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Full Length Research Paper

A study on the extraction and purification technology of tea sapogenin

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In order to extract and purify tea sapogenin, first the extraction liquid is obtained using mechanically pressed tea-seeds as raw material, which will be decreased by organic solvent. When the conditions like the methanol volume fraction is 75v/v, solid-liquid is 1:4 and the ultrasonic frequency is 25.8 Hz, extract them for 30 min. After this, collect the extraction liquid and slowly add in Hz-841 macroporous resin column, then elute it with 0.3% NaOH. The third step is to elute with ethanol of different concentrations after the coloring pigment is got rid of, and then detect it with thin layer chromatography (TLC). It is discovered that there is only tea sapogenin in the 35 – 95v/v ethanol elution liquid. The last step is to collect the 95% ethanol elution liquid and vacuum condense it; and then tea-Tea sapogenin with a purity of 96% can be obtained.

Key words: Tea sapogenin, macroporous resin, purification, ultrasonic, extraction.

INTRODUCTION

Tea sapogenin, a natural non-ionic surfactant, not only has a good emulsifying, dispersing and moisturizing capability, but is also a good foam stabilizer with a great and cleaning capacity, having a hydrophile-lipophilic balance value of 16 (Guo, 2007). Thus, it can be used as foam stabilizer for the building of concrete (Pu and Zhu, 1999), potentiating agent (Li et al., 2005), soil amendment (Hong and Tokunaga, 2000), oxidation preventive (Venukumar and Latha, 2002; Si et al., 2003); so it is widely used in daily chemical industries, building materials, food industries and agriculture. There is 10-13% tea sapogenin in tea seeds, which makes it an ideal resource for the extraction of tea sapogenin. Usually tea sapogenin is extracted by using solvent like water, ethanol and methanol. Among these, water produces a high percentage of impurity, which makes separation and purification difficult; and methanol is too poisonous, bringing serious pollution to the environment.

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Accordingly, this experiment will use pressed tea seed meal as raw material, ethanol as the solvent and ultrasonic extraction technique will also be employed. Meanwhile, the impact brought about by different ultrasonic frequencies, ethanol of different concentrations, various extraction durations and different solid -liquid ratios will be studied and used to select the best extraction technique. The purity of the tea sapogenin is the bottle-neck for the exploitation of the tea sapogenin. Though purifying methods like precipitation involving Ca^{2+}, Ba^{2+}, organic solvent (acetone), silica gel column chromatography (Liu et al., 2008; Li et al., 2008; Yuan et al., 2008) are used, the products still have the problem of having a dark luster color, lack of texture and low purity. To surmount the above limitations, macroporous resin will be used as the absorption agent, thin layer chromatography (TLC) detection technique will also be used, and the focus is to study the elution behaviors of ethanol at different concentrations. When the 0.3% NaOH solution is used to elute, the impurity and pigment is absorbed in the macroporous resin, and if 95v/v ethanol elution liquid is collected and vacuum condensed, then tea- Tea sapogenin of 96% can be acquired.

Table 1. Different treatment methods and their individual effects on tea sapogenin extraction.

Treatment methods	Desiccation situation	Characters of the dry substance	The concentration of tea sapogenin in the residual extraction liquid /mg/ml	The mass of tea sapogenin (g)
Pretreated	Easy	Off-white powder	0.02390	11.9340
Not treated	Difficult	Reddish brown thick solid	0.02079	10.3950

The mass of the tea sapogenin refers to the quality of mass sapogenin extracted from 100 g of tea seeds.

MATERIALS AND METHODS

Materials and main equipment

Pressed tea seed meal, Yuxi Biological Pharmacy Enterprise, Yun Nan Province; ethanol, vanillin, sulphuric acid, normal butanol and glacial acetic acid are all purchased from reagent companies in Xi'an; HZ-841 macroporous resin, purchased from Mosu Science Equipment Co., Ltd in Shanghai.

