

Full Length Research Paper

Study on the extraction of dioscin by the ultrasonic-assisted ethanol

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With *Dioscorea zingiberensis* as raw materials, and with the yield of diosgenin as assessment criteria, the effect on extraction yield of dioscin of frequency of ultrasonic, the period of ultrasonic and solid-liquid ratio (*D. zingiberensis* : alcohol) was studied via orthogonal test. A new and unique method to accomplish this was by utilizing the technology of ultrasonic assisted ethanol extraction. The optimal processing parameters of this method were confirmed. The method was compared with solvent extraction process for the effect on extraction yield of dioscin. It was shown that the technology of ultrasonic assisted ethanol extraction which can significantly increase the extraction yield and extraction efficiency of dioscin. The ultrasonic did not destroy *D. zingiberensis* cell structure, but decreased the boundary layer thickness between *D. zingiberensis* (solid phase) and alcohol (medium), and accelerated cells inside and outside the material exchange. International rectifier (IR) further demonstrated that ultrasonic merely increased extraction yield of dioscin instead of destroying the cell structure.

Key words: *D. zingiberensis*, ultrasonic waves, extraction, diosgenin.

INTRODUCTION

Dioscin, a steroid compound that is formed when diosgenin and glucides connect through β -1,3 glycosidic bond can be found mainly in the root of *Dioscorea zingiberensis* C.H. Wright, *Dioscorea nipponica* Makino, *Dioscorea panthaica* prain et Burkil and *D. nipponica* Makinovar rosthani prain et Burk (Yang et al., 2003). The hydrolysate of dioscin-diosgenin is a very important fundamental material to make steroidal hormonal drugs. Steroidal hormone has effective pharmacological capabilities such as resisting infection, hypersusceptibility, viruses and shock. Thus, it is a drug that can be used to cure rheumatism, cardiovascular disease, lymphocytic leukemia, cellularity encephalitis, and dermatosis; it is

also an important anti-tumor drug and an important drug (Evans, 2002; Hu and Yao, 2002) to salvage patients at critical stages.

A traditional way of producing diosgenin was through direct acid hydrolysis. That is to say that the *D. zingiberensis* was pestled firstly, then hydrolyzed with acid, filtered, dried, and lastly extracted with petroleum ether. There are two biggest disadvantages of this process. One is usage of large amount of acid, which leads to serious pollution. Another is the process of acid hydrolysis; the starch in the *D. zingiberensis* is converted into reducing sugar, whereas it is hard to recycle, thus results in resource waste and increased the difficulty

Table 1. The orthogonal experiment's factors and Levels.

Level	Solid-liquid ratio	Extraction time/min	Frequency of the ultrasonic/KHz	Volume fraction of the alcohol/v/v
Level 1	1:8	20	14.52	65
Level 2	1:10	30	25.80	75
Level 3	1:12	40	35.74	85

of treatment of the acid liquor (Link, 2006). To reduce the quality of acid in the process of producing diosgenine, Xiang et al. (2008) adopted mechanical separation technology; separated starch and cellulose in *D. zingiberensis*, hydrolyzed turbid liquid (which contains mainly dioscin), effectively reduced the amount of acid used, but the whole process had excessive water use and pollution problem still was not solved. Chen et al. (2007) adopted alcohol fermentation, hoping to convert starch in the *D. zingiberensis* into alcohol, but because of dioscin bacteriostasis, it resulted in low alcoholic strength in the fermentation liquid (6.5 v/v).

On the other hand, steroids of the diosgenine might be broken in the process of alcohol fermentation, which results in low purity of the diosgenine. Ultrasonic assisted ethanol extraction was used in this paper, to extract dioscin from *D. zingiberensis* (Which contains 2 to 3% of diosgenin); hydrolyzed dioscin with acid, washed residues, then dried and diosgenin was gotten from it. Yeast was added into *D. zingiberensis* which was extracted for further fermentation. Also, this method reduced the amount of acid which is used in the process of hydrolysis to get diosgenin from dioscin as well as reduced the pollution of this process. This method made full use of starch resource in the *D. zingiberensis*.

