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Full Length Research Paper

Orthogonal test design for optimization of the ultrasonic extraction of naringin from *Citrus grandis* tomentosa

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***Citrus grandis* tomentosa is a premature or maturescent dried pericarp of *C. grandis* tomentosa or *C. grandis* (L.) Osbeck, and it is one of the most important crude drugs in traditional Chinese medicine. In this article, *C. grandis* tomentosa rich in naringin was used as an experimental material. This research, study the effects of ultrasonic extraction temperature, ratio of solvent to raw material, extraction time, and ultrasonic power for the extraction yield of naringin through a single-factor exploration. Then, through an orthogonal experiment ($L_9(3)^4$), it was investigated to get the best extraction conditions. The results showed that the effect of these factors on the extraction yield decreases in the order: extraction time> ultrasonic power> extraction temperature> ratio of solvent to raw material. The highest yield was obtained when temperature, ratio of solvent to raw material, ultrasonic time and ultrasonic power were 40℃, 20:1, 50 min, and 280 W, respectively. the experiment was repeated three times with the selected condition, the average extraction yield of naringin from the *C. grandis* tomentosa was 82.11%. Compared with conventional extraction methods, ultrasonic extraction is time-saving, energy saving, and in the low temperature.**

Key words: Ultrasonic extraction, orthogonal experiment, *Citrus grandis* tomentosa, naringin.

INTRODUCTION

Citrus grandis tomentosa is a premature or maturescent dried pericarp of *C. grandis* tomentosa or *C. grandis* (L.) Osbeck (Chen et al., 2009; Li, 2009), it is one of the most important crude drugs in traditional Chinese medicine, used in getting rid of cold, eliminating wet and apophlegmatic. It is also frequently used as an herbal drug for its reputed medicinal properties, for example, as a remedy for cough and phlegm. Flavones is one of the main effective substances of *C. grandis* tomentosa (Guang et al., 2004; Pang and Yang, 2007), they contain naringin, rhoifolin (Huang and Shen, 1990), poncirin and neohesperidin, etc. Among the flavones, naringin is more than 70% and has significant effect on anti-inflammation, and the index of evaluation (Wei et al., 2005; Sudto et al., 2009; Zhen et al., 2010). In recent years, a large number of articles have been published on the study of the contents of *C. grandis* tomentosa using the conventional extraction technology of decoction with water (Li et al., 2008; Huang and liang, 2005; Pan et al., 2007). From the large amount of concerned literature, it was found that few reports have been published regarding ultrasonic extraction of naringin from *C. grandis* tomentosa. However, ultrasonic extraction is a new and excellent technology for crude drugs, especially for traditional Chinese medicine. The mechanical effect, thermal effect, and cavitation effect are three principles to ultrasonically extract the active substances of plants. The thermal effects of ultrasound can enhance the audio medium temperature and speed up the dissolution of the active ingredients. The main effect of mechanical effects is to appear in second-order phenomena such as diffusive

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pressure. Cavitations effect can make a variety of substances into small particles and formulate emulsions; they can also increase the processing of active ingredients into the solvent and improve the extraction yield (Niu et al., 2007; Huang and Ma 2007). Naringin from *C. grandis* tomentosa was usually extracted with the solvent extraction method, which had the shortcomings of time-consuming, costly, easily damaged the active ingredients, and low extraction yield. So, the aim of this study was to carry out a more exhaustive analysis of the naringin from *C. grandis* tomentosa. In this article, the method of ultrasonic extraction of naringin from *C. grandis* tomentosa was studied. Also, compared with the conventional extraction methods and making reference to creating a new method of short extraction time, high efficiency, low cost, simple process, and easy expansion of production.

MATERIALS AND METHODS

Plant and reagents

C. grandis tomentosa were collected from Guangdong Province of Southern China, which was identified by Traditional Chinese Medicine Department of Institute for Drug Control of Guangzhou city. Naringin reference substance was obtained from National Institute for Control of Pharmaceutical and Biological Products and its batch number was 110722-200309. All other solvents and chemicals were analytically graded and purchased from Tianjin Fu-Yu Chemical Ltd., Co (Tianjin, China).

