

Study on Tea Saponin Extraction from Shell of Oil-tea Camellia Seeds

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Abstract—In order to effectively use the waste oil-tea camellia seed shell and reduce environmental pollution, this paper presented a tea saponin extraction method by using tap water as the extraction agent and precipitation method from oil-tea camellia seed shell. The best extraction conditions and parameterize the factors were optimized for tea saponin extraction from oil-tea camellia seeds: the extraction temperature is 80°C, ratio of liquid to solid is 6:1, pH value for extraction is 9 and the extraction time is 6h. The final tea saponin extraction rate could reach 94.66%. The best precipitate and tea saponin release agent recipe are obtained: Poly aluminum chloride (30% concentration) is the suitable flocculant and the optimum amount is 8% of preliminary extracted liquid by weight and placed still for 2 hrs. The optimum amount of CaO, as precipitate agent, is 10% of the weight of the raw materials, and stirred for 4h. The release agent, ammonium bicarbonate, was used in an amount of the 30% of the tea saponin calcium in order to achieve a chemical equilibrium reaction rate of 50%.

Keywords—oil-tea camellia seeds; tea saponin; extraction

I. INTRODUCTION

Tea saponin, consisting of triterpenoid saponins, structural sugar and acid structure, is traditionally extracted from oil-tea camellia seeds;^[1]. The pure tea saponin is milky white or pale yellow solid amorphous powder, and its melting point ranges from 223 to 224 °C. The molecular formula is $C_{57}H_{90}O_{26}$ and the relative molecular mass is 1203^[2-3]. The crystallized saponin is soluble in water content of methanol, ethanol, butanol and glacial acetic acid, and it can be dissolved in hot water, hot alcohol, but insoluble in cold water, ethanol, can not be dissolved in ether, chloroform, petroleum ether and benzene^[4,5]. Tea Saponin is not only an excellent natural non-ionic surface active agent, which can be used for emulsification, dispersion, wetting, detergency, forming, foaming stability and other surfactants^[6,7]. Tea saponin has biologically active roles, such as hemolysis, fish toxicity, impermeability of anti-inflammatory, cough analgesic, antibacterial, insecticidal and inhibition of alcohol absorption, the class of biological hormones, etc^[8,9].

Oil-tea camellia is very rich of resources in China, the cultivation area is over 45 million mu, and annual output of oil-tea camellia seeds is more than 100 million tons^[10-11].

Traditionally, the shells of oil-tea camellia seeds were entirely discarded as by-products during the oil-tea camellia oil extraction process from oil-tea camellia seeds^[12-13]. There is 5-8% of tea saponin in oil-tea camellia seed shell, and the tea saponin could be extracted and be widely used in chemical, pharmaceutical, building materials, food, textile, printing, pesticides, aquaculture and other industries, thus increase substantially the accessory value of the oil-tea camellia^[14-16]. However, few studies so far, to our knowledge, have been reported to extract tea saponin from shell of oil-tea camellia seeds. To increase the tea saponin extraction ratio from shell of oil-tea camellia seeds, The tea saponin extraction conditions and parameter of extraction process were optimized from oil-tea camellia seed shell by using the tea saponin extraction technology from oil-tea camellia seeds as reference.

II. MATERIALS AND METHODS

A. Main materials and reagents

The shell of oil-tea camellia seeds was obtained from Hunan Academy of Forestry. The materials for tea saponin are listed as follows: tap water, distilled water, concentrated sulfuric acid, 77% sulfuric acid, 8% vanillin and 30% poly aluminum chloride (analytical grade), calcium oxide (industrial grade) and ammonium bicarbonate (analytical grade).

B. Main instruments

- The following instruments were used for tea saponin extraction:
- Desktop pipe rack centrifuge TDL-5Z from Hunan Star Scientific Scientific Instruments Co., Ltd.
- Electric blast oven from Shanghai Chongming Experimental Instrument Factory
- UV-2550 UV-Vis spectrophotometer from Shimadzu consumables sales company
- JJ-1 precision electric mixer from Changzhou Sino Instrument Co., Ltd.
- Significant number of electric water bath pot from Shanghai Yuejin Medical Instrument

This work was funded by the National Eleventh-Five-Year scientific and technological support project "the comprehensive utilization and demonstration of by-products of *Camellia oleifera*" (2009BADB1B10) and Hunan National Camellia standardization demonstration area (SFQ-1316).

