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Extraction of Anthraquinone Compounds from Chinese Chestnut by Using Ultrasonic-assisted Technology

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Abstract: Ultrasonic assisted method was used to extract total anthraquinone compounds from non-enzymatic Browning chestnut kernel. The chestnut was heated in the microwave on 640w by a stainless steel knife for 60 s. Then fried it by heating 120°C for 30 min method result in non-enzymatic until browning of chestnuts fully. The anthraquinone in Chinese chestnut was extracted by ultrasonic cleaner with frequency of 80 kHz and power percentage of 80%. The absorption value was measured and calculated at 254 nm by ultraviolet spectrophotometer. The effects of ethanol concentration, ultrasonic time, extraction temperature and liquid-solid ratio on extraction efficiency were studied. The effect level of different factors on the extraction efficiency of anthraquinone compounds was as follows: liquid-solid ratio>Ultrasonic time > ethanol concentration > extraction temperature. The extraction rate of anthraquinone increased and reached the hightest point when liquid-solid ratio was between 2/1 and 4/1, and it decreased as the liquid-solid ratio rise. The extraction rate of anthraquinone increased with the increase of ultrasonic time, which was the maximum when ultrasonic time was 30 minutes, and the extraction rate remained stable. The extraction rate of anthraquinone increased with the increase of ethanol concentration. The extraction rate reached the maximum when the ethanol concentration was 80%, and tends to be stable with the increased of the concentration. The extraction rate of anthraquinone was increased sharply before the temperature reached 60°C. And it maintained stable at the highest point between 60°C and 70°C. According to the regression equation and the single factor experiment, the optimum extraction technology were ethanol concentration (85%) ultrasonic time (35min) extraction temperature (60°C) and liquid-solid ratio was 4.5:1. The extraction rate of chestnut anthraquinones was up to 77.31μg/g dry weight.

Keywords: Chinese Chestnut, Anthraquinone, Extraction, Ultrasonic

1. Introduction

Chinese chestnut is a *Castanea Miller* plant in the Fagaceae family. China is the country of origin of Chinese chestnuts. The cultivation of chestnut has a long history which dating back to the western zhou dynasty. Now, it has been widely cultivated by artificial, mainly distributed in Taiwan, Vietnam and the Chinese mainland. Chinese chestnut is called woody grain, hardcore crop. [1]

Non-enzymatic browning of chestnut mainly includes maillard reaction, Caramelization reaction, ascorbic acid oxidationand, automatic oxidation of polyphenols. [2, 3] In his study, Wu Y H [4] showed that the content of anthraquinone was very low before non-enzymatic browning but after full browning of chestnut, the content of anthraquinone increased by 70.11%, indicating that anthraquinone was one of the important products of browning.

Anthraquinones and their derivatives are widely distributed

in plants, such as polygonum cuspidatum, polygonum multiflorum, semen cassiae, madder, aloe and rhubarb. There are 3 kinds of anthraquinone in nature, The main types are emodin type and alizarin type [5, 6].

Liu Q D et al. showed that aloe vera extract of anthraquinone compounds had a certain ability to scavenge DPPH free radicals. And the scavenging ability showed a significant dose-effect relationship with the concentration [7, 8].

Agarwal S K et al. [9, 10] showed that the rhubarb acid in anthraquinone had a relatively effective inhibitory effect on staphylococcus aureus, streptococcus aureus, diphtheria, subtilis, typhoid and paratyphoid bacillus.

Emodin has a cathartic effect on experimental rats. It inhibited of absorption and the stimulation of water secretion by the large intestine. It would cause an increase in water capacity [11] and lead to watery diarrhea [12].

The water extraction and the organic solvent extraction are the most commonly used methods in daily life and in the laboratory. For example, the water extraction method is used for cassia seed tea, and the alcohol extraction method is used for medicinal liquor.

Ultrasonic extraction technology is used ultrasound radiation pressure to produce a new extraction method of cavitation effect. Then by mechanical stirring, it promotes the dissolution of diffusion. Ultrasonic assisted extraction method used ultrasonic cleaning instrument or ultrasonic tissue crushing device. Ultrasonic extraction technology is more convenient operation which compared with the traditional extraction and extraction with high efficiency with short extraction time [13].