MATERIALS AND METHODS

D. zingiberensis was provided by An Kang Institute of *D. zingiberensis*, and appraised by Professor Hu Zhenghai from Northwest University. Petroleum ether (boiling point 60-90°C), hydrochloric acid, and ethanol were purchased from Xi'an Chemical Reagent Company. A multi-frequency sonochemical reactor (SC-III) from Jiu Zhou Mechanical and Engineering Research Center, XT5 Microscope Melting Point Inspect from Shanghai Laboratory Instrument Works Co., Ltd.; FTIR Spectrum (VECTOR-22) from the German BRUKER Company; Soxhlet Extractor from Chongqing Beibang Glass Instrument Factory and Scanning Electron Microscope (CS3400), from Beijing Elaborate Technology Development Ltd were used for the study.

To simplify the experiment, dioscin was replaced by solid matter in the filter liquid in this paper; it is not accurate, but it can reflect *D. zingiberensis* nature to some extent. Generally speaking, the more content of solid matter in the extract liquid, the more amount of extraction of the dioscin. 60-mesh *D. zingiberensis* of 100 g was collected, treated with ultrasonic assisted ethanol to get the filter liquor, 1 ml of it was collected into the tube, then dried under 105°C for 2 h to get its quality. The quality was calculated according to the formula:

The amount of the solid matter in the extract liquor = the volume of

the extract liquor (ml) × the quality of the 1 ml filter liquor (g/ml);

The content of the solid matter in the extract liquor = the solid matter in the extract liquor (g) / the content of the *D. zingiberensis* (100).

The filter liquor (100 *D. zingiberensis*) was vacuum concentrated which was treated with ultrasonic extraction to a paste (about 10 g), 30 ml 1.5 mol/L sulfuric acid was added, then hydrolyzed under 108°C for 4 h, the residue was washed to neutral after filtering, dried, extracted with petroleum ether, crystallized to get the diosgenin, and dried until constant weight. The yield of the diosgenin was calculated according to formula α:

Yield of the diosgenin = the content of the diosgenin (g) / the content of the *D. zingiberensis* × 100%... α

The hydrolysis product of the dioscin is diosgenin, the extract yield of the dioscin and the yield of the diosgenin had linear correlation. For convenience, the two concepts may probably change with each other in this paper.

RESULTS AND DISCUSSION

The optimization of the conditions of the ultrasonic assisted ethanol extraction

On the basis of single factor experiments, the orthogonal experiment was applied to optimize the extraction. 9 shares of the 60-mesh *D. zingiberensis* (100 g) was collected, then volume fraction of 65, 75, and 85 v/v alcohol, was added respectively, according to the solid-liquid ratio of 1:8, 1:10, 1:12. Under the frequency of the ultrasonic of 14.52, 25.80, and 35.74 KHz, ultrasonic extraction, time were respectively 20, 30 and 40 min. Lastly, it was filtered to get filtrate, the filtrate was vacuum dried until paste was obtained, hydrolyzed for 4 h under 108°C after adding 30 ml 1.5 mol/L sulfuric acid, filtered and the residue was washed until neutral, which was dried, extracted with petroleum ether and crystallized to get diosgenin which was dried to constant weight. Every experiment was repeated for 3 times, and average yield of the diosgenin was calculated. The experimental arrangement is shown in Table 1, and the experimental results are shown in Table 2. Influencing factors of the yield of diosgenin are alcohol volume fraction, ultrasonic extraction time, solid-liquid ratio, and frequency of the ultrasonic. The optimal extraction condition was solid-liquid ratio of 1:10, alcohol volume fraction of 65%, extraction time of 30 min, and frequency of the ultrasonic of 25.80 KHz. Dioscin was extracted under this condition,

Table 2. The result of the orthogonal experiment.