- Chinese medicine grinder from Tianjin Teste Instrument Co., Ltd.
- Trace continuously adjustable pipette from Beijing Qingyun Zhuoli Precision Equipment Co., Ltd.
- DZF-OB vacuum oven from Shanghai Yuejin Medical Instrument Factory,
- SHB-III recycled water use of the vacuum pump from Zhengzhou Great Wall Division Industry and Trade Co., Ltd.
- Electronic Analytical Balance from Australia House Instruments (Shanghai) Co., Ltd.

C. Extraction methods

1) Tea saponin standard curve

a) Preparation of standard tea saponin solution

The standard tea saponin solution (0.9 mg/ml) was prepared with following protocol: 0.1000g standard tea saponin powder (containing 90% tea saponin) was weighted precisely and placed in a 100 ml volumetric flask, then dissolved with appropriate amount of 80% ethanol, and then more ethanol were added and set the volume to the mark to make it 0.9 mg/ml and shake evenly.

b) Measurement method

The precise amount of the standard solution (0.5 ml) were gauged and placed in a 10 ml test tube with stopper, which was put in a ice-water bath, then 0.5 ml 8% vanillin solution and 5 ml 77% sulfuric acid were added. After fully shaking the test tube, the test tube was transferred to an intermittent warming water bath (60°C) for coloring for 20 min, then taken to cool down in a ice-water bath for another 10 min. Then the absorbance of the solution was measured with 1 cm cuvette at the wavelength of maximum absorption 545 nm (VU-2008 UV-visible spectrophotometer scanning) by using the blank one as reference after the tube was taken off the ice-water bath and resumed to room temperature.

c) Graphing the standard curve and the determination of formula

A series of the standard solution (0 ml, 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml and 0.5 ml) were taken and the mixed solutions were prepared with method described in 1.3.1.2 and the absorbance values were measured. Hereby, the standard curve was made and the calculation formula was assessed with excel.

2) Tea saponin extraction procedure with water solvent method

Shell of oil -tea camellia seeds→grinding and sieving→soaking in solvent→centrifugal separated liquid→flocculation→centrifugal separated liquid→precipitation agent added→centrifugation for saponin cake→saponin release→vacuum filtration→vacuum condensing→ tea saponin crystal

The shells of oil -tea camellia seeds were picked by hands and ground with Chinese medicine grinder and sieved with 20 meshes sieve. Sieved oil -tea camellia seed shell powder was sucked and stirred in hot water for a certain time and

centrifugated with Desktop pipe rack centrifuge TDL-5Z to get the preliminary extracted liquid. To remove the impurities in the preliminary extracted liquid, a certain amount of flocculants were added to the preliminary extracted liquid and placed at room temperature for about 4 hour, until there are no suspended solids. Here the purified extracted liquid was obtained after centrifugation. A certain amount of precipitation agent (CaO) were added to the purified extracted liquid and stirred sufficiently to get the saponin precipitate after centrifugation. Then ammonium bicarbonate, the tea saponin release agent, was added to the saponin precipitate. When the reaction of tea saponin with calcium was fully conducted, the mixed liquid was filtered with SHB-III recycled water use of the vacuum pump twice, one is the mixed liquid and the other is the vacuum suction filtered liquid residue, which was washed with hot water again. These two sets of filtered liquid were merged as the crude tea saponin extracts. After the vacuum concentration process, here is the crude tea saponin crystal. In the process of the tea saponin extraction, the solvent and extraction process is the key part of the whole experiment, which determines the extraction ratio of the tea saponin from shell of oil -tea camellia seeds. The extraction parameters, including extraction time, temperature, ratio of liquid to solid and pH, were determined with the single factor experimental design.

a) Trail for Ratio of liquid to solid

8 parts ground and sieved oil -tea camellia seed shell powder, each 50 g, were prepared and mixed with solvent. The ratio of liquid to solid were 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, respectively. The pH is adjust to 7, and all the tubes were placed at a 80 °C water bath for 4 Hours. The extracted tea saponin content of each treatment was measured through the process of centrifugation, flocculation, centrifugation etc., according to the extraction protocol as described above.

