Extraction and Dyeing Property of Juglone from Walnut Green Husk

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Abstract. Ultrasonic method for the extraction of juglone from walnut green husk was performed. The content of juglone was determined by spectrophotometry at wavelength 421 nm based on juglone as standard. Ultrasonic time, the solvent to sample ratio were selected for the single-factor experiment. By orthogonal experiment, the optimum condition was determined as solvent to sample ratio was 40:1, extracting temperature was 25℃, extracting time was 25min. In this condition, the actual content of juglone could reach to 2.5574mg/g. In this paper, the crude extracts of juglone from walnut green husk was used to dye something. The results had shown that when sodium sulfate was used to as a mordant, the coloring degree would be the deepest. The coloring degree became deeper and deeper with the dyeing time increasing. There was no significant difference in coloring degree between 120 min and 160 min.

1. Introduction
Juglone was found in walnut of walnut family and penicillium[1], its name is called 5-hydroxy-1,4-naphthoquinone, moreover, the content of juglone in walnut green husk was higher than that in bark and leaf [2]. Juglone was unstable in nature and could be degraded by high temperature, pH as well as illumination[3]. At present, the main methods to obtain juglone were extraction, separation from plants and chemical synthesis. The extracting methods of juglone had been reported, such as cold immersion, ultrasonic extraction, reflux extraction, decompression distillation[4-5]. The structure of juglone were purified and identified in 1985[6]. Some researchers determined the content of juglone in walnut green husk by HPLC[7]. The chemical synthesis methods mainly included 5, 8-dihydroxyl-1-tetrahydronapththone intermediate synthesis, synthesis of naphthalene by nitration and oxidation, synthesis of naphthalene by sulphonation oxidation, ozone regenerated chromic acid oxidation[8]. Studies had shown that juglone had significant activities of antibacterial, antitumor, anti hypertensive
and enzyme inhibition \cite{9-10}.

In recent years, more and more studies had been done on the antitumor effect of juglone in vitro\cite{11}. It was reported that juglone had inhibitory effect on various tumors in vitro, such as leukemia HL-60\cite{12}, liver cancer BEL-7402\cite{13}, lung cancer A549\cite{14}, colon cancer CT-26\cite{15}, and the like. In recent years, cultivation area of walnut in China increased by 10\%, it was expected to reach to 2.67 million hm\(^2\) by 2020, and the annual output would reach to 3 million tones. Therefore, it was of high value to study the extraction of juglone by simple method\cite{16}.

2. Materials and Reagent

2.1 Materials
The walnuts were harvested from luancheng district, shijiazhuang city, hebei province in 2017.

2.2 Reagents
Ethyl Acetate and Methanol was purchased from Beijing Chemical Works, China. Juglone was purchased from Shangai YuanYe Biotechnology co. LTD, China. Ethyl acetate and methanol were of analytical grade, Juglone were of HPLC grade.

3. Experimental Methods

3.1 Preparation of Standard Solution
The 2.00mg juglone sample was placed in a 50mL brown flask, dissolved in methanol, diluted to scale and shook well, then got the standard solution.

3.2 Linear Relation Survey
The sample of 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 mL were added in 10mL brown bottle, added anhydrous methanol to scale, measured the light absorption value at wavelength 421 nm. The standard curve was drawn with the mass concentration of juglone c(mg/mL) and the absorbance value A\cite{17-18}.

3.3 Preparation of Walnut Green Husk Powder
The fresh walnut green husk were cut into 1cm×1cm pieces and added ethyl alcohol according to the solid-liquid ratio of 1:30, and then, extractions were performed at 25°C for 30min, the filtered residues was placed in a setting that avoided light and ventilation until they dried out, shredded the dried walnut green husk, which was sieved with 60 mesh, then got the powder of walnut green husk.