2. Materials and Methods

2.1. Sample

The chestnut which used in the experiment was Yanshan chestnut. And it came from Funing county in Hebei province. Firstly, the fresh chestnut was treated with enzyme killing method by Wu Y H [4]. The chestnut was heated in the microwave on 640w by a stainless steel knife for 60 s. Then fried it by heating 120°C for 30 min method result in non-enzymatic until browning of chestnuts fully. After cooling, remove the shell and astringent were peeled, cut into pieces and put into sealing bag for later use.

2.2. Ultrasonic Assisted Extraction

Samples from 25 g chestnuts were prepared in a 250 ml conical flask with 100 ml 70% ethanol (Beijing chemical factory) in ultrasonic cleaners (KQ-600 vde, Kunshan ultrasonic instruments co., LTD.). The temperature was set to 50°C, the frequency was 80 kHz, and the power percentage was 80%. After 1 h of assisted extraction, the mixture was filtered and steamed with ethanol (Beijing chemical factory). The mixture was extracted with chloroform (Beijing chemical factory) until the chloroform layer became colorless, and then chloroform solution was combined, and then chloroform was recovered by rotary distillation. The concentrate was made up to 25 mL of methanol. The absorbance was measured at 254nm using adual-beam UV-Visspectrophotomete (Tu-1910, Beijing purdue general instruments co., LTD.), and three experiments were performed in parallel [14].

2.3. The Single Factor Extraction Test for Ultrasonic Assisted

In the process of ultrasound-assisted extraction of anthraquinones, extraction temperature, ultrasonic time, liquid-material ratio, ethanol concentration and other factors which have a great impact on the extraction rate. In this experiment, the single factor experiment was used to determine the best extraction conditions, and then the orthogonal experiment was conducted to determine the best extraction conditions of the anthraquinone compound.

2.3.1. Extraction Temperature Effect on Extraction

The five samples which weighted 25 g of the pretreatment of chestnut in the 250mL. Conical flask were add 100 mL 70% ethanol in ultrasonic cleaning machine. The temperature in 30, 40, 50, 60, 70°C condition, the ultrasonic assisted extraction of 30 min. Then, according to the "2.2" test method above, the absorbance of the extract was measured and the extraction rate of the anthraquinone compound was calculated.

2.3.2. The Effect of Ultrasonic Time on Extraction

The five samples which weighted 25 g of the pretreatment of chestnut in the 250mL conical flask were added to 100 mL 70% ethanol in ultrasonic cleaning machine. Under the environment of the temperature which at the condition of 50°C, respectively, the ultrasonic assisted extraction was applied for 10, 20, 30, 40, 50 min. According to the "2.2" test method above and the extraction rate of the anthraquinone compounds was calculated.

2.3.3. The Effect of Liquid - Material Ratio on Extraction

The five samples which weighted 25 g of the pretreatment of chestnut in the 250mL conical flask were added to 50, 75, 100, 125, 150 mL of 70% ethanol in ultrasonic cleaning machine. The temperature under the condition of 50°C and the ultrasonic assisted extraction of 30 min. According to the "2.2" test method above and the extraction rate of the anthraquinone compounds was calculated.

2.3.4. The Effect of Ethanol Concentration on Extraction

The five samples which weighted 25 g of the pretreatment and chestnut in the 250 mL conical flask were added to 100 mL of 40, 50, 60, 70, 80% ethanol ultrasonic cleaning machine, then ultrasonic-assisted extraction was carried out for 30 min at the temperature of 50°C. According to the "2.2" test method above and the extraction rate of the anthraquinone compounds was calculated.

2.4. Orthogonal Test Design

On the basis of single factor experiment, the optimal parameters of each factor were selected to extract factors. Such as temperature, ultrasonic time, ethanol concentration

and liquid-material ratio. And the orthogonal experiment of four factors and three levels, namely L9 (3^4) which was designed as shown in Table 1.

