, which can only save resources, but also bring certain economic benefits, and to some extent, can also reduce environmental pollution.

Pigment is widely used as food additive in the food industry. Edible pigment is usually divided into two categories: natural pigment and synthetic pigment [6]. Due to its strong coloring, bright color, good stability, insulation from odor and taste, uniform texture, easy to dissolve, good fineness effects, low cost, and other advantages, synthetic pigment is widely used [7]. In recent years, the media has exposed stained bread, pork with lean, milk with melamine and other events, pushing food additives to the cusp, resulting in consumption of food additives to be debated. Meanwhile, the harm of synthetic pigment to human health also caused consumers' more attention with the development of toxicology and analytic techniques and people's more attention to their health. Therefore, people are looking forward to the replacement of synthetic colors with safe natural pigment; additionally, food processing industries, food industries and cosmetics manufacturing industries greatly demand natural pigment, thus making the extraction process of orange peel pigment is practically significant.

EXPERIMENTAL SECTION

Materials and instruments

Orange peel powder; 95% ethanol; WFJ7200 visible spectrophotometer; TDL-5C large-capacity low-speed desktop centrifuge; D8023CTL-K4 microwave oven; DK-98-1 constant temperature water bath pot; A1104 electronic balance

Experimental methods

Solvent extraction method：Orange peel were dried at 35 ~ 40 ℃, crushed and screened. 1.000g were weighed in a beaker, adding 20ml 95% ethanol, and heated in a constant temperature pot, poured the solution, a yellow liquid was obtained by filtration, then concentrated by transferring into a constant temperature water bath distillation apparatus under reduced pressure. As 10% to 15 % solution remained, drying them in the oven to get the crude pigment.

Microwave-assisted extraction：Orange peel were dried at 35 ~ 40 ℃, crushed and screened. 1.000g were weighed in a beaker, adding 20ml 95% ethanol, and 80s under microwave irradiation, poured the solution, a yellow liquid was obtained by filtration, then concentrated by transferring into a constant temperature water bath distillation apparatus under reduced pressure. As 10% to 15 % solution remained, drying them in the oven to get the crude pigment.

Single factor test

Solvent extraction method：Selecting five factors, including extraction agent concentration, extraction temperature, solid-liquid ratio, extraction time, extraction times for study, and each factor should select variables of different gradients.

Microwave-assisted extraction：Selecting five factors, including extraction agent concentration, microwave irradiation power, microwave irradiation time, solid-liquid ratio, extraction time, extraction times for study, and each factor should select variables of different gradients.

Orthogonal test

Solvent extraction method：Selecting three factors and three levels experiments of the experimental results obtained by single factor test. Test factors and levels were shown in table 1.

Table 1 Factor and level of orthogonal experiment

	Factors		
Levels	Extraction time (h)	Extraction Temperature (℃)	Extraction Number
1	1	45	1
2	1.5	55	2
3	2	65	3

Microwave-assisted extraction：Selecting four factors and three levels experiments of the experimental results obtained by single factor test. Test factors and levels were shown in table 2.

Table 2 Factors and levels of orthogonal experiment

	Factors			
Levels	Ratio of material to liquid	Microwave radiation time (s)	Microwave radiation power	Extraction Number
1	1:20	60	Low frequency	1
2	1:30	70	middle	2
3	1:40	80	middle and high frequency	3

RESULTS AND DISCUSSION

Single factor test with solvent extraction

It can be seen from Figure 1, the absorbance increases with the increase of the ethanol concentration, indicating that the greater the concentration, the better the extraction; while the ethanol concentration was 95%, the absorbance reached 1.047, subsequently, the absorbance was flat and even reduced with further increase of ethanol concentration. Based on the above analysis, the subsequent extraction method of single factor experiments were conducted with 95% ethanol.