Parameter	Solid-liquid ratio	Extraction time (min)	Frequency (k)	Volume fraction of ethanol %	Yield of diosgenin/%
1	1:12	20	14.52	65	1.267
2	1:12	30	25.80	75	1.789
3	1:12	40	35.74	85	1.142
4	1:10	20	25.80	85	1.204
5	1:10	30	35.74	65	2.062
6	1:10	40	14.52	75	1.828
7	1: 8	20	35.74	75	1.312
8	1: 8	30	14.52	85	1.205
9	1: 8	40	25.80	65	1.756
k1	1.40	1.26	1.43	1.70	/
k2	1.70	1.69	1.58	1.64	/
k3	1.42	1.58	1.51	1.18	/
R	0.30	0.43	0.15	0.52	/

Table 3. The effect of extraction methods on extraction yield of dioscin and diosgenin yield.

Parameter	The content of the solid matter/%				The yield of the diosgenin/%			
	1th	2th	3th	Total	1th	2th	3th	Total
Ultrasonic-assisted extraction	12.255	3.428	1.844	17.527	1.8445	0.8415	0.4485	3.1345
Ethanol extraction	9.474	0.37	0.17	10.014	1.3621	0.5650	0.4952	2.4223
Acid hydrolysis	/	/	/	/	/	/	/	2.7765

hydrolyzed and dried to get the diosgenin, then the experiment was repeated for three times; the mean yield of the diosgenin was 2.081%.

Comparison of the method of ultrasonic assisted ethanol extraction with that of solvent extraction

The effect of extraction methods on extraction yield of dioscin and diosgenin yield

60-mesh *D. zingiberensis* (100 g) was collected, volume fraction of 65 v/v alcohol 1000 ml was added, which was extracted at 30 min at the frequency of 25.80 KHz, and the content of the solid matter and diosgenin yield was calculated. The first, second and third extraction of the residue over diosgenin, was collected the above operation was repeated; the second, third and fourth content of solid matter in the extract and the yield of diosgenin was calculated; each experiment was repeated for three times. 60-mesh *D. zingiberensis* (100 g) was collected, 1.5 mol/L 300 ml sulfuric acid was added, hydrolyzed at 4 h at 108°C, filtered, the residue was washed until neutral, was dried, and extracted with petroleum ether to get diosgenin.

The results are shown in Table 3. Table 3 shows that it

is clear that the method of ultrasonic assisted ethanol extraction can obtain a higher extraction yield of dioscin and diosgenin yield than that of the solvent extraction. Extraction yield of dioscin with ultrasonic assisted ethanol extraction for 30 min was 2.781% higher than that with solvent extraction for 30 days. Thus, ultrasonic assisted ethanol extraction not only increases the extraction yield of the dioscin and diosgenin yield but also increase the extract efficiency of the dioscin.

The diosgenin yield with ultrasonic assisted ethanol extraction (3.1345%) was 0.7122%; higher than that of solvent extraction (2.4223%), and was 0.358% higher than that of direct acid hydrolyze (2.7765%).

The effect of extract methods on morphological structure of *D. zingiberensis*

D. zingiberensis was collected which had been extracted before, then extracted for 3 times with ultrasonic assisted ethanol extraction and solvent extraction, respectively which was vacuum dried for 24 h at 50°C. Morphology structure was analysed with SEM and the results are shown in Figure 1. Figure 1 shows that the cytoarchitecture of the *D. zingiberensis* before and after the ultrasonic extraction did not change much, and the cells