b) Trail for extraction temperature

8 parts ground and sieved oil -tea camellia seed powder, each 50 g, were prepared and mixed with solvent at ratio of 8:1. After adjusting pH to 7, the tubes filled with solvent and shell powder were sucked and extracted for 4 hrs in 40°C, 55°C, 65°C, 70°C, 75°C, 80°C, 85°C and 90°C water bath, respectively. Then the extracted tea saponin content of each treatment was measured through the processes of centrifugation, flocculation, centrifugation etc., according to the extraction protocol as described above.

c) Trial for extraction time

6 parts ground and sieved oil -tea camellia seed shell powder, each 50 g, were prepared and mixed with solvent at ratio of 8:1 and put at a 80°C water bath. After adjusting pH to 7, the tubes filled with solvent and shell powder were sucked and extracted for 1h, 2h, 3h, 4h, 5h and 6h, respectively. Then the extracted tea saponin content of each treatment was measured through the processes of centrifugation, flocculation, centrifugation etc., according to the extraction protocol as described above.

d) Trail for pH value

8 parts ground and sieved oil -tea camellia seed shell powder, each 50 g, were weighed and mixed with solvent at

ratio of 8:1 and place them at a 80°C water bath for 4 hrs after the pH values were adjusted to 4, 5, 6, 7, 8, 9, 10 and 11, respectively. Then the extracted tea saponin content of each treatment was measured through the processes of centrifugation, flocculation, centrifugation etc., according to the extraction protocol as described above.

e) Orthogonal experiment for extraction

According to the previous results from the single factor trial for tea saponin extraction, orthogonal experiments, $L_9(3^4)$, were designed with four factors (temperature, ratio of liquid to solid, pH value and extraction time) and three levels for the tea saponin extraction from shell of oil -tea camellia seeds, as shown in Table 1.

TABLE 1 LIST OF FACTORS AND LEVELS OF ORTHOGONAL TEST $L_9(3^4)$

levels	Factors			
	A	B	C	D
1	70	5:1	9	4
2	80	6:1	10	5
3	90	7:1	11	6

f) Selection and dosage of flocculant

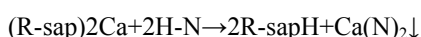
6 parts preliminary extracted liquid, each 200 g were weighed. 5%, 8% and 10% of the weight of extracted liquid, that is 10 g, 16 g and 20 g poly aluminum chloride (concentration 30%) were added to the former three parts of preliminary extracted liquid, respectively, and 1%, 2% and 3% of the weight of extracted liquid, that is 2 g, 4 g and 6 g alum were added to the latter three parts of preliminary extracted liquid. The tea saponin yields were measured after placed at room temperature for 2 hrs and centrifugation separation.

g) Determination of the CaO amount as precipitate agent

7 parts preliminary extracted liquid after extracting from 50 g ground and sieved oil -tea camellia seed shell each, were prepared. Then 6%, 7%, 8%, 9%, 10%, 11% and 12% of the weight of oil -tea camellia seed shell, that is 3g, 3.5g, 4g, 4.5g, 5g, 5.5g and 6g CaO powder were added, respectively. After placed for 4 hrs at room temperature and separated the liquid from saponin calcium through centrifugation, the saponin obtained was measured and the relationship between the added amount of CaO and tea saponin conversion ratio was assessed.

h) Determination of ammonium bicarbonate amount as tea saponin release agent

According to the reaction equation



R-sapH represents the tea saponin molecules (R=sapogenin, H=the carboxy hydrogen atom), HN represents saponin release inhibitor (N is -I price).

6 parts of the same weight of saponin calcium, each 100g were prepared. Then 10%, 20%, 25%, 30%, 35% and 40% of

the weight of saponin calcium, that is 10g, 20g, 25g, 30g, 35g and 40g ammonium bicarbonate were added, respectively. After placed and stirred at a 60°C water bath for 2 hrs, and the liquid was obtained through vacuum filter. The solid residues were washed a small amount of hot water and vacuum filtered again. These two sets of filtered liquid were mixed and the saponin content was measured to evaluate the impact of saponin release agent amount on the saponin release rate.

