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Full Length Research Paper

A study on the ultrasonic-assisted ethanol extraction of dioscin

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In order to solve the pollution problem in the production of diosgenin. Adopting ultrasonic-assisted ethanol extraction technology to study the effects that different ultrasonic frequencies, various volume fractions of ethanol, different extraction durations and extraction times and various solid-liquid ratios have on the dioscin and its hydrolysant in the extraction liquid. What is more of a test on the melting point, as well as on an IR test will be carried out. In addition, a SEM observation of the cytoarchitecture of *Dioscorea zingiberensis* before and after the extraction will also be completed. The best condition for the ultrasonic-assisted ethanol extraction of dioscin is when the ultrasonic frequency is 35.74 KHz, solid-liquid ratio (*D. zingiberensis*: ethanol) 1:10, ethanol volume fraction is 75%, and when it is extracted for 30 min and for 3 times; under these conditions the hydrolysant will be the purest, the acceleration of the extraction is because the boundary layer between the cells and solvent is thinned by ultrasonic, which in turn speeds up the exchange inside and outside the cells.

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

INTRODUCITON

Dioscin, a steroid compound (Yang et al., 2003) 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 and Burkil and *D. nipponica* Makinovar rosthani prain and Burk (Yang et al., 2003). The hydrolysant 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 anit-tumor drug and an important drug (Evans, 2002; Aradhana et al., 1992; Hu and Yao, 2002; Moalic et al., 2001) to salvage patients at critical stages. The pollution problem brought along by the traditional method using acid has become the bottle-neck for the development of the diosgenin production and has limited the development (Link, 2006) of the industry. Recently, though separation technology (Han et al., 2007) and Pretreatment technology using ethanol fermentation (Chen, 2007) are successful in decreasing pollution to a certain degree, the clean production of diosgenin still has not been achieved.

The main reason is that the above two technologies both used acid to hydrolyze. This study will use ultrasonic as assistant to the solvent to extract, macroporous resin to separate and purify the dioscin and the enzymolysis of the dioscin is used to solve the pollution problem completely. Using ultrasonic as the assistant to the ethanol for the extraction of dioscin will be the center of this study and is also the premise to realize the clean production of diosgenin. This article will adopt non-agent and orthogonal experiment methods to study the effects the ultrasonic frequency, ultrasonic duration, solid-liquid ratio and extraction times have on the extraction of dioscin and its hydrolysant (diosgenin), so the best condition to use ultrasonic as the assistant to the ethanol to extract diosgenin can be selected. Also a study on the

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melting point and an IR test of the hydrolysant will be carried out, which shows that the substance is highly pure diosgenin and the SEM result shows that ultrasonic wave will not destroy the cytoarchitecture of *D. zingiberensis*. It can be deducted that the reason why ultrasonic wave accelerate the extraction of the usable components of the plants is because it can thin the boundary layer between the cells and the solvent and speeds up the exchange between the substances inside and outside the cells.

MATERIALS AND METHODS

D. zingiberensis, Provided by An Kang Institute of *D. zingiberensis*, appraised by Professor Hu Zhenghai from Northwest University; Petroleum ether (boiling point 60-90℃), hydrochloric acid, ethanol are purchased from Xi'an Chemical Reagent Company. A Multi-frequency Sonochemical Reactor (SC-Ⅲ), Jiu Zhou Mechanical and Engineering Research Center, XT5 Microscope Melting Point Inspect, Shanghai Laboratory Instrument Works Co., Ltd. FTIR Spectrum (VECTOR-22) from the German BRUKER Company; Soxhlet Extractor, Chongqing Beibang Glass Instrument Factory; BS224S elctronic balance, Beijing Sartorius Mechotronic Co., Ltd. R206D Rotary Evaporator, Shanghai SENCO Instrument Co., Ltd. Glass Instrument air dryer, Zhenzhou Dufu Instrument Factory, Scanning Electron Microscope (CS3400), Scanning Electron Acoustic Microscope, Beijing Elaborate Technology Development Ltd.