Table 1. *Factors and levels of L9 (3^4) orthogonal test.*

Level	A Extraction temperature (°C)	B Ultrasonic time (min)	C Ethanol concentration (%)	D Liquid-to-solid ratio
1	55	25	75	3.5:1
2	60	30	80	4:1
3	65	35	85	4.5:1

The data analysis

SPSS software was used to analyze the data in this experiment, and the significance level of ANOVA was 0.05. When $P < 0.05$, the difference was significant.

3. Results and Discussions

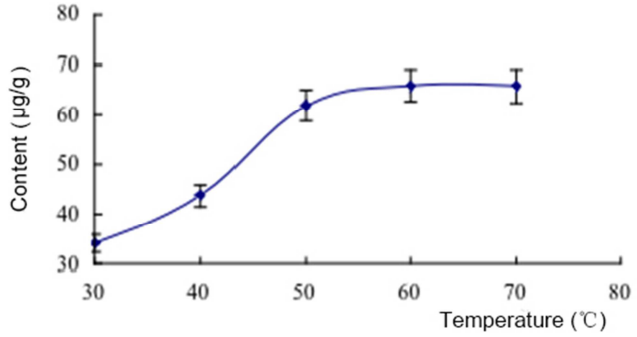

Figure 1. *Effect of extracting temperature on the concentration of anthraquinones.*

In Figure 1, the extraction rate of anthraquinone from non-enzymatic browning chestnut was increased rapidly at first and then became stable with the high temperature. At the temperature of 60°C, the amount of extraction of the content of anthraquinone compounds reached the highest. No significant difference ($P>0.05$). Thus, from the comprehensive consideration was chosen 60°C for the extraction of the optimal temperature. Therefore, 55°C, 60°C, 65°C were used for orthogonal experiment of three levels.

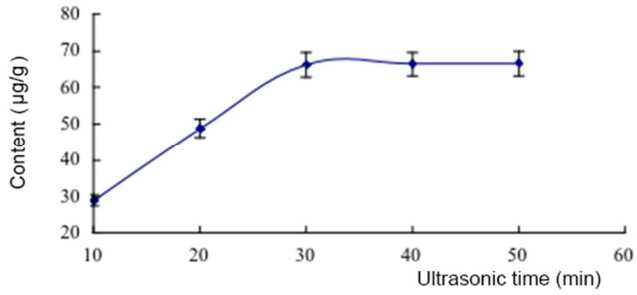

Figure 2. *Effect of extracting time on the concentration of anthraquinones.*

In Figure 2, the extraction rate of anthraquinone from non-enzymatic browning chestnut was increased with the increasing time. After 30 minutes, the overall trend was flat in a slight decline. Because the extraction had reached the equilibrium state which was extracted within 30 minutes. When the time was increased for 40min, under the condition of ultrasonic extraction, there was no significant difference between the extraction effect at 40min and 30min. $P > 0.05$. Therefore, the optimal ultrasonic time was selected for 30min. And 25 min, 30 min and 35 min were selected as the three levels of the orthogonal test.

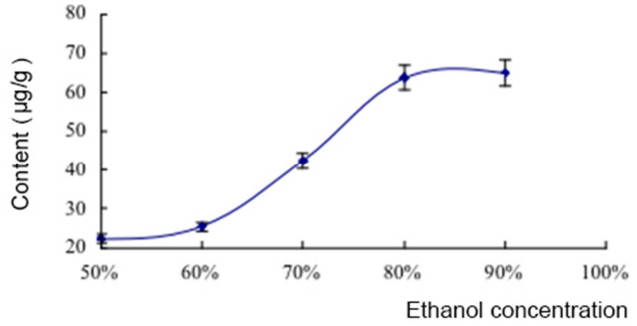

Figure 3. *Effect of ratio on the concentration of anthraquinones.*

In Figure 3, the extraction rate of anthraquinone from non-enzymatic browning chestnut was within the range of 2/1~4/1, it increased with the high material-liquid ratio. When the material to liquid ratio reaches a quarter, the extraction rate reached the maximum. After that, the solid-liquid ratio continued to increase and the extraction rate decreased. The increasing of the solid-liquid ratio accelerates the mass transferring process of the solvent and contributed to the dissolution of the extract. However, when the ratio of material to liquid increased to a certain extent, the solvent leaching of the extract reached an equilibrium state. Further increasing the solid-liquid ratio will caused a large amount of impurities to be dissolved, which was disadvantageous for extraction [15]. Therefore, 4/1 was selected as the optimal liquid-material ratio. And 3.5/1, 4/1 and 4.5/1 were selected as the three levels of the orthogonal test.