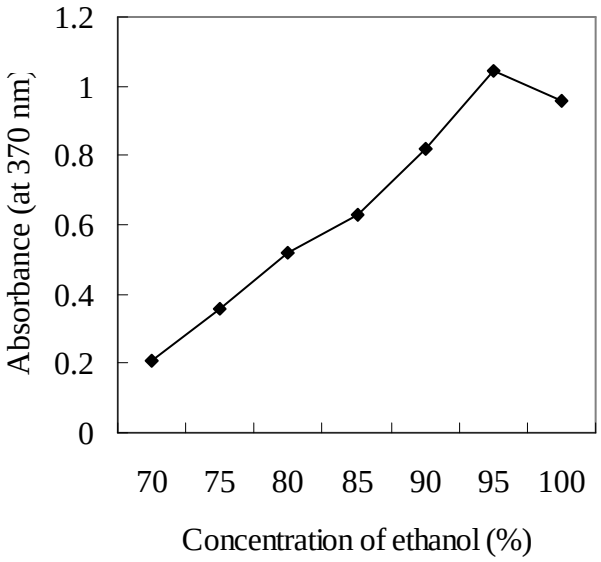

Fig. 1 Effects of ethanol concentrations on absorbance value

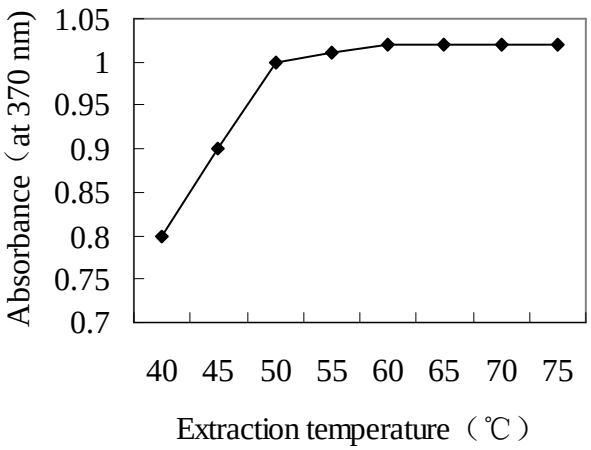

Fig. 2 Effects of temperature of extraction on absorbance value

It can be seen from Figure 2, when temperature was in the range of 40 ℃ to 60 ℃, the absorbance increases with the temperature increases, and after 60 ℃, the absorbance begins to decrease with increasing temperature. The reason may be that, the stability of peel pigment decreased, leading to the increase of rate loss, impacting extraction effects.

It can be seen from Figure 3, when solid-liquid ratio of 1:10 changed to 1:20, the absorbance increased to 1.013, and when the solid-liquid ratio increased to 1:30, 1:40 and 1:50, the absorbance was increasing but more slowly. Considering from the perspective of the environmental and the economic efficiency, the best solid-liquid ratio should be set to 1:20.