Apparatus

Spectrophotometer studies were performed on a UV1101 UV-visible spectrophotometer. A LC10ATVP liquid chromatograph was used. The ultrasonic cleaner was used for extraction, and other apparatus were also used for the extraction.

High performance liquid chromatography (HPLC) systems

Separations were carried out with a TSK gel ODS-80TSQA. The mobile phase was a mixture of methanol, acetic acid, and water (35: 4: 61, v/v/v) and was delivered at a flow rate of 1 ml/min. The detection wavelength was set at 283 nm for quantitative determination. The injection volume was 10 μl. Theoretical plate number of naringin peak should be no lower than 5000.

Preparation of reference solution

An amount of 20 mg of dried to constant weight of naringin reference substance was accurately weighed and put into a 100 ml measuring flask, add methanol to the mark, then shake up. Take 3 ml of the solution accurately to a 100 ml measuring flask, add methanol to the mark, shake up, and the reference solution was obtained.

Preparation of sample solution

Accurately measured 10 ml of the extract was poured into 100 ml measuring flask, and was allowed to condense to a constant weight, and methanol was added up to the mark, and was shake up. 5 ml of the solution accurately taken and was poured into a 25 ml measuring flask, and methanol was add to the mark, shake up, and the sample solution was obtained.

Determination of sample solution

Accurately measured 1 ml of the naringin sample was added to a 25 ml measuring flask, and methanol was added to the mark, and was shaken up. Then, another 6 ml of the sample was accurately taken to a 10 ml measuring flask, add methanol to the mark, shake up, and the sample solution was obtained. Accurately taken reference solutions of 10 μl, respectively were injected into liquid chromatograph, testing it at 283 nm and the content of naringin was calculated. The chromatogram is as shown in Figure 1.

Optimization of naringin ultrasonic extraction

An orthogonal $L_9(3)^4$ test design in the ultrasonic extraction mode was used for optimization of the extraction conditions. In the single-factor test, according to experimental methods, relative literatures were used to inspect single factor effect. Nine extractions were carried out at the ratios of solvent to raw material 10:1, 15:1, and 20:1. Extraction time of 30, 40, and 50 min, temperature of extractions 30, 40, and 50°C, ultrasonic power 240, 280, and 320 W were used on the basis of the single-factor test. Table 1 shows the experimental conditions for the extraction of naringin from *C. grandis* tomentosa.

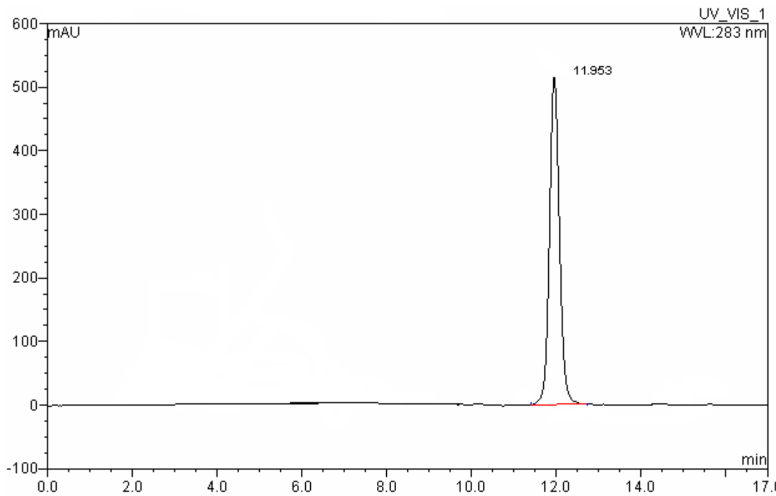
RESULTS AND DISCUSSION

Effect of temperature on extraction yield of naringin from *C. grandis* Tomentosa