Measurement method

Dioscin: The goal of employing extraction method using solvents is to obtain dioscin from *D. zingiberensis*, but because during the extraction process the dissoluble components inside the *D. zingiberensis* will also be extracted, so in order to be accurate this article will use the solid instead of dioscin as the standard to evaluate the extraction results. Take *D. zingiberensis* with a granularity of 60, first make sure of a certain solid-liquid ratio (*D. zingiberensis*: solvent), a stated ultrasonic frequency and ethanol volume fraction, then extract for a certain time, vacuum filter, record the volume of the filtrate. After this go through the following procedure: take 1 ml of the filtrate in a weighing utensil, and dry in the temperature between 105 ～ 110℃ till the weight is constant, and compute the quantity of the solid substance inside the 1 ml filtrate, work out the solid quantity inside the filtrate according to the following equation:

Solid (g) =the volume of the extraction liquid (ml) x the solid contentment in 1 ml of the extraction liquid (g)

D. zingiberensis: first vacuum condense the filtrate, reclaim the ethanol and put in 300 ml of 4mol/L hydrochloric acid 300 ml; then hydrolyze in 110℃ for 4 h, filter and elute the residue with deionized water till it is neutral and dry and filter the sediment in 105 ～ 110℃; the next thing to do is to put it into the soxhlet extractor after it is bagged in the filter paper, add 80 ml of petroleum ether to the soxhlet flask, extract four hours in the primary boiling state; the last thing is to take out the sediment and reclaim the petroleum ether, cool it till there is only *D. zingiberensis* separating out, crystallize repeatedly and then dry to get *D. zingiberensis*. The purpose of using acidolysis is to appraise the purity of dioscin extracted with the assistance of ultrasonic, and select the best extraction conditions.

The arrangement of mon - agent experiment

Take 100 g of *D. zingiberensis* with a granularity of 60, add ethanol of different volume fractions according to a certain solid-liquid ratio, then at different ultrasonic frequencies, extract for a certain time, filter and do the experiment according to measurement method in the solid substance and *D. zingiberensis* in the extraction liquid.

The arrangement of orthogonal experiment

On the basis of the Mon-agent experiment, using ultrasonic frequency, the volume fraction of ethanol, solid-liquid ratio, and extraction time as factors and design the experiment levels. After this arrange an orthogonal experiment to decide the best extraction conditions.

Extraction times and its effects on the solid and dioscin to be extracted

Take 100 g of *D. zingiberensis* with a granularity of 60, extract under the best conditions; collect the extraction liquid from the 1st, 2nd, 3rd and 4th extraction. Then study the effects that extraction times have on the solid and *D. zingiberensis* in the extraction liquid and compare with the traditional extraction method. The traditional extraction method for *D. zingiberensis*: take 100 g of *D. zingiberensis* with a granularity of 60, add in 300 ml of 4 mol/L hydrochloric acid, and hydrolyze for 4 h in 110 ℃ and then proceed the rest of the procedure as the same as that of measurement method.

Extraction methods and their effects on the solid and *D. zingiberensis* in the extracted substance

Take 100 g of *D. zingiberensis* with a granularity of 60, do the experiment according to the best extraction conditions from 1.2.2 and compare it with the solid and *D. zingiberensis* obtained from the method without using ultrasonic and extracted in room temperature.

Ultrasonic-assisted ethanol extraction and its effects on the structure of *D. zingiberensis*

Take *D. zingiberensis* before and after the extraction and without being processed by ultrasonic and do a gold spout scan and observe.

The performance tokens of the hydrolysate of dioscin

Take little amount of the dioscin (solid) from the multiple crystallization; do a melting point and infrared detection and develop its characters.

RESULTS AND DISCUSSION

The results from the mon-agent experiment

Do the experiment according to the arrangement of mon - agent experiment and the results are shown in Table 1, Figures 1, 2 and 3. It can be seen from Table 1 that when other conditions are the same but the ultrasonic frequency is 25.8 KHz, the solid and *D. zingiberensis* will be the most. The reason is that, the strong cavitation,

Table 1. Ultrasonic Frequency and its Influence on the Solids and *D. zingiberensis*.

