Ultrasonic extraction of anthraquinone from walnut green husk

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Abstract. Anthraquinone is the main component of walnut green husk. In order to measure the content of anthraquinone from walnut green husk, ethanol extraction with assisted 40 kHz ultrasonic and ultraviolet spectrophotometer method were used in this study. The extracting effect of anthraquinone by ultrasonic wave and its influenced factors were studied. The best extracting scheme and process parameters were discussed. The different extracting conditions for anthraquinone were studied. The results showed that the extracting rate of anthraquinone was related to ethanol concentration, ultrasonic time, extracting temperature and other factors. The effect of different factors on the extraction of anthraquinone was as follows: extracting temperature > ultrasonic time > ethanol concentration. According to experiments, the best extracting parameters were temperature (60 °C), ethanol concentration (70%), ultrasonic time (30 minutes). The anthraquinone content could reach 5.7 mg/g.

1. Introduction
Walnut, a perennial deciduous tree of the genus Juglanaceae [1]. It is widely distributed in China with many varieties and rich in resources [2]. China is the world's largest country for producing walnut and is now mainly distributed in Xinjiang, Yunnan, Shanxi and Hebei [3]. Walnut green husk, also known as Qinglongyi, is a thick layer of immature green peel on the outside of walnut, without special smell and bitter taste [4-5]. Walnut green husk, as a valuable reusable resource, is valuable for further development and utilization. The main chemical components in walnut green husk are quinones, terpenoids, polyphenols, diarylheptanes, and flavonoids [6]. At present, the research on extracting methods of anthraquinone in walnut green husk is less [7]. Anthraquinones had anticancer, analgesic, antioxidant, and antibacterial effects [8]. Zhong J M et al. [9] used ethanol to extract anthraquinone from walnut green husk. Ding Y et al. [10] showed that the extraction rate of anthraquinone from walnut green husk was (4.12 ± 0.01) mg/g. At present, the main methods for determining anthraquinone content were colorimetry, thin-layer chromatography, and ultraviolet spectrophotometry [11]. In this paper, the content of anthraquinone in walnut green husk was determined by ultraviolet-visible spectrophotometry. The method was simple, convenient and fast, and had high accuracy.
The traditional extracting method of anthraquinone compounds was solvent extraction, which used organic solvents such as chloroform, which polluted the environment and had high toxicity [12]. The hot reflux method was easy to break the active ingredients because of the long heating time [13]. Microwave extraction compared with ultrasonic extraction, the efficiency of microwave extraction was lower and microwave was harmful to human body [14]. Ultrasonic extraction had the advantages of low extracting temperature, high extracting rate and short extracting time [15], and there was little damage to the active ingredients and little loss in the extracting process. In this paper, ultrasonic extraction was chosen. This method provided a reference for the extraction of effective components and laid a foundation for further research.

2. Materials and methods

2.1. Sample
The walnut used in this research was harvested in Shijiazhuang City, Hebei Province in 2019.

2.2. Ultrasonic extraction of anthraquinone
The ultrasonic frequency was 40 kHz and the ultrasonic power was 100 W. The absorption value of the sample was determined at 510 nm for ultraviolet photometer.

2.3. Anthraquinone standard curve
10.0 mg 1,8-dihydroxyanthraquinone standard solution was weighed accurately, and dissolved in an appropriate amount of ether by shaking well, and was added with methanol to make the volume to 50 mL. 5 mL solution was placed in a 25 mL volumetric flask. The standard solutions of 1.0 mL, 2.0 mL, 3.0 mL, 4.0 mL, 5.0 mL and 6.0 mL were respectively transferred into a 10 mL brown bottle, which was added 0.5% magnesium acetate-methanol solution to scale. Besides, 0.5% magnesium acetate-methanol solution was used as blank control. After that, the absorption value of the standard solution at 510 nm was measured and then the standard curve was drawn with anthraquinone mass concentration \(c (\text{mg/mL})\) and absorbance value \(A\).

1 mL anthraquinone extract was used and 0.5% magnesium acetate-methanol solution was added to the scale and the absorbance value was measured. The determination was performed three times in parallel and the average value was taken to obtain the contents of anthraquinone in the extract from the standard curve.

2.4. Single factor experiment
The extraction rate of anthraquinone was related to ethanol concentration, ultrasonic time, extracting temperature and other factors. The independent and dependent variables were determined, and the single factor experiment was carried out.

2.4.1. Effects of different ethanol concentration on the content of anthraquinone. Five portions of 10 g fresh walnut green husk were accurately weighed, and 30 mL ethanol solutions of 50%, 60%, 70%, 80% and 90% were added in turn, then filtered at 60 °C for 60 min under ultrasound. The volume of the solution was fixed to 25 mL with the corresponding concentration of ethanol, 1 mL was taken and added to the methanol solution of magnesium acetate in a 25 mL volumetric flask, then shaken and left to stand still. Finally, the absorption value was measured at 510 nm.