A multi-frequency sonochemical reactor (SC-β, Jiu Zhou Mechanical and Engineering Research Center, Chengdu; UV Spectrophotometer (Spr756), Shanghai Spectrum Instruments Co., Ltd; Rotary Evaporator (R201BL), Shiyuan Science Equipment Co., Ltd, Shanghai; Medicinal Herb Grinders (WKX-130), Jingcheng Medical Equipment Manufacture Co., Ltd, Qingzhou Shandong Province; 30 × 100 SG13 thin-layer chromatography silica gel plate, Qingdao Makall Group; Spectrophotometer (722), Shanghai Precision and Scientific Instrument Co., Ltd.

Experiment methods

Pretreatment of the tea seed meal

Take 80 g of pressed tea-seed meal with a particle size of 60 mesh with 250 ml of acetone, and put them both in a Soxhlet extractor. After this, heat it and reflux till there is no fat; then take it out to be used.

Take pretreated and not treated tee-seed powder of 100 g each. When the ultrasonic frequency is 25.80 KHz, the ethanol volume fraction is 75v/v, and the solid-liquid ratio is 1:5, extract them both for 40 min; filter and record the volume of the filtrate. Then take 1 ml of the filtrate, and weigh after drying it. After this put 1 ml of the filtrate into a 100 ml volumetric flask and measure the absorption value. Then compute the mass fraction of the tea sapogenin.

The tea sapogenin = the volume of the extraction liquid × drying weight of 1 ml filter × the tea sapogenin of 1 ml filter.

The extraction of tea sapogenin

Take 100 g of pretreated pressed tea-seed meal with a 250 ml flask. Add in a certain volume of ethanol and then fix the flask onto the reaction frame; activate the ultrasonic pulverizer. Then study the impact the ultrasonic frequency, the volume of ethanol, the extraction time, solid-liquid ratio and the extraction times have on the extraction of tea sapogenin.

Purrifaction of the tea sapogenin

Take 200 g of HZ-841 macroporous protease and put it in a 500 ml beaker; then add proper amount of ethanol and soak it for 2 h. Load column and then elute it with ethanol till the liquid and the water coming out is no longer turbid. After this get rid of the ethanol with water and get it ready for use by soaking it in water.

Take the extraction liquid of tea sapogenin that has a mass fraction of 35 mg/ml, 200 ml, and then slowly put it in a separation column; after which wait for 10 h, then elute it with NaOH and ethanol solution of different concentrations. Collect the ethanol elution liquid of different concentrations and Point boardby capillary. Then use developer made up of n-butanol, acetic acid and water in the ratio of (4:1:5), color developing reagent (Yi and Tatsuya, 2000) made of ethanol with 1% $AlCl_3$ and 10% H_2SO_4. And do a TLC analysis and make a comparison with the standard sample of tea sapogenin to decide the best combination of the all these liquids.

RESULTS AND DISCUSSION

The extraction of the tea sapogenin

The Pretreatment of the tea seed meal and its effect on the extraction of tea sapogenin

Do the experiment according to 1.2.1 and compare with the results of the sapogenin without pretreatment; the testing results are shown in Table 1.

It is shown in Table 1 that each of the criteria of the tea sapogenin acquired when the tea-seeds are pretreated is far better than that gotten when the seeds are not pretreated. The main reason lies on the fact that the tea seed meal still has about 10% of tea seed oil and this will have negative effects on the extraction of tea sapogenin. So the tea seed powder should better be pretreated to get rid of as much of the fat as possible when used to extract tea sapogenin.

The effect ultrasonic frequency has on the extraction of tea sapogenin

When the volume of ethanol is 75%, the solid-liquid ratio is 1:5 and the extraction time is 20 min. The effect that ultrasonic frequency has on the extraction is studied in Table 2.

As can be seen from Table 2 that when the ultrasonic frequency is 25.8 KHz, not only is the concentration of the tea sapogenin in the extraction liquid the highest but it also contains the most tea sapogenin. Thus the best ultrasonic frequency should be 25.8 KHz. The main reason for this is that the strong cavitation effect, mechanical vibration, high acceleration, emulsification and stirring effect brought about by the ultrasonic frequency

Table 2. Effect Ultrasonic frequency has on the extraction of tea sapogenin.