Ultrasonic frequency /KHz	14.52	25.80	35.74
Quantity of the solid/g	8.730	9.849	8.874
The quantity of *D. zingiberensis* /g	1.4891	1.9674	1.6760

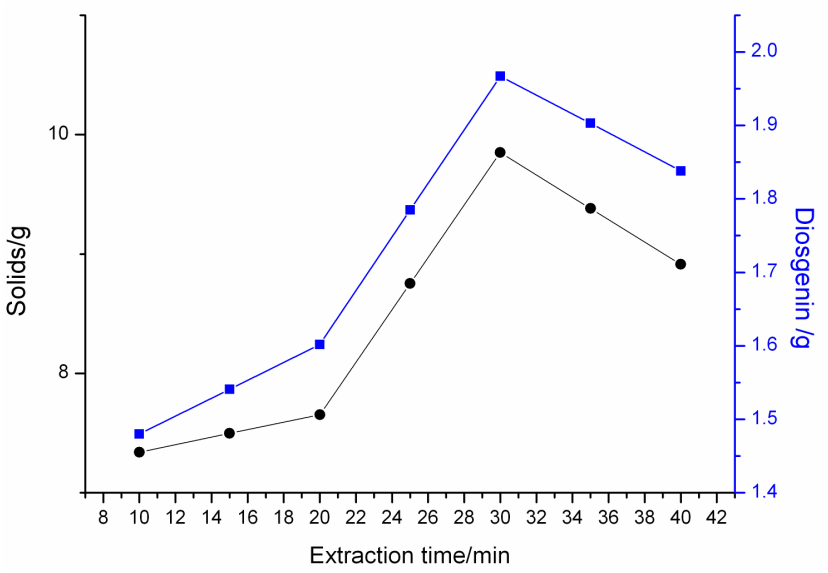

Figure 1. The effects ultrasonic extraction time has on the solids and diosgenin. This experiment is done when the volume fraction of ethanol is 70%, solid-liquid ratio is 1:10, the ultrasonic frequency is 25.80 KHz and various ultrasonic durations.

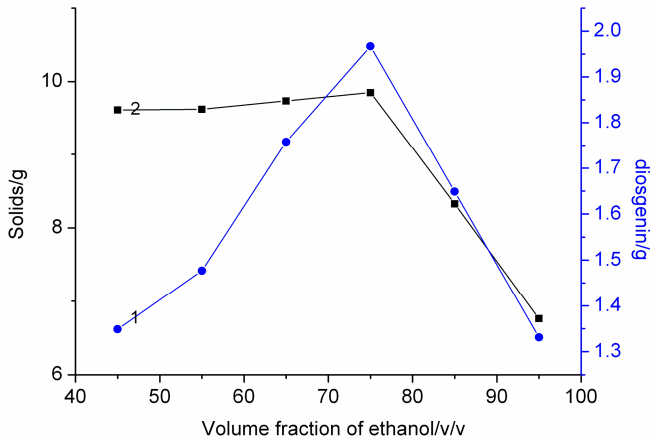

Figure 2. Effects volume fraction of ethanol has on the solids and diosgenin in the extracted. This experiment is done when the ultrasonic frequency is 25.80KHz, solid-liquid ratio is 1: 10 and extracted for 30 min with ethanol of various volume fractions.

mechanical vibration and accelerated motion came from the ultrasonic wave increases the motion frequency and speed of the molecule, specifically when the ultrasonic frequency and the motion frequency of the molecule are the same, the solid-liquid boundary layer between the cell and the solvent is greatly thinned and the penetration