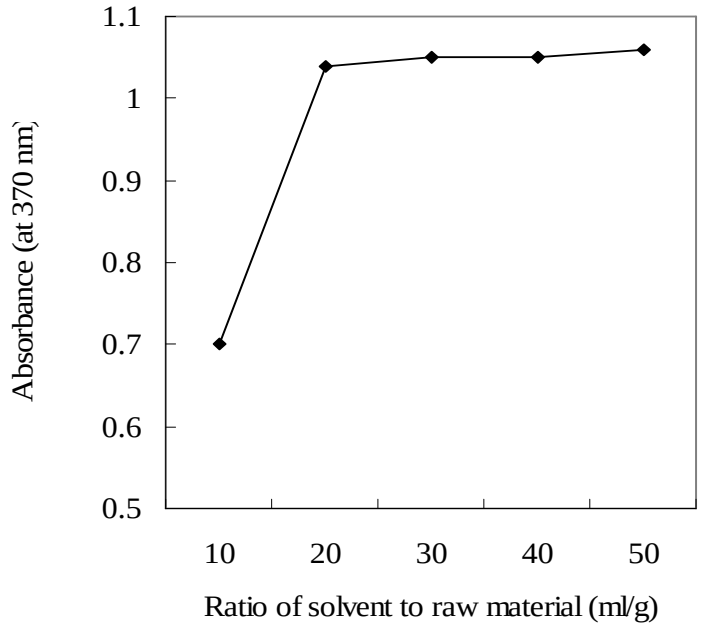

Fig. 3 Effects of ratio of solvent to raw material on the absorbance value

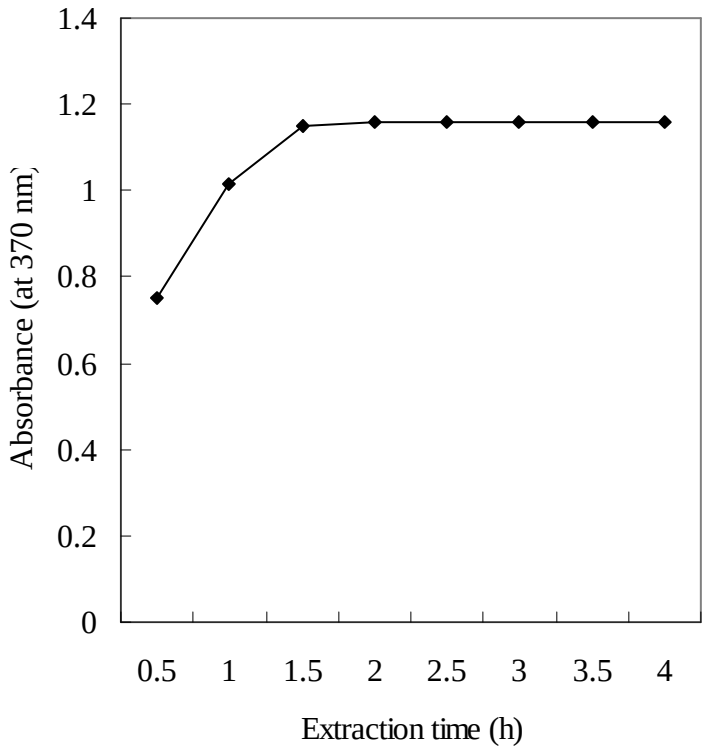

Fig. 4 Effects of extraction time on the absorbance value

Figure 4 showed that, when the extraction time was in the range of 1h ~ 2h, the absorbance had been increased, and when the extraction time was over 2h, the absorbance increased slowly, probably due to solvent had been exhausted extraction or extraction of the pigment had been basically completed after two hours.

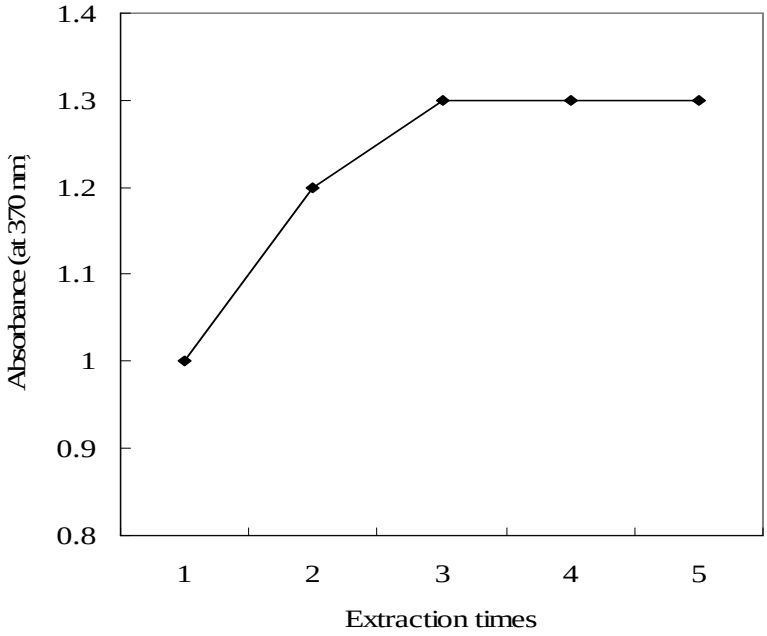

Fig. 5 Effects of extraction times on the absorbance value

Figure 5 showed when extraction times increased to twice and 3 times, there was a significant change in absorbance, and when extraction times increased to 4 times and 5 times, no further increase in absorbance, indicating that extraction was completed at this time.