III. RESULTS AND DISCUSSION

A. Graphing the standard curve and determination of the formula

1) The standard curve

TABLE 2 THE TEA SAPONIN CONTENT AND ABSORBANCE FOR THE STANDARD CURVE

Tea saponin content (ug)	0	90	180	270	360	450
Absorbance(A)	0	0.123	0.181	0.268	0.319	0.412

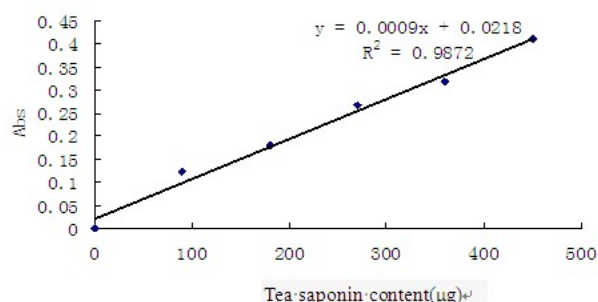


Fig-1 Standard curve of oil tea saponin (Vanillin-oil of vitriol color reaction)

2) Determination of the formula

According to the absorbance of the standard tea saponin solution and the standard curve with vanillin sulfuric acid colorimetric method, which is obtained at the maximum absorption wavelength of 545 nm at different concentration of tea saponin, the regression equation is as follow:

$$A=0.0009x+0.0218.$$

then combined with the standard curve, tea saponin content is calculated with equation:

$$\text{Tea saponin \%} = (A-0.0218) / 0.0009V \cdot N/W$$

Where A represents absorbance

V represents the volume of the solution

N represents dilution times

W represents the weight of the samples (ug)

B. Influence of ratio of liquid to solid on the extraction rate

The previous results showed that the greater the ratio of liquid to solid, the smaller the solution concentration, so is the mass transfer driving force and extraction speed. However, the amount of solvent would increase as the ratio of liquid to solid increases and increase the cost of condense afterward. Besides, the higher ratio of liquid to solid could also come up with more impurities in solution. Therefore, it is necessary to select

and determine the appropriate ratio of liquid to solid. Here the relationship between extraction rate and ratio of liquid to solid was shown in Figure 2.

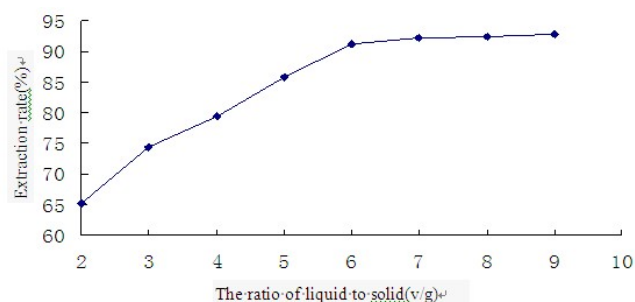


Fig-2 The relation between ratio of liquid to solid and extraction rate

The results showed that extraction rate increased with ratio of liquid to solid substantially, until the extraction rate reached 91.27% when the ratio comes to 6:1. Then increase trend slowed down and maintained in a stable level. Considering the costs of energy consumption and the risk of increasing impurity, 6:1 could be the optimum ratio for the tea saponin extraction.

C. Influence of temperature on the extraction rate

As shown in figure 3, extraction rate of tea saponin increased to 90.54% when the temperature rises from 40°C to 80°C. However, the extraction rate began to decline when the temperature is higher than 80°C, this is probably the accelerated decomposition rate when the temperature is higher than 80°C.

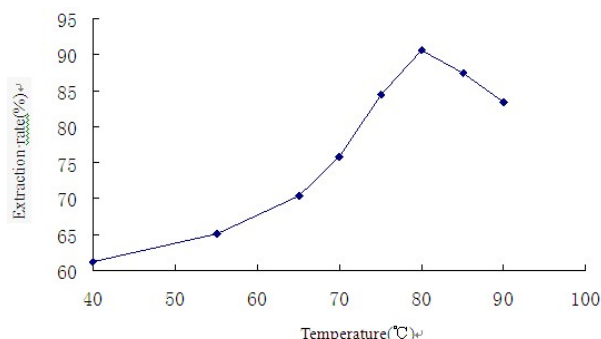


Fig-3 The relation between extraction temperature and extraction rate

D. Influence of extraction time on the extraction rate

Theoretically, the leaching process of solute diffused from the inside of the oil -tea camellia seed shell particles to the solution requires a certain time, and thus the extraction rate would increase over prolonged extraction rate, until the mutual transform speed reaches the balance point, the extraction rate will also maintain in a stable level. In order to find out the optimum extraction time, several different extraction time tests were conducted. The results showed that the tea saponin extraction rate increased over time and it reached 89.97% after 4h of extraction. This increase trend slowed down over prolonged time, suggesting balance point of the mutual solute transform could reached at 4h, which could also be the optimum extraction time for saponin from

shell of oil -tea camellia seed shell.