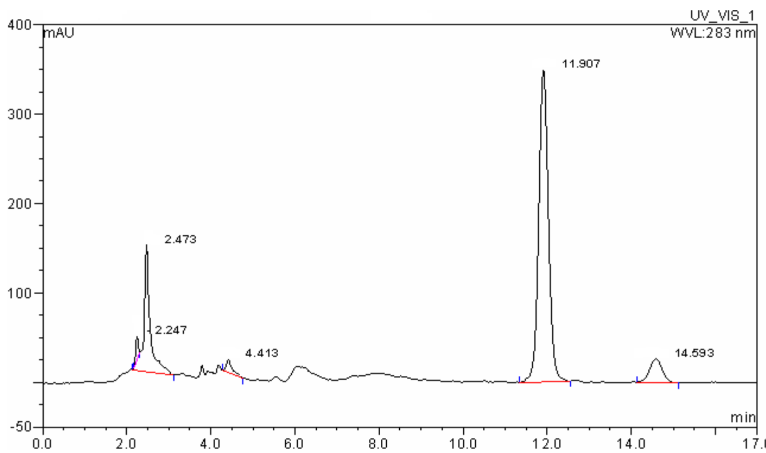
In this work, the effect of temperature on extraction yield of naringin from *C. grandis* tomentosa was investigated, and the results are as shown in Figure 2. First, the other extraction conditions of naringin from *C. grandis* tomentosa, that is, ratio of solvent to raw material, extraction time, and ultrasonic power were fixed at 1:15, 40 min, and 280 W, respectively, and the temperature was slightly changed. As shown in Figure 2, the extraction yield of naringin from *C. grandis* tomentosa increased from 57.43 to 82.21% significantly with temperature increase from 30 to 50°C. However, the temperature reached 60°C, the extraction yield of naringin from *C. grandis* tomentosa was simply raised up to 83.55%. In order to keep the active ingredients of the plant, the experimental temperature was no longer raised.

Effect of ratio of solvent to raw material on extraction yield of naringin from *C. grandis* Tomentosa

Fix the other extraction conditions of naringin from *C. grandis* tomentosa, that is, extraction temperature, extraction time, and ultrasonic power at 50°C, 40 min, and



A



B

Figure 1. HPLC (A) reference solution and (B) sample solution.

Table 1. Factors and levels of the orthogonal analysis.

Level	A	B	C	D
	Extraction temperature (℃)	Ratio of solvent to raw material	Extraction time (min)	Ultrasonic power (W)
1	30	10:1	30	240
2	40	15:1	40	280
3	50	20:1	50	320

and 280 W, respectively, and the ratio of solvent to raw material was 5:1, 10:1, 15:1, and 20:1. The effect of ratio of solvent to raw material on extraction yield of naringin from *C. grandis* tomentosa was investigated, and the results are as shown in Figure 3. As shown in Figure 3, the extraction yield of naringin from *C. grandis* tomentosa significantly increased from 70.59 to 82.34% with the ratio of solvent to raw material increased from 5:1 to 15:1. However, the ratio of solvent to raw material reached 15:1, the extraction yield of naringin from *C. grandis* tomentosa was simply raised up to 83.37.

Effect of ratio of extraction time on extraction yield of naringin from *C. grandis* Tomentosa

Fix the other extraction conditions of naringin from *C. grandis* tomentosa, that is, extraction temperature, ratio of solvent to raw material, and ultrasonic power at 50℃, 15:1, and 280 W, respectively, and the extraction time was