Ultrasonic frequency/KHz	The mass fraction of the tea sapogenin in the extraction liquid	The mass of tea sapogenin (g)
14.52	0.0224	11.1725
25.80	0.2314	11.5700
35.74	0.0228	11.3985

The mass of the tea sapogenin refers to the quality of mass sapogenin extracted from 100 g of tea seeds.

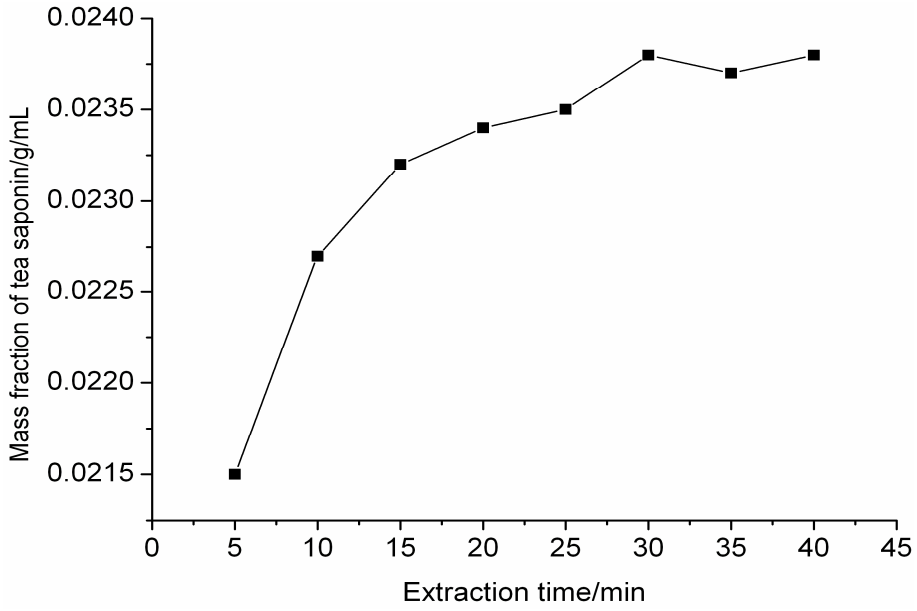

Figure 1. Effect of ultrasonic extraction time on the extraction of tea sapogenin in ultrasonic process.

will increase the frequency and speed of the movement of the molecule. And specifically, when the ultrasonic frequency is the same as the movement frequency of the molecule, a strong resonance will greatly thin the solid and liquid boundary layer, thus increasing the penetration ability of the solvent; therefore it strengthens the diffusibility, solvency of the substance inside the cell, which in turn improves the extraction efficiency of the tea sapogenin (Riera et al., 2006; Li et al., 2004; Hromadkova et al., 2002).

Ultrasonic extraction time and the extraction of tea sapogenin

When the volume fraction of the methanol is 75%, the solid-liquid ratio is 1:5 and the ultrasonic frequency is 25.8Hz. The effect that extraction time has on the extraction of tea sapogenin is seen in Figure 1.

It can be seen from the figure that with the increase of the ultrasonic extraction time, the mass fraction of the tea sapogenin in the extraction liquid will first increase and then decrease, and when the extraction time is 30 min the mass fraction of tea sapogenin will be and 0.238 g/ml, which is the highest. The reasons for this are: 1) With the extension of the extraction time and when the concentration of the tea sapogenin reaches a certain level, the dynamics of transfer unit decreases and 2) Under the huge pressure created by the cavitation effect brought about by the ultrasonic, the structure of tea sapogenin extracted will be broken (Rom adkova et al., 1999).

The volume fraction of ethanol and its effect on the extraction of tea sapogenin

When the ultrasonic frequency is 25.80 KHz and the solid-liquid ratio is 1:5, set the extraction time at 30 min. The effect the volume fraction of ethanol has on the extraction of tea sapogenin is seen in Figure 2.