2.4.2. Effects of different ultrasonic time on the content of anthraquinone. Five portions of 10 g fresh walnut green husk were accurately weighed, and 30 mL of 50% ethanol was added in turn, then filtered at 60 °C for 20 min, 30 min, 40 min, 50 min and 60 min respectively. The corresponding concentration of ethanol solution was added to 25 mL, and then 1mL was made up to 25 mL with magnesium acetate methanol solution, mixed and allowed to stand. Next, the absorption value was measured at 510 nm.
2.4.3. Effects of different extraction temperature on the content of anthraquinone. Five portions of 10 g fresh walnut green husk were accurately weighed, and 30 mL of 50% ethanol was added in turn, and the impurities were filtered by ultrasound at 40 °C, 50 °C, 60 °C, 70 °C and 80 °C for 60 min. The corresponding concentration of ethanol solution was used to make up to 25mL and 1mL was removed to make up to 25mL with magnesium acetate methanol solution, mixed and left for a while. Last, the absorbance value was measured at 510 nm.

2.5. Orthogonal experiment design

Through the single factor test, the better treatment condition parameters were selected and the orthogonal test design was carried out. There were 3 factors and 3 levels in table 1.

| Levels | Ethanol concentration(A)/% | Ultrasonic time (B)/min | Extracting temperature(C)/℃ |
|--------|---------------------------|-------------------------|-----------------------------|
| 1      | 70                        | 30                      | 40                          |
| 2      | 80                        | 40                      | 50                          |
| 3      | 90                        | 50                      | 60                          |

3. Results and discussion

3.1. Standard curve of anthraquinone

The results analysis showed that the regression equation was $Y = 0.0478X + 0.0097$, $r = 0.9978$. The linear relationship of the regression equation was good.

3.2. Results of single factor experiment

As could be seen from Figure 2, with the increase of ethanol concentration, the anthraquinone extraction content gradually increased. When the ethanol concentration reached 80%, the anthraquinone content reached the highest. The ethanol concentration was very significant for the yield of anthraquinone. Therefore, 70%, 80% and 90% ethanol concentration were selected.
It could be seen from Figure 3 that the solubility of anthraquinone increased with time increased. When the ultrasonic extraction time was 40 min, the anthraquinone extraction content reached the highest level firstly. With the change of ultrasonic time, the content of anthraquinone fluctuated, but the change was not obvious. Too short ultrasonic time was not conducive to the dissolution of anthraquinone, but too long time could increase the amount of substances, which might lead to impurities. The ultrasonic time was 30 min, 40 min and 50 min, and the following experiments were carried out.

As shown in Figure 4, when the extraction temperature was 50 °C, the anthraquinone extraction content was the highest. When the extraction temperature was higher than 50 °C, the yield of anthraquinone decreased with the increase of temperature. However, when the extracting temperature rose to a certain level, the amount of extraction no longer increased and the active ingredients were destroyed. Further increase in temperature was not conducive to extraction. Thus, the appropriate extracting temperature should be controlled. The extracting temperature was 40 °C, 50 °C and 60 °C, and the orthogonal experiment was carried out to obtain the best extraction effect.

3.3. Results of orthogonal experiment

| Experimental Groups | Extracting Temperature(A)/°C | Ultrasound Time (B)/min | Ethanol Concentration(C)/% | Content (mg/g) |
|---------------------|-------------------------------|-------------------------|----------------------------|----------------|
| 1                   | 1                             | 1                       | 1                          | 4.48           |
| 2                   | 1                             | 2                       | 3                          | 5.35           |
| 3                   | 1                             | 3                       | 2                          | 4.37           |
| 4                   | 2                             | 1                       | 2                          | 4.79           |
| 5                   | 2                             | 2                       | 1                          | 3.52           |
| 6                   | 2                             | 3                       | 3                          | 4.76           |
| 7                   | 3                             | 1                       | 3                          | 5.65           |
| 8                   | 3                             | 2                       | 2                          | 5.15           |
| 9                   | 3                             | 3                       | 1                          | 3.38           |
| k1                  | 4.74                          | 4.98                    | 3.90                       |
| k2                  | 4.36                          | 4.68                    | 4.78                       |
| k3                  | 4.73                          | 4.17                    | 5.26                       |
| R                   | 0.37                          | 0.80                    | 1.46                       |

The range analysis of orthogonal experiment showed that the best technology condition of ultrasonic assisted ethanol extraction of anthraquinone was A1B1C3, that was, the extraction of anthraquinone with 70% ethanol for 30 minutes at a temperature of 60 °C. As a result, The content of anthraquinone
was 5.7 mg/g under the optimum conditions. The primary and secondary order of the influence of the three factors on the yield of anthraquinone was: extracting temperature > ultrasonic time > ethanol concentration.

4. Conclusion

The results showed that ultrasonic assisted ethanol extraction was an effective method for the extraction of anthraquinone. In conclusion, when the extracting temperature was 60 ℃, the ethanol concentration was 70% and the ultrasonic time was 30 min, the extraction content of anthraquinone could reach 5.7 mg/g. The results showed that the extraction rate of anthraquinone was related to ethanol concentration, ultrasonic time, extracting temperature and other factors. It could be seen that this study provided a complete theoretical basis for the extraction of anthraquinone from walnut green husk, which was of great significance.

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