Orthogonal test with extraction method

Using 95% ethanol as extraction agent, factors and levels were shown in Table 1, results were shown in Table 3.

Table 3 the results of orthogonal experiment

No. of experiment	A	B	C	Absorbance
1	1	1	1	0.871
2	1	2	2	1.101
3	1	3	3	1.214
4	2	1	2	1.119
5	2	2	3	1.3
6	2	3	1	0.911
7	3	1	3	1.297
8	3	2	1	0.901
9	3	3	2	1.12
K_1	1.062	1.096	0.894	
K_2	1.11	1,101	1.113	
K_3	1.106	1.081	1.27	
R	0.048	0.019	0.376	
Major and minor factors	C	A	B	
Optimal level	A_2	B_2	C_3	

It can be seen from Table 3: the order of factors affecting orange peel pigment extraction rate is: extraction times> extraction time> extraction temperature, optimum process was $A_2B_2C_3$, so the extraction time was 1.5h, extraction temperature was 55 ℃ and 3 times for extraction .

Single factor test with microwave-assisted extraction method

It can be seen from Figure 6, the absorbance increases with the increase of the ethanol concentration, indicating that the greater the concentration, the better the extraction; while the ethanol concentration was 95%, the absorbance reached 0.659, subsequently, the absorbance increase slowly with further increase of ethanol concentration. Based on the above analysis, the extraction method of single factor experiments were conducted with 95% ethanol.

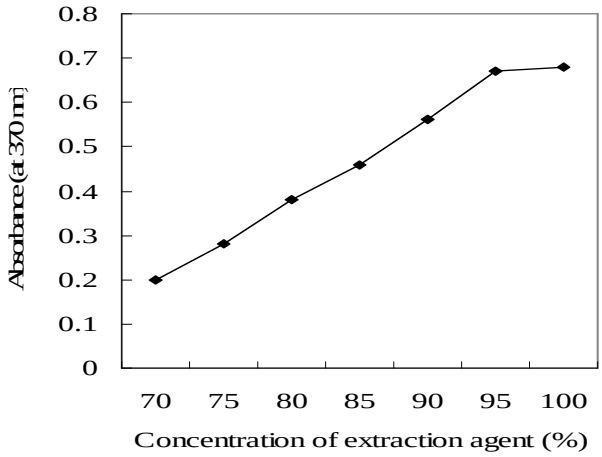

Fig. 6 Effects of ethanol concentrations on the absorbance value

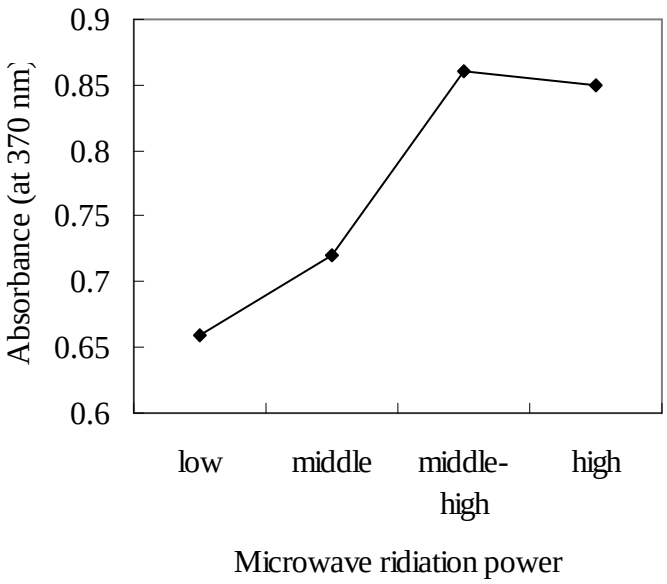

Fig. 7 Effects of microwave radiation power on the absorbance value

It can be seen from Figure 7, along with the increase of microwave radiation power, the absorbance value also increased, while absorbance was no longer increasing and even a slight decreasing with the high-frequency microwave radiation power, it might be that, when microwave irradiation power increased, the temperature rose, increasing the loss rate of pigment.