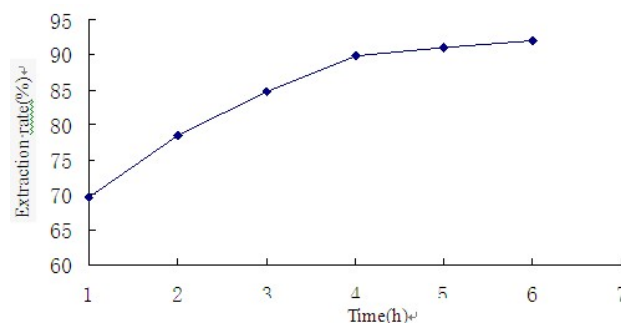


Fig-4 The relation between extraction time and extraction rate

E. Influence of pH on the extraction rate

As shown in Figure 5, the extraction rate increased with pH value when the pH is located between 3 and 10. The highest extraction rate reached 91.23% and then the tea saponin extraction rate showed downward trend from pH 10 to 11. It is notable that tea saponin is a weak acidic substance, and it is conducive for extraction of tea saponin under suitable conditions. However, on the one hand the dissolution of some alkali soluble impurities also excessive, on the other hand some tea saponin decomposition rate would be accelerated under alkaline conditions, which is confirmed from our result that the amount of tea saponin extract declined when the pH exceeds 10.

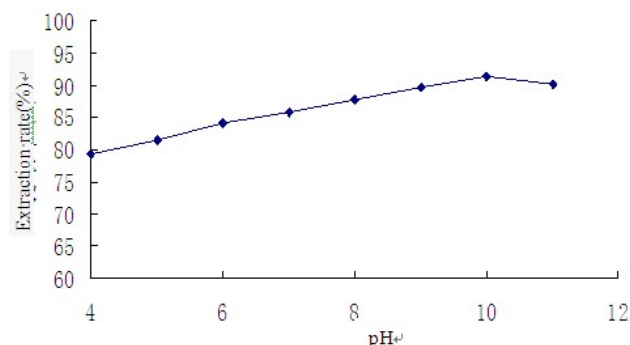


Fig-5 The relation between pH value and extraction rate

F. Optimization of tea saponin extraction conditions and selection of the optimum level

Orthogonal analysis showed that the main determinants order is A>B>C>D. The extraction temperature showed greatest impact on the tea saponin extraction rate, followed by ratio of liquid to solid and pH value. While the extraction time showed slightest impact on extraction rate. Therefore, the extraction temperature should be strictly controlled in the optimum level. From the results of orthogonal experiment, the best condition for tea saponin extraction is A2B2C1D3, namely the extraction temperature is 80°C, ratio of liquid to solid is 6:1, pH value for extraction is 9 and the extraction time is 6h.

TABLE 3. RESULT OF ORTHOGNAL TEST DESIGN AND TEA SAPONINE EXTRACTION RATE

No.	A	B	C	D	extraction rate(%)
1	1	1	1	1	91.43
2	1	2	2	2	89.79
3	1	3	3	3	85.87
4	2	1	2	3	92.68
5	2	2	3	1	94.66
6	2	3	1	2	92.23
7	3	1	3	2	86.14
8	3	2	1	3	91.74
9	3	3	2	1	84.95
K ₁	267.09	270.25	275.40	271.04	
K ₂	279.57	276.19	267.42	268.16	
K ₃	262.83	263.05	266.67	270.29	
k ₁	89.03	90.08	91.80	90.35	
k ₂	93.19	92.06	89.14	89.39	
k ₃	87.61	87.68	88.89	90.10	
R	5.58	4.38	2.91	0.96	

G. Determination of the selection and dosage of flocculant

As shown in Table 4, both poly aluminum chloride and alum could precipitate out the impurities to some extent, but effect of 30% poly aluminum chloride is better and faster than alum. Furthermore, the size of precipitate particles is also larger with 30% of poly aluminum chloride than that with alum. The results also showed that 16g poly aluminum chloride (30% concentration), that is 8% of the total preliminary extracted liquid, is best option for flocculant as the amount of precipitate produced is low and the saponin rate is also maintained at high level.