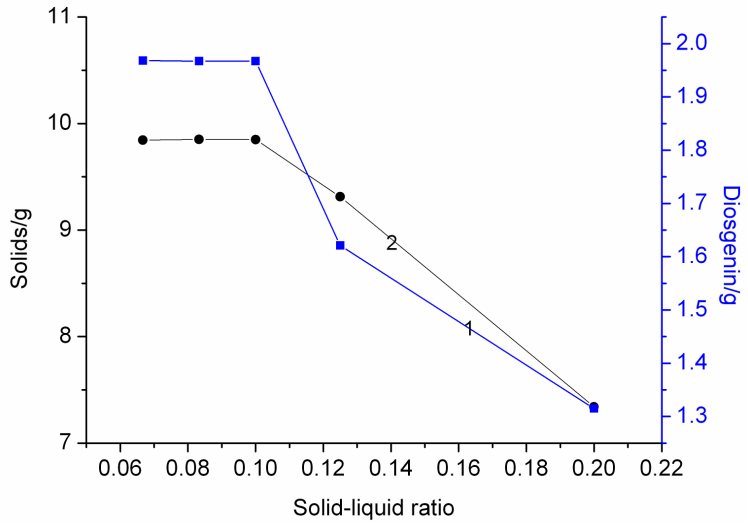

Figure 3. The effects solid-liquid ratio has on solids and diosgenin. The experiment is done when the ultrasonic frequency is 25.80 KHz, ethanol volume is 75v/v, and the extraction duration is 30 min with different solid-liquid ratio.

ability of the solvent is increased thus increases the release, pervasion and dissolution of the substance inside the cells and in this way the extraction efficiency (Hromadkova and Ebringerova, 1999; Hromadkova et al., 2002) of the dioscin is improved.

It can be seen from the Figure 1 that with the extension of the extraction time, the solids from the extraction and also the *D. zingiberensis* will both first increase and then decrease. Especially when the extraction time is 30 min, the quantity of the solids and *D. zingiberensis* will be both the largest (9.8490 and 1.9671 g). The reason is that with the extraction carried on, the concentration of the extracted substance increases, which in turn reduces the driving force for the extraction, and with the extraction carried further on the heat produced by the ultrasonic will denature the protein extracted, which will also close the dioscin in, which in turn again decreases the content of solids in the diosgenin filtrate, and this means the diosgenin hydrolyzed form the solids will also decrease.

It can be seen from the Figure 2 that when the volume fraction of the ethanol is 75 v/v, the content of the solids in the extraction liquid and the dioscin will be the highest. When the ethanol volume is between 45 to 75%, with the increase of the volume fraction, the amount of the solids does not show any dominant changes, but the content of diosgenin drastically increases. This is because it is easy for dioscin to dissolve ethanol, so with the increase of the volume of ethanol, the dissolution ability of the dioscin will also get better. The starch in the *D. zingiberensis* and protein are denatured easily. When the ethanol volume isover 75 v/v, on one side with the increase, the starch and protein are denatured drastically. On the other, the heat produced from the ultrasonic will urge a further denaturalization of the starch and protein. Then the dioscin is clothed inside the network of the solids, so it will not enter the filtrate, then the solids and diosgenin decreases. To make it easier to make the figure, the solid-ratios (diosgenin: ethanol) is numeralized, 1-diosgenin, 2-solids.

It can be seen from Figure 3 that with the increase of the ratio, the solids and diosgenin both first increase and then stabilize. When the ratio is 1: 10 (0.1), the content of the solids and diosgenin will be the highest (9.849 and 1.9671, respectively). This is because as the ratio increases, the concentration difference of the solids inside and outside the diosgenin cells also increases.

However, the impetus of the medium will increase but the viscosity and resistance of the medium both deduce, which makes it easier for the solids inside the cells to dissolve. But when the solid-liquid ratio reaches a certain number, the concentration difference of the solids inside and outside the cells will only influence the speed of the extraction and not much of extraction of the solids.

Orthogonal experiment results

Mon-agent experiment shows that the best conditions for the ultrasonic to assist the extraction of dioscin with ethanol are: when the ultrasonic frequency is 25.80 KHz, the solid-liquid ratio is 1:10 and volume fraction of ethanol is 75v/v, extract using ultrasonic as assistance for 30 min. Considering the reciprocity among different factors, to simplify the problem, using diosgenin as the evaluation factors, do the orthogonal experiment according to the arrangement of orthogonal experiment.

Table 2. Arrangement of the orthogonal experiment.