It can be seen from Figure 8, the absorbance increased as the microwave irradiation time increased, and the absorbance reached 0.980 at 70s, followed by increasing the time of microwave radiation, but there was no increase in absorbance and even decreased, this might be pigment loss rate increased with the increase of irradiation time, and then absorbance decreased.

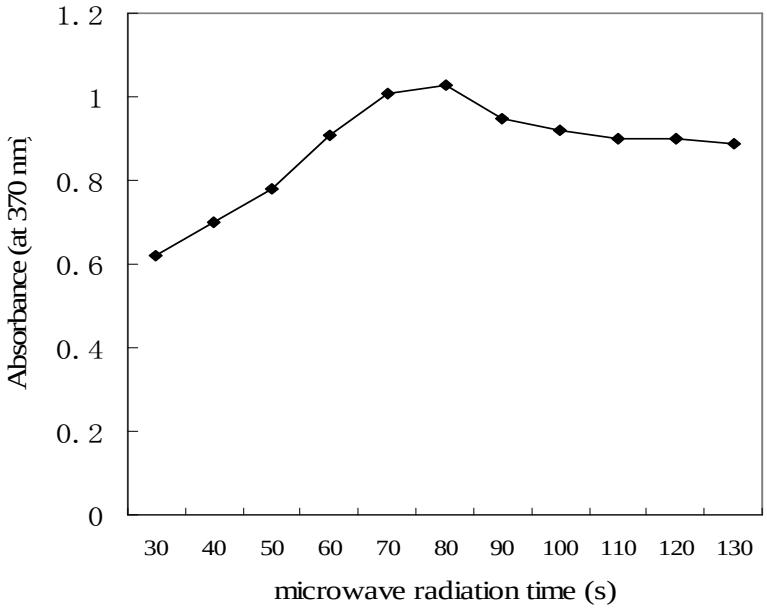

Fig. 8 Effects of microwave radiation time on the absorbance value

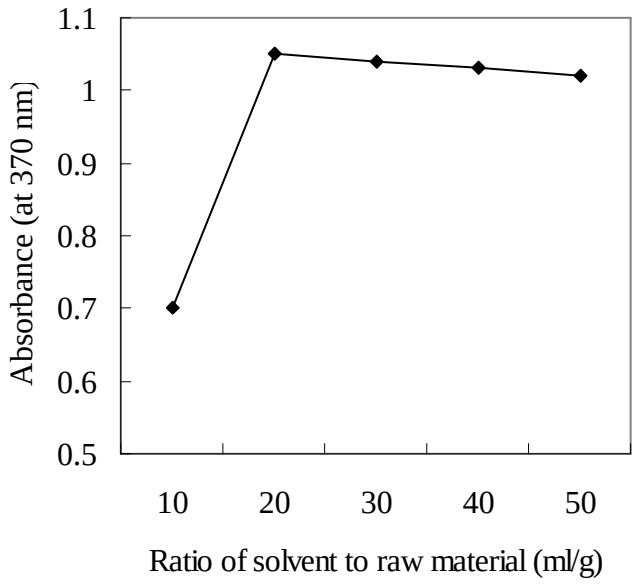

Fig. 9 Effects of ratio of solvent to raw material on the absorbance value

It can be seen from Figure 9, when solid-liquid ratio of 1:10 changed to 1:20, the absorbance increased to 0.978, and when the solid-liquid ratio increased to 1:30, 1:40 and 1:50, the absorbance was changing slowly. Considering from the perspective of the environmental and the economic efficiency, the best solid-liquid ratio should be set to 1:20.