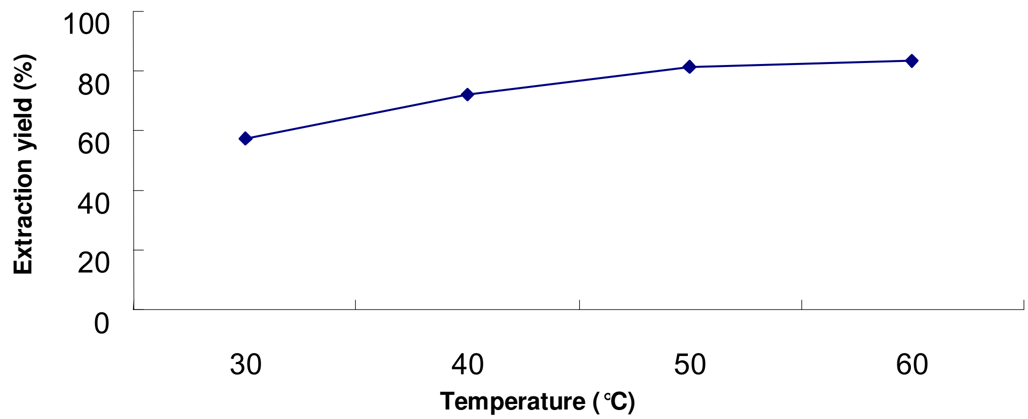


Figure 2. Effect of temperature on extraction yield.

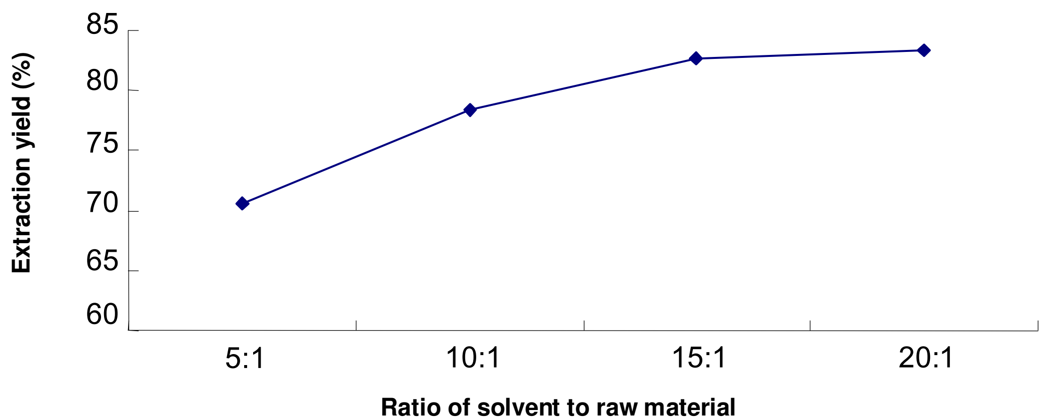


Figure 3. Effect of ratio of solvent to raw material.

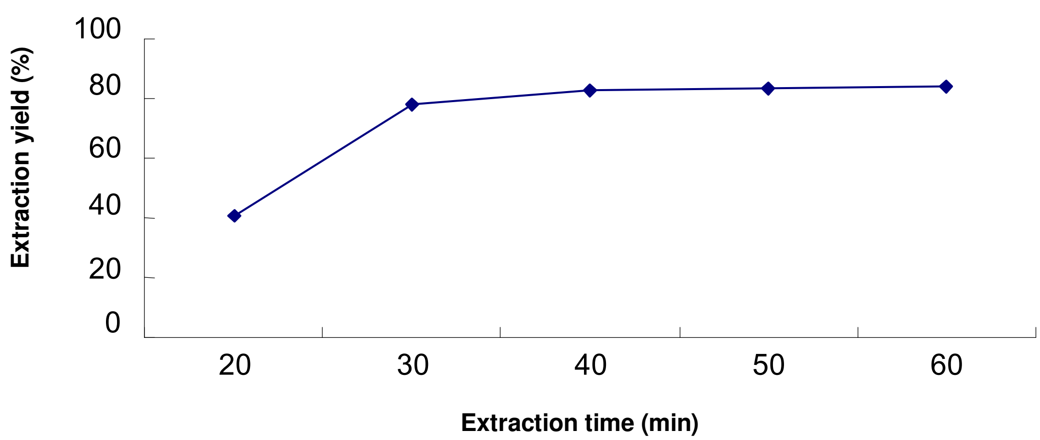


Figure 4. Effect of extraction time on extraction yield.