TABLE 4 COMPARISON AMONG DIFFERENT FLOCCULANTS

Flocculants	Tea saponin rate (%)	Precipitate color	precipitate amount
30% poly aluminum chloride 10g	22.74	brown	a little
30% poly aluminum chloride 16g	29.23	brown	a little
30% poly aluminum chloride 20g	31.67	brown	a lot
Alum 1%	15.31	light brown	a little
Alum 2%	21.78	light brown	a little
Alum 3%	25.43	light brown	a lot

H. Determination of CaO amount as precipitate agent

As shown in Figure 6, saponin precipitation rate showed a higher sedimentation rate as the amount of CaO increased. However, the precipitate rate decreased as the amount of CaO exceeded 10%, where the precipitate rate was the highest, reaching 82.86%. This is likely the result of the accelerated saponin decomposition under alkaline conditions induced by the excessive CaO addition.

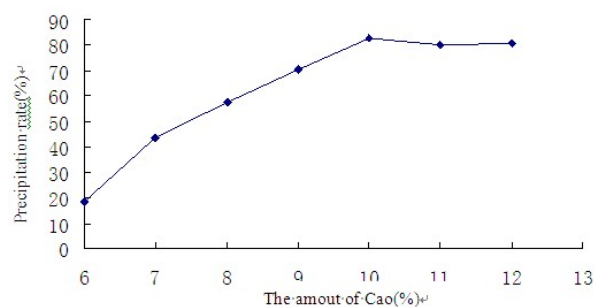


Fig. 6 Effect of CaO addition on precipitate rate

I. Determination of ammonium bicarbonate amount as tea saponin release agent

As shown in Figure 7, the saponin release rate increase with amount of release agent ammonium bicarbonate in the range of 30% of saponin calcium, in which the saponin release rate reach 72.36%. When the chemical equilibrium reaction rate reached 50% and the saponin release rate did not improve significantly even if more ammonium bicarbonate was applied.

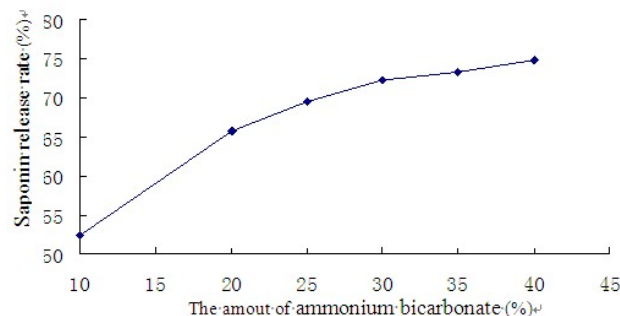


Fig-7 Effect of ammonium bicarbonate on saponin release rate

IV. CONCLUSIONS AND OUTLOOK

Through this experiment, The most suitable conditions and parameterize the factors were optimized for tea saponin extraction from shell of oil -tea camellia seeds: the extraction temperature is 80°C, ratio of liquid to solid is 6:1, pH value for extraction is 9 and the extraction time is 6h. Poly aluminum chloride (30% concentration) is the suitable flocculant and the optimum amount is 8% of preliminary extracted liquid by weight and placed still for 2 hrs. The optimum amount of CaO, as precipitate agent, is 10% of the weight of the raw materials, and stirred for 4h. The release agent, ammonium bicarbonate, was used in an amount of the 30% of the saponin calcium in order to achieve a chemical equilibrium reaction rate of 50%. In this study, we mainly focused on the extraction methods from raw material, not on the saponin purification. Therefore, there are some drawbacks on the process of tea saponin extraction on this aspect, for example, the saponin was also removed with impurities, while some impurities, such as some pigments, tannins, sugars, etc, is still in the solution during the flocculant process. What's more, it is valuable and deserves further investigation as oil -tea camellia seed shell contains many function polysaccharide (e.g. xylose), in addition to tea saponin. Regarding tea saponin, many difficulties are in the way of tea

saponin extraction and product development as the cost of industrial production cost is high, purification is very difficult and the content is quite low. In order to meet the industrial production requirements, therefore, more efforts are needed to enforce the researches and studies on the technology of tea saponin extraction and purification process.

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