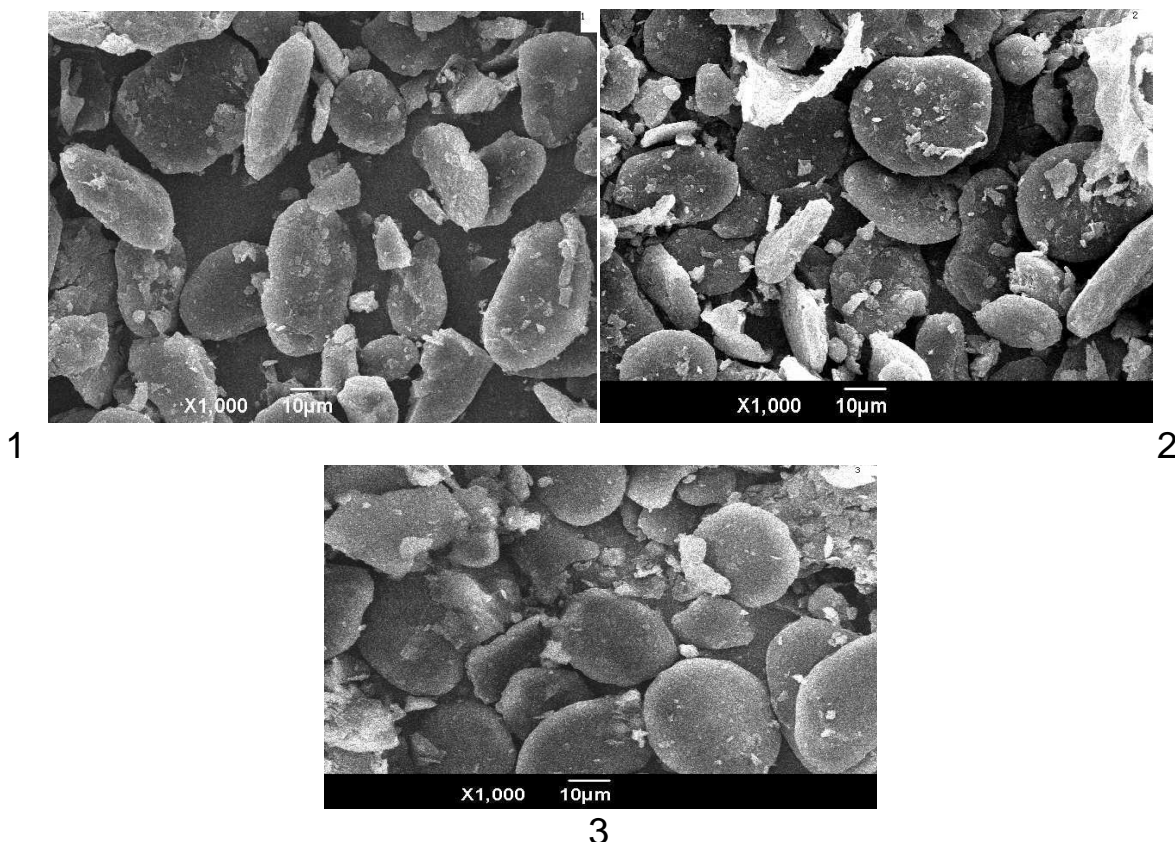


Figure 1. The effect of extract methods on morphological structure of *D. zingiberensis*. 1, Ultrasonic assists ethanol extraction; 2, solvent extraction; 3, *D. zingiberensis* without any treatment.

were integrate, and the edges were trim, which means that the ultrasonic does not break the cytoarchitecture during the extraction process, and this result does not agree with the findings from the former experiments (Hromadkova et al., 2002). Therefore, it can be conferred that the principle for ultrasonic to assist the solvent extraction is that the ultrasonic field through the ultrasonic oscillation, ultrasonic cavitation and cavitation will effectively intensifies the liquid to perfuse outside the membrane, within the pores and a quickens surface diffusion, and all of these will bring a reduction of the boundary layer between the cells and the solvent, so that the velocity of the medium is increased.

Properties identify of diosgenin

The collected diosgenin which was obtained from the method of ultrasonic assisted ethanol extraction (10 g), was recrystallized, and the measured melting point was 203 to 207°C. 99% crystalline purity of diosgenin melting was 204 to 207°C. The collected diosgenin was obtained from recrystallization (0.2 g), the right amount of potassium bromide was added, ground, and then measured with IR; the results are shown in Figure 2.