20, 30, 40, 50, and 60 min. The effect of extraction time on extraction yield of naringin from *C. grandis* tomentosa was investigated, and the results are listed in Figure 4. The extraction yield of naringin from *C. grandis* tomentosa significantly increased from 40.77 to 82.38% with the extraction time increasing from 20 to 40 min as shown in Figure 4. However, there was no significant change on extraction yield of naringin from *C. grandis*

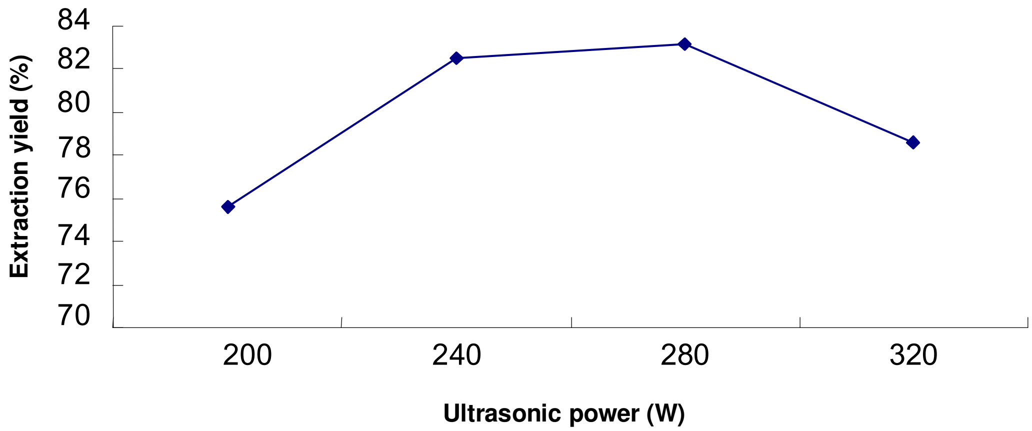


Figure 5. Effect of ultrasonic power on extraction yield.

tomentosa when the extraction time increased to 50 and 60 min.

Effect of ratio of ultrasonic power on extraction yield of naringin from *C. grandis* Tomentosa

Fixing the other extraction conditions of naringin from *C. grandis* tomentosa, that is, extraction temperature, ratio of solvent to raw material, and extraction time at 50℃, 15:1, and 40 min, respectively, and the ultrasonic power was 200, 240, 280, and 320. The effect of ultrasonic power on extraction yield of naringin from *C. grandis* tomentosa was investigated, and the results are listed in Figure 5. The extraction yield of naringin from *C. grandis* tomentosa increased from 75.62 to 83.12% significantly with the ultrasonic power increasing from 200 to 280 W as shown in Figure 5. However, extraction yield of naringin from *C. grandis* tomentosa was not increased, but it decreased when the ultrasonic power increased to 280 W.

Optimization of the ultrasonic extraction parameters of naringin from *C. grandis* Tomentosa

The first step in the extraction procedure of naringin from *C. grandis* tomentosa is to optimize the operating conditions to obtain an efficient extraction of the target compounds and avoid the co-extraction of the undesired compounds. Since various parameters potentially affect the extraction process, the optimization of the experimental conditions is a critical step in the development of a solvent extraction method. In fact, extraction temperature, ratio of solvent to raw material, extraction time and ultrasonic power are generally considered to be the most important factors. Optimization of the suitable extraction conditions in the naringin extraction can be carried out by using an experimental design. In the present study, all the selected factors were examined using an orthogonal $L_9(3)^4$ test design. The total evaluation index was analyzed by statistical method. The results of orthogonal test and extreme difference analysis are shown in Tables 2 and 3. The analysis of variance was performed by statistical software SPSS 12.0 (SPSS Inc.) and the result is listed in Table 3.

As shown in Table 2, it can be found that the effect of factors on the extraction yields of naringin decreases in the order: C> D> A> B according to the R values. The extraction time was found to be the most important determinant of the yield, while ratio of solvent to raw material least. According to the outcomes and analyses, the optimized extraction methods was C_3D_2 A_2B_2, namely, when temperature, ratio of solvent to raw material, ultrasonic time, and ultrasonic power were 40℃, 15:1, 50 min, and 280 W, respectively, the extraction yield was the highest.

Table 3 showed that the extraction time had a noticeable effect on the extraction yield of naringin from *C. grandis* tomentosa. It demonstrated that the longer the extraction time, the better the extraction effect, while the ratio of solvent to raw material had a least effect on the extraction yield.