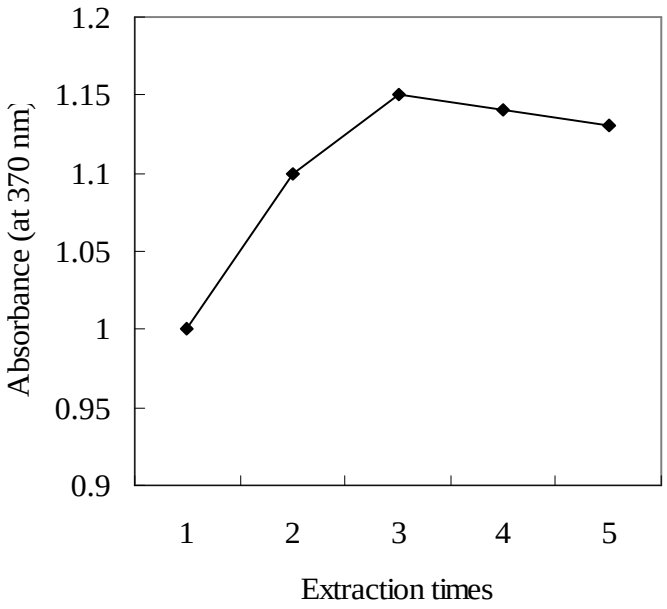

Fig. 10 Effects of extraction times on the absorbance value

Figure 10 showed when extraction times increased to twice and 3 times, there was a significant change in absorbance, and when extraction times increased to 4 times and 5 times, no further increase in absorbance, indicating that extraction was completed at this time.

Orthogonal test with microwave-assisted extraction

Using 95% ethanol as extraction agent, factors and levels were shown in Table 2, results were shown in Table 4.

Table 4 Results of orthogonal experiment

No. of experiment	A	B	C	D	Absorbance
1	1	1	1	1	0.978
2	1	2	2	2	1.053
3	1	3	3	3	1.132
4	2	1	2	3	1.101
5	2	2	3	1	1.001
6	2	3	1	2	1.072
7	3	1	3	2	1.088
8	3	2	1	3	1.117
9	3	3	2	1	1.012
K_1	1.054	1.056	1.056	0.997	
K_2	1.058	1.057	1.055	1.071	
K_3	1.072	1.072	1.073	1.117	
R	0.018	0.016	0.018	0.119	
Major and minor factors	D	C	A	B	
Optimal level	A_3	B_3	C_3	D_3	

It can be seen from Table 4: the order of factors affecting orange peel pigment extraction rate is: extraction times > microwave radiation power, influence of microwave radiation power and solid-liquid ratio on the extraction rate were equivalent, and influence of solid-liquid ratio was a little larger then that of microwave irradiation time. It can also be seen from Table 4 that, optimum process was $A_3B_3C_3D_3$, so the solid-liquid ratio was 1:40, microwave irradiation time was 80s, microwave radiation power was high-frequency, and 3 times for extraction.

CONCLUSION

In this study, based on the results of single factors experiment of ethanol extraction and microwave-assisted ethanol extraction, orthogonal experiments of two extractions, the following conclusions can be drawn:

The optimum process of ethanol extractions were: ethanol concentration was 95%; ratio of raw material to solvent was 1:20 (g/ml); extraction time was 1.5 h; extraction temperature was 55 ℃and extraction times were 3 times.

The optimum process of microwave-assisted ethanol extraction is: ethanol concentration was 95%; the ratio of raw material to solvent was 1:40 (g/ml); microwave irradiation time was 80s; microwave radiation power was medium-high frequency and the extraction times were 3 times.

Both methods were consistent on two parameters, the concentration of extraction agent and the extraction times. The biggest advantage of microwave-assisted extraction method is that the short extraction time, i.e. solvent extraction takes 1.5 hours, while microwave-assisted extraction only takes 80s. Currently, extraction process for active ingredients of natural plant still faces some limitations, apart from low content of natural pigment, the time-consuming, large solvent consumption, low extraction rate are the problems that many scholars generally try to solve, and microwave-assisted extraction method provides a good foundation for the follow-up study in time shortening.

On the other hand, microwave-assisted extraction method takes a large solvent consumption, and from the perspective of environmental and economic point, it possesses small advantages. These data above are only completed in the experiment, and due to certain influences of experimental error and laboratory equipment on the results, further studies should be considered in optimizing experiments.

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