Figure 2 shows that the sample in 1237, 1050 cm^{-1} ($\text{C}_3\text{-OH}$ and $\Delta^{3,5}$, 978, 917, 896 and 860 cm^{-1} (25 spironoalkyl) all made an appearance, and this is the same (Zhang and Wu, 2007) as that of the diosgenin. All of the above proves that the diosgenin obtained when ethanol is used as the assistance had a comparatively higher purity.

Conclusion

The technology of ultrasonic assisted ethanol extraction can significantly increase the extraction yield and extraction efficiency of dioscin. The ultrasonic did not destroy *D. zingiberensis* cell structure, but could decrease the boundary layer thickness between *D. zingiberensis* (solid phase) and alcohol (medium), and could accelerate cells inside and outside the material exchange. Dioscin, which is the hydrolyzate of *D. zingiberensis* not only is the main raw material of three synthetic hormones, but also is the essential treatment drugs of cardiovascular and cerebrovascular diseases. *D. zingiberensis* contains only 2 to 3% dioscin. In the extraction process, though a number of polar impurities (starch and protein) were extracted, the extraction volume

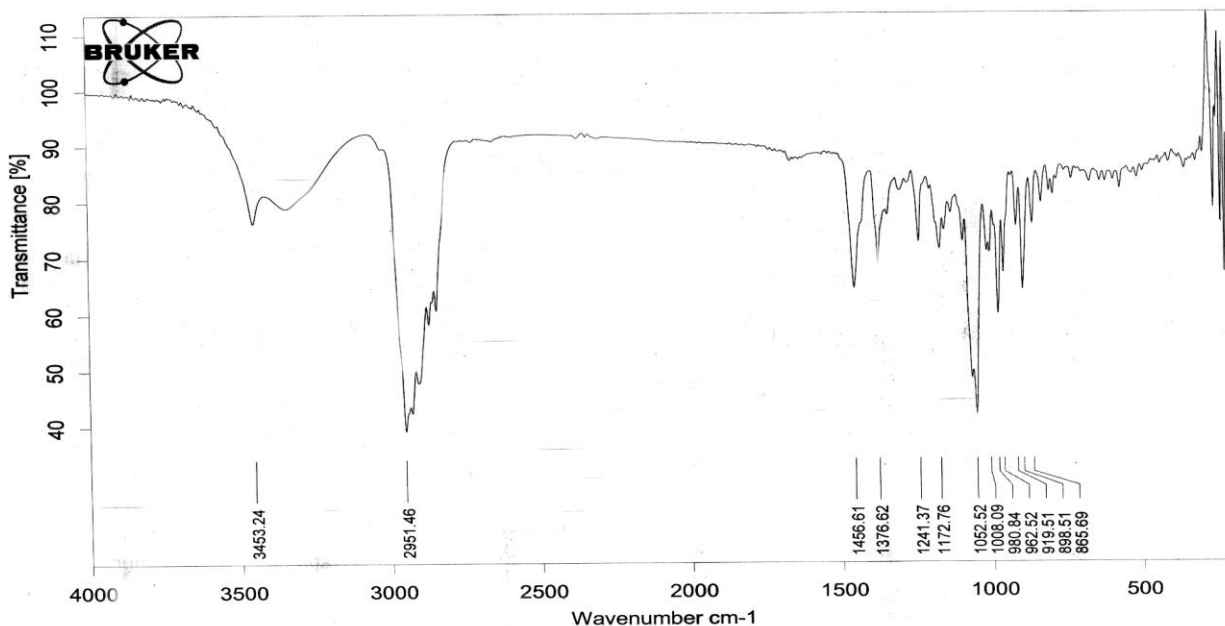


Figure 2. The IR of diosgenin.

(solid matter) was also less than 10%, used acid hydrolysis and acid consumption was also 10% of direct acid hydrolysis. To a certain extent, this method reduced pollution and achieves a clean production of *D. zingiberensis*.

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