It can be seen from the above figure that when ethanol volume fraction is 75v/v, the concentration of the tea sapogenin is also the highest (0.0234 g/m). The reason for this is that the lower the concentration of ethanol is,

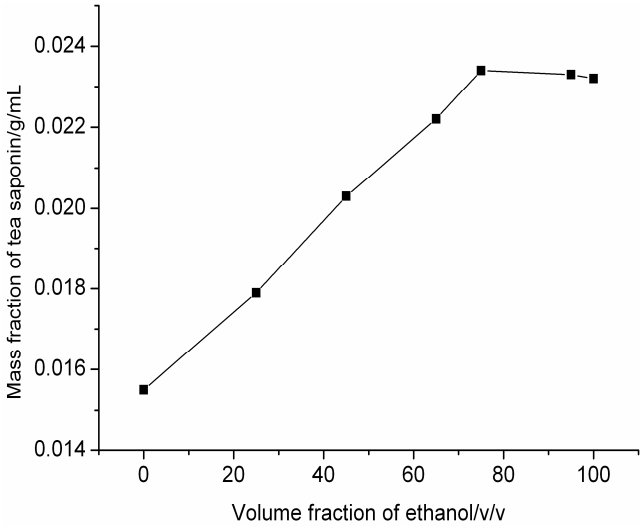

Figure 2. Effect of the volume fraction of ethanol on the extraction of tea sapogenin.

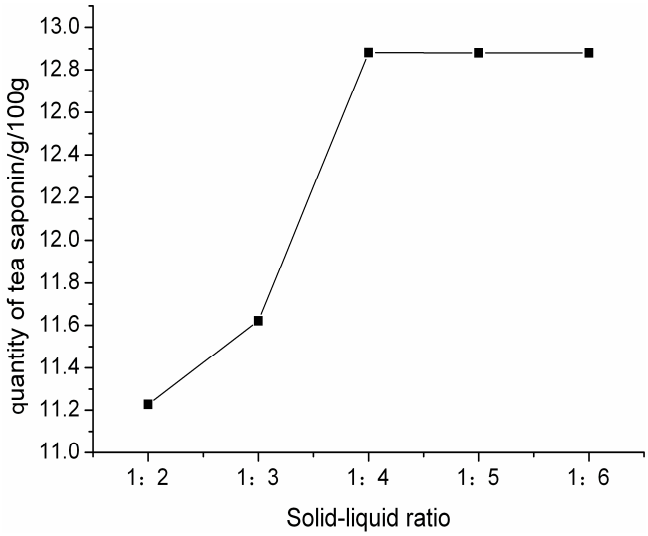

Figure 3. Effects solid-liquid ratio has on extraction of tea sapogenin.

the easier it gets for the soluble glucide, protein and starch to dissolve, which leads to the increase of the concentration and the viscosity of the medium, which is bad for the extraction of the tea sapogenin. In addition, when the concentration of the ethanol is low, it is easy for the tea sapogenin to reach the critical micelle concentration with its surface activity being increased and its foam stabled but it is difficult to concentrate. And when the concentration of ethanol is too high, the protein will denature, the tea sapogenin will be cuddled in the net made up of protein and starch, which makes it difficult for the tea sapogenin to extract. Therefore it is the best to use ethanol with a volume fraction of 75v/v when extracting tea sapogenin.

Solid-liquid ratio and its effect on the extraction of tea sapogenin

When the volume fraction is 75% and the ultrasonic frequency is 25.80 KHz. With an extraction time of 30 min to eliminate the influence the solid-liquid ratio has on the mass fraction of the tea sapogenin in the extraction liquid, the mass of the tea sapogenin will be used as the quota to study how solid-liquid ratio influences the extraction of tea sapogenin (Figure 3).

It can be seen from Figure 3 that the mass of the tea sapogenin in the extraction liquid increases as the ratio does, then it goes stable, because the higher the ratio gets the stronger the driving force of the medium gets. And this makes it easier for the tea sapogenin to be extracted, but when the solid-liquid ratio gets to a certain level, the solid-liquid ratio will only affect the extraction speed without increasing the mass of the tea sapogenin.

When the ratio is too big, the cost for the extraction of tea sapogenin increases, so it is best to keep the ratio at 1:4 for the best extraction using ultrasonic.