Verification test

C. grandis tomentosa powder was extracted with a ratio of solvent to raw material of 15:1 of the optimized condition on an ultrasonic machine of 280 W for 50 min. When the experiment was repeated three times, the extraction yield of naringin from *C. grandis* tomentosa is shown in Table 4. Thus, the selected method can be used for the extraction of naringin from *C. grandis* tomentosa. The method proved to be of short extraction time, high efficiency, low cost, simple process, and easy expansion of production.

Table 2. Analysis of $L_9(3)^4$ orthogonal experiments results.

Number	A	B	C	D	Extraction yield (%)
1	1	1	1	1	45.20
2	1	2	2	2	59.35
3	1	3	3	3	83.45
4	2	1	2	3	62.24
5	2	2	3	1	81.33
6	2	3	1	2	65.35
7	3	1	3	2	82.87
8	3	2	1	3	58.69
9	3	3	2	1	49.65
K_1	62.67	63.44	56.41	58.73	-
K_2	69.64	66.46	57.08	69.19	-
K_3	63.74	66.15	82.55	68.13	-
R	6.97	3.02	26.14	10.46	-

Table 3. Variance analysis results.

Origin of variance	SS	V	Ratio F	Threshod F	P
A	84.62	2	5.105	19.00	-
B	16.58	2	1.000	19.00	-
C	1332.29	2	80.370	19.00	-
D	198.97	2	12.003	19.00	-
Blank deviation	16.58	2	-	-	-

Table 4. Results of every batch of extraction yields.

Extraction time (min)	Extraction temperature (℃)	Ratio of solvent to raw material	Ultrasonic power (W)	Extraction yield (%)	Average (%)
50	40	15:1	280	82.11	-
50	40	15:1	280	83.12	-
50	40	15:1	280	81.27	-
50	40	15:1	280	82.11	-
50	40	15:1	280	83.33	82.25
50	40	15:1	280	81.31	-
50	40	15:1	280	82.34	-
50	40	15:1	280	82.15	-
50	40	15:1	280	82.51	-

Comparison of ultrasound extraction and conventional method

Table 5 shows the comparison of ultrasound extraction and conventional extraction method. Comparing with the boiling extracting method, ultrasound extraction had the advantages of short extraction time, low extraction temperature, small volume of solvent, high extraction rate, etc.

Conclusion

Naringin is one of the active constituents in *C. grandis* tomentosa, and its content is the evaluation index of *C. grandis* tomentosa in Chinese pharmacopeia. So, naringin was selected as the index of *C. grandis* tomentosa for optimizing ultrasonic extraction method in this study. How it has affected phlegm is a further study. By comprehensive assessment of extraction time, ultrasonic power,

Table 5. Comparison of ultrasound extraction and conventional extraction method.

Factor	Ultrasound extraction	Decoction method
Time (min)	50	90
Ratio of solvent to raw material	15:1	40:1
Temperature (℃)	40	100°
Extraction yield (%)	82.11	65.23

extraction temperature, and ratio of solvent to raw material, the effects of these factors on the extraction yields decrease in the order: C> D> A> B. It was shown that extraction time affected the extraction most and ratio of solvent to raw material least. According to the outcomes and analyses, the optimized extraction methods was C_3D_2 A_2B_2, namely, when temperature, ratio of solvent to raw material, ultrasonic time, and ultrasonic power were 40℃, 15:1, 50 min, and 280 W, respectively, extraction yield was the highest. When the experiment was repeated three times under the same condition, the average extraction yield of naringin from *C. grandis* tomentosa was over 80%. The method was proven to be of short extraction time, high efficiency, low cost, simple process, and easy expansion of production. Meanwhile, the contents of naringin were determined by HPLC. Besides, from the large amount of concerned literature, it was found that few reports have been published regarding the naringin from *C. grandis*. However, this article has done the preliminary research of the naringin from *C. grandis* tomentosa. So, it can be used as a reference to do further research on the naringin from *C. grandis* tomentosa for other research works.

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