	Solid-liquid ratio	Duration of ultrasonic extraction (min)	Frequency (kHz)	Volume fraction of ethanol %	Experiment results
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
k_1	1.40	1.26	1.43	1.46	
k_2	1.69	1.69	1.34	1.64	
k_3	1.19	1.34	1.58	1.18	
R	0.50	0.43	0.15	0.46	

It can be seen from Table 2 that, the factors that influence diosgenin are and in the order of solid-liquid ratio, the volume fraction of ethanol, the extraction duration and the ultrasonic frequency, and the best combination of these factors are: ultrasonic frequency is 35.74KHz; the volume fraction is 75 v/v, solid-ratio is 1: 10 and extraction duration is 30 min, which is different from the results from mon-agent, and this is because the orthogonal test put the reciprocity among all the factors into consideration.

Extraction times and its effects on the solids and diosgenin in the extracted substance

Do the experiment according to extraction times and its effects on the solid and dioscin to be extracted; the results are shown in Figure 4. It can be seen from Figure 4 that as being extracted more and more times, the solids and the diosgenin in the extraction both decrease drastically.

If the solids and diosgenin from the forth extraction are used as the base, then the solids from the first, second, third and forth takes up 60.93, 22.05, 11.86, 0.52 and 56.93% of the total amount of solids and diosgenin from the first, second, third and forth takes up 25.97, 13.84 and 0.33% of the total amount of the diosgenin. Considering the cost of extraction, three extractions will achieve the purpose. If the experiment is done according to the above requirement, there will be 3.1345 g of diosgenin, which is 1.1289 times more than the diosgenin (2.7765 g) got using traditional acidolysis.

Extraction methods and its effects on the solids and diosgenin in the extraction liquid

Do the experiment according to extraction methods and their effects on the solid and *D. zingiberensis* in the extracted substance; extract 3 times; the results are shown in Table 4. As shown in Table 4 that the diosgenin (3.1345 g) obtained when the extraction is assisted by ultrasonic is 1.294 times more than that got in room temperature extraction (2.4223 g), so it is safe to come to the conclusion that ultrasonic assisted extraction method will not only increase the efficiency of the extraction greatly but also the production rate of the extraction.

If a comparison is made of the data in Figure 4 and Table 4, it will be seen that the pattern that

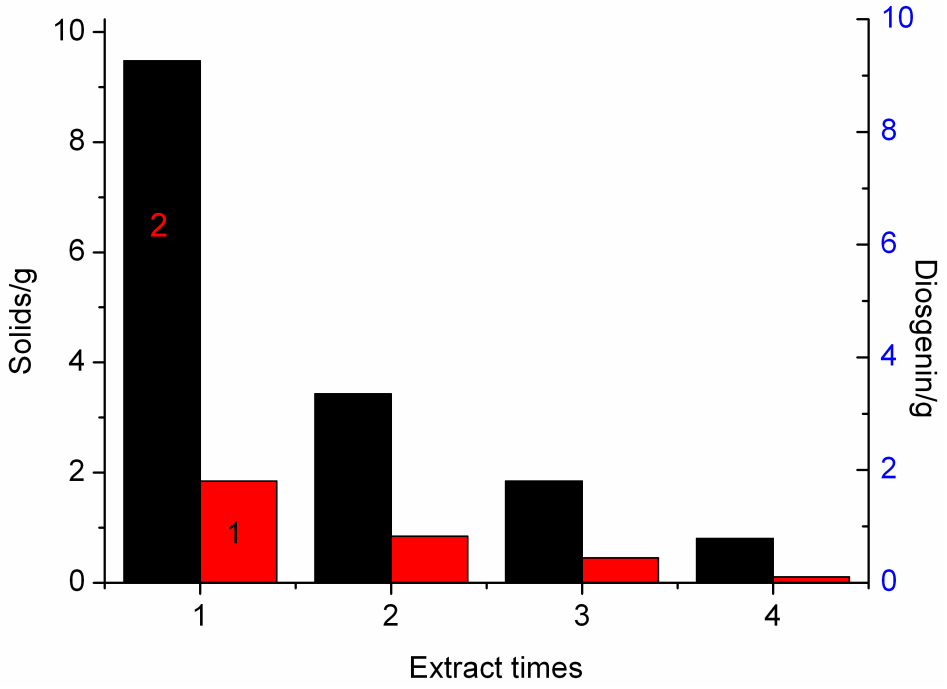

Figure 4. Effects extraction times have on the solids and diosgenin. This experiment is done when the ultrasonic frequency is 35.74KHz, the volume fraction of ethanol is 75v/v, solid-liquid ratio 1:10 and extracted for 30 min and for 4 times. 1-Diosgenin, 2-Solids.