Extraction times and its effect on the extraction

When the mass fraction of the ethanol is 75v/v (the ultrasonic frequency is 25.80 KHz) extraction time is 30 min and the solid-liquid ratio is 1:4, the quantity of the tea sapogenin in the extraction liquid is used as the quota to study the influence that extraction times have on the extraction of the tea sapogenin (Figure 4).

It can be seen from the figure that the more the liquid is extracted, the more the quantity of the tea sapogenin decreases drastically. If the quantity of the tea sapogenin gotten after being extracted for three times is used as the base, then the tea sapogenin from the first round, second and the third is 65.33, 22.23 and 12.40% of the total quantity of tea sapogenin, respectively. Considering the cost of the extraction of tea sapogenin, it is best to extract for three times.

The purification of tea sapogenin

The Influence that NaOH has on the mass of tea sapogenin

After the tea sapogenin in the extraction liquid is absorbed by the macroporous resin, first elute with NaOH of different concentrations. It will be seen that the quantity and purity of the tea sapogenin is closely connected with the mass fraction of NaOH (Table 3).

Table 3 shows that when the mass fraction of the NaOH is 0.3%, NaOH elutes the impurity, bringing out the best color pigment; and so the tea sapogenin will be the purest. When the mass fraction of the NaOH is 0.1 or