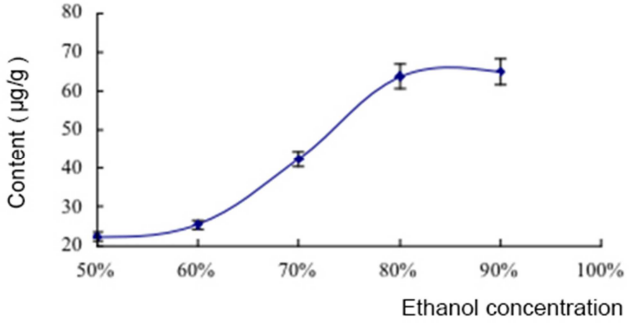

Figure 4. *Effect of ethanol concentration on the concentration of anthraquinones.*

In Figure 4, when the ethanol concentration was 50% and 60%, the extraction rate of anthraquinone was very low. Then, as the ethanol concentration increased, the extraction rate began to increase rapidly. When the ethanol concentration is 80%, the extraction rate reached a maximum. After analysis of variance, there was no significant difference in the content of terpenoids between 80% and 90% (P>0.05). This was the reason that the terpenoids are less polar and hardly soluble in water. When the concentration of ethanol was about 80%, the polarity of the solvent was consistent with the polarity of the anthraquinone, and the extraction rate was the highest, so the principle of similar compatibility was satisfied. Therefore, 80% was selected as the optimal ethanol concentration. And 75%, 80% and 85% were selected as the three levels of the orthogonal test.

On the basis of single factor experiment, four factors and three levels orthogonal experiments were conducted on extraction temperature, solvent concentration, ultrasonic time and liquid-material ratio, with the content of anthraquinone compounds (g/g dry weight) as the experimental index. Experimental scheme and result analysis were shown in Table 2.

Table 2. *Analysis of results of L9 (3^4) orthogonal test.*

Experimental Group	Factors				Content (μg/g Dry Weight)
	A Temperature (°C)	B Ultrasound Time (min)	C Ethanol Concentration (%)	D Liquid-to-SolidRatio	
1	1	1	1	1	64.37
2	1	2	2	2	62.82
3	1	3	3	3	76.36
4	2	1	2	3	72.59
5	2	2	3	1	65.74
6	2	3	1	2	66.55
7	3	1	3	2	65.84
8	3	2	1	3	66.86
9	3	3	2	1	65.83
k1	67.85	67.60	65.93	65.31	
k2	68.29	65.14	67.08	65.07	
k3	66.18	69.58	69.31	71.94	
R	2.11	4.44	3.38	6.87	
Optimal Combination	A2	B3	C3	D3	

According to the orthogonal experiment and range analysis, for the anthraquinone compound, the influence of various factors on the extraction effects were as follows: solid-liquid ratio> ultrasonic time> ethanol concentration> extraction temperature. The optimum process condition was A2B3C3D3. The best extraction condition was: ethanol concentration of 85%, ultrasonic time was 35 min, extraction temperature of 60°C, Material to liquid ratio: 4.5:1.

According to the orthogonal experiment, there are some factors to determine the condition: ethanol concentration of 85%, ultrasonic time was 35 min, extraction temperature of 60°C, solid-liquid ratio of 4.5:1. Methods from the content of anthraquinone compounds in the extract was 77.31±0.32 μg/g. Therefore, the extraction conditions optimized by orthogonal test could be used as the best conditions for ultrasonic assisted extraction of anthraquinone compounds.

4. Conclusion

Four factors including extraction temperature, ultrasonic time, ethanol concentration, and solid-liquid ratio were considered for the extraction rate. And the orthogonal experiment was carried out. In the end, the optimum of technological conditions were ethanol concentration of 85%, the extracting time of 35 min, extraction temperature of 60°C and solid-liquid ratio of 4.5:1.

Under these conditions, the extraction yield of anthraquinone compounds reached 77.31 μg/g dry weight.

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