Table 4. The extraction methods and its effects on solids and diosgenin.

	Solids/g			Diosgenin/g			
	First	Second	Third	First	Second	Third	Total
Ultrasonic-assisted extraction	9.474	3.428	1.844	1.8445	0.8415	0.4485	3.1345
Extraction in room temperature	12.255	0.37	0.17	1.3621	0.5650	0.4952	2.4223

extraction in room temperature and using ultrasonic wave for assistance is different. It is probably because during the extraction process assisted by ultrasonic, because the duration is short, so that there is not enough time for the cells to expand, so the extraction is mainly accomplished relying on the ultrasonic to speed up the molecule motion. The diosgenin (2.4422 g) got from the extraction in room temperatue is 87.96% of the quantity of diosgenin (2.7765 g) using acidolysis and 77.9% of the diosgenin (3.1345) using ultrasonic as assistance.

Ultrasonic-assisted ethanol extraction and its influence on structure of *D. zingiberensis*

It can be seen from the above Figure that the cytoarchitecture of the *D. zingiberensis* before and after the ultrasonic extraction did not change much, and the cells are integrate, and the edges are trim, which means that the ultrasonic does not break the cytoarchitecture during the extraction process, and this result does not agree to the findings from the former experiments (Zhang and Wei, 2007) 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.

The performance tokens of the dioscin

The melting point of the dioscin hydrolysate

The hydrolysate of the dioscin obtained using ethanol as solvent and ultrasonic as assistant in acid condition has a melting point of 208-214℃, which is consistent with the

standard sample of diosgenin, which has a purity of≥99% and melting point between 204-207℃ and the result shows that the diosgenin got using this method is highly pure.

The IR of diosgenin hydrolysate

The IR is that of the sample obtained using ultrasonic assisted ethanol extraction of diosgenin after acidolysis. It can be seen from the above figure that the sample in 1237 cm^{-1}, 1050 cm^{-1} (C_3-OH and Δ^5) 978, 917, 896 and 860 cm^{-1}（25 spironoalkyl）all made an appearance, and this is the same (Jau-tien et al., 2006). as that of the Diosgenin. All of the above proves that the diosgenin obtained when ethanol is used as the assistance has a comparatively higher purity.

Conclusion

The application of ultrasonic technology in the extraction of the targeted components in the plants (*D. zingiberensis*) will remarkably improve the efficiency and production rate of the traditional extraction method. The efficiency of the technique involving the assistance of ultrasonic is 240 times than that of using only ethanol as solvent. The production rate of ultrasonic assisted extraction is 1.28 times more than that using ethanol and 1.1289 times more than the traditional acidolysis method. Though using ethanol as extractant will increase the cost but because of the improvement of production rate of the diosgenin and the consistency of the components of *D. zingiberensis* before and after the extraction, which creates the conditions for the comprehensive use of the waste residue (production of ethanol, protein feed) so technically and economically using this method is applicable.

Because of the connection between the solid (dioscin) in the extraction liquid and the hydrolysate (diosgenin), to a degree we can use the solid (diosgenin) to represent diosgenin (the solid) and this conclusion provides convenience for future researches. The dioscin obtained from the ultrasonic assisted extraction method is about 10% of the total amount of solids. This is probably because of the low efficiency of the enzymolysis of the dioscin. The separation and purification of dioscin is the key to improve the efficiency of the enzymolysis of dioscin. This technology is an important step of the whole technology and also the premise of the realization of the clean production of diosgenin.

In this experiment when the ethanol extraction is assisted by ultrasonic, the cytoarchitecture of *D. zingiberensis* will not be destroyed, and the cavitation effect of the ultrasonic will make the lingering boundary layer formed between the grains of the solid (*D. zingiberensis*) and the solvent (ethanol) get thinner, and even renews constantly, so the extraction is sped up.

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