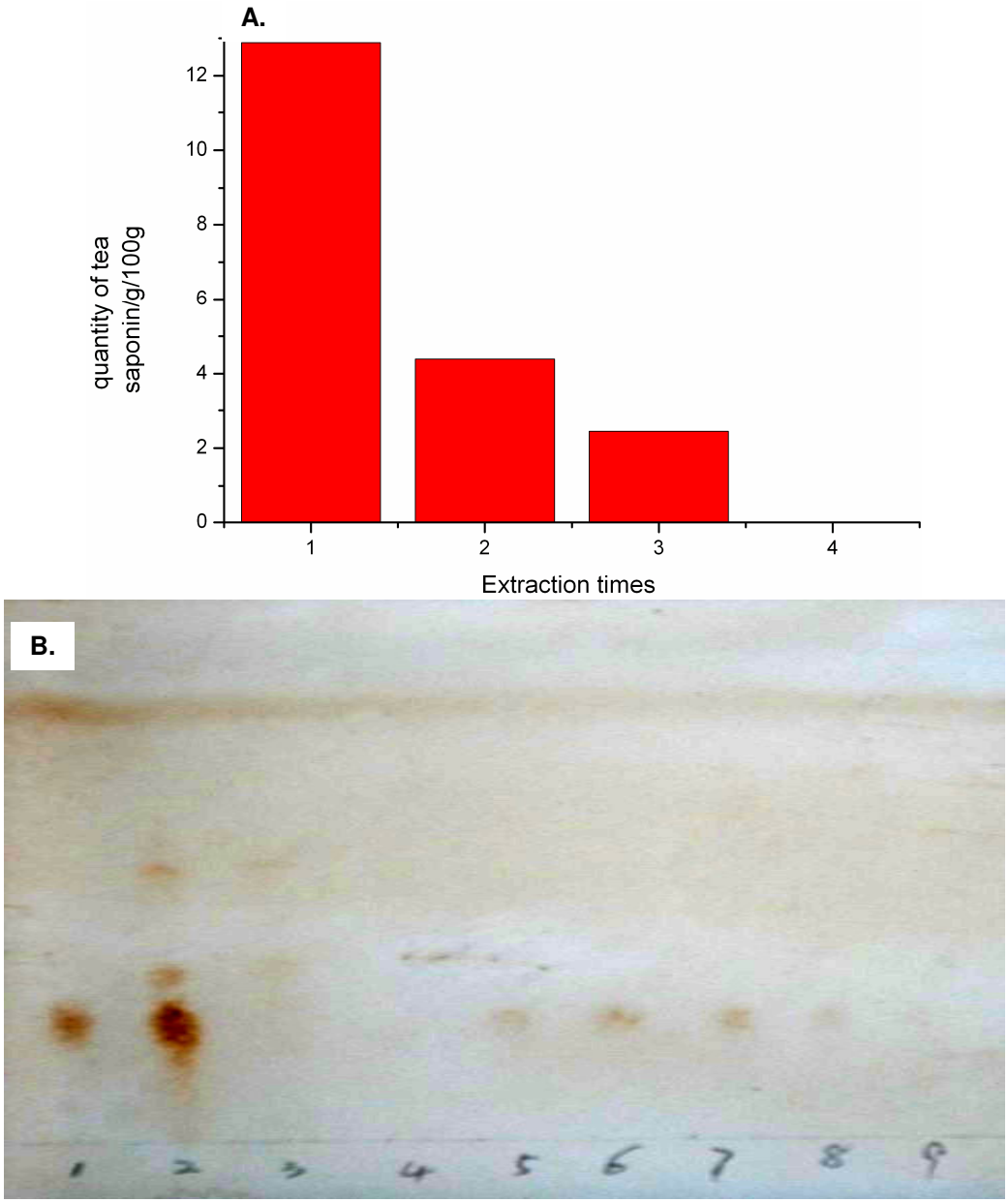

Figure 4. A. Effects of extraction times on the extraction of tea sapogenin. **B.** TCL results of ethanol elution. Sample 1 is the standard sample; sample 2 is the elution liquid after being eluted by 0.3% of NaOH; samples 5, 6, 7 and 8 are elution liquid eluted by 5, 55, 75 and 95% ethanol, respectively.

Table 3. NaOH mass fraction and its effect on the quality of tea sapogenin.

NaOH mass fraction (%)	0.1	0.2	0.3	0.4	0.5
The quantity of tea sapogenin (g)	4.48	3.92	3.64	2.8	1.68
The color and luster of the tea sapogenin	Light yellow	Light yellow	Off white	Off white	Off white
The purity of the tea sapogenin (%)	80	90	96	96	96

The mass of the tea sapogenin refers to the quality of mass sapogenin extracted from 100 g of tea seeds.

0.2%, NaOH elutes the impurity, bringing about the worst purity. But when the moss fraction of the NaOH is 0.4 or 0.5%, which means it is too alkaline. This makes part of color pigment; so the tea sapogenin will have the lowest

the tea sapogenin turns into soluble saline, which will be eluted; so some of the tea sapogenin will be lost, with lower productivity.

Ethanol of different concentrations and its effect on the components of the elution liquid

After being eluted by NaOH and then elute it with water till it is neutral. Then elute again with ethanol of different concentrations and collect the elution liquid, using developer made up of n-butanol, acetic acid and water in the ratio of (4:1:5), color developing reagent 10% H_2SO_4 and do a TLC analysis (Figure 4).

It can be seen in Figure 4 that 5, 6, 7 and 8 are the TCL results of ethanol elution liquid of 35, 55, 75 and 95%, respectively, which are in accordance with the standard sample, which proves that there is only tea sapogenin in the ethanol elution liquid of 35, 55, 75 and 95%. In the 3rd, 4th and 9th might because there is too little of Point-like volume; there is no Color Spotted, and further research shows that with the increase of the volume fraction of ethanol, it gets easier for the tea sapogenin to elute. This is because when the mass of volume of the ethanol is the highest; its polarity gets close to that of the tea sapogenin, so it is best to use ethanol of 95v/v as the elution agent.

DISCUSSION

Ultrasonic is widely used in the extraction of usable elements of both plants and animals because of its unique mechanical (Dietrich et al., 2004), cavitation and chemical effects. Compared with other common extraction solvent, ultrasonic wave can ensure the efficiency and quality of the tea sapogenin. Ultrasonic apparatus is ideal assistant equipment for the extraction of plants. The frequency of the ultrasonic wave is directly connected to the quality and quantity of tea sapogenin, but it is not when the frequency is the highest, the easiest it will be for the extraction; it is only when the frequency of the ultrasonic is the same as that of the plant, then the ultrasonic will be the most effective. At the same time, when the polarity of the liquid is almost the same as the extracted substance, then the amount of the wanted substance would be the highest.

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