3.4 Preparation of Experimental Solution
The 1.00g powder of walnut green husk was mixed with 25mL ethyl acetate, and then, they were accurately weighed and placed in 100mL conical flask, the extractions were carried out for 30min at 25°C, shook well, filtered after static setting, the suspensions were then filtered to no ethyl acetate through a rotary evaporator, dissolved in methanol, filtered out insoluble matter, and transferred to 50 mL brown solution bottle, then got the solution.

3.5 The Precision Experiment
The 3.0mL solution of the experimental product was accurately placed in 10mL volumetric flask, followed the standard curve making method, and then, the absorbance value was measured for 8 consecutive times, RSD was 0.21\%, it indicated that the instrument that we used had a good precision.

3.6 The Stability Experiment
The 3.0mL solution of the experimental product was accurately placed in 10mL volumetric flask, followed the standard curve making method, and then the absorbance values were measured at 20, 40, 60, 80, 100, 120, 150, 200 min respectively, 8 parallel values were measured each time. RSD was 3.42%, this indicated that the sample solution was stable within 3.5h.

3.7 The Reproducibility Experiment
The 1.0g walnut green husk of the same batch was added in 9 conical flasks respectively, followed the standard curve making method, and then the absorbance value was measured for 8 consecutive times. Average content of juglone was 2.5574 mg/g, RSD was 3.98%.

3.8 Single-Factor Experiment
Ultrasonic time and sample to solvent ratio on the extraction of juglone from walnut green husk were assessed.

The 1.0g powder of walnut green husk was placed in a flask in ultrasonic synergistic response instrument, extractions were performed at 25°C and different time (10, 20, 30, 40, 50min), at different sample to solvent ratio (1:15, 1:25, 1:35, 1:45, 1:55); the suspensions were then filtered through a rotary evaporator at 30°C; extracts be dissolved by 40mL methyl alcohol; filtered to remove insoluble matter; metered volume to 50 mL with methyl alcohol; 3 mL extracting solution was added in 10mL flask, followed the standard curve making method; and then, measured the light absorption value three times at wave length 421 nm; then, the light absorption value was taken into the standard curve to calculate the content of juglone.

3.9 Orthogonal Design
The 1.0g walnut green husk was added in 9 conical flasks respectively, the extracting rate of juglone was taken as the index, and the sample to solvent ratio and time was selected as the factors. The experiment was carried out according to L9(34) orthogonal table to investigate the extracting technology of juglone from walnut green husk[19]. Each factor was set at 3 levels, as shown in table 1.

Table 1. Orthogonal Experimental Factors of Extracting Technology of Juglone from Walnut Green Husk.

| Levels | A Time/min | B the Sample to Solvent Ratio |
|--------|------------|-----------------------------|
| 1      | 25         | 1:30                        |
| 2      | 30         | 1:35                        |
| 3      | 35         | 1:40                        |

3.10 Preliminary Study on Dyeing Effects
This paper focused on the influence of different metal ions and different time on dyeing effects to provide a reference for developing new dyeing products.

3.10.1 Preparation of Hair Dyes and Influence of Different Dyeing Time on Dyeing Effects
Ultrasonic extraction was carried out according to the best extracting technology of juglone, we could obtain the crude powder over filtered, the evaporated was at 30°C. 1.0g powder was dissolved in 50mL ethyl alcohol, and then hair dyes could be obtained by adding 1.5g of ferrisulphas, magnesiu acetate, sodium sulfate, copper sulfate, potassium chloride respectively.

7.5g grey dog’s hair was divided into 5 parts on average, then they were added in the dyeing liquid, and were performed at 40°C for 1h.

3.10.2 Influence of Different Dyeing Time on Dyeing Effects
The 8.0g powder of walnut green husk with 7.5g sodium sulfate was added in 400mL ethyl alcohol,
and it was divided into 8 parts on average, and were performed at 40℃and at different time (20, 40, 60, 80, 100, 120, 140, 160 min).

4. Results and Discussion

As shown in figure 1, the regression equation: $Y = 0.0349X - 0.0065$ ($r = 0.9993$), it showed that juglone had a good linear relationship between 0 and 16.00μg·mL$^{-1}$.

According to figure 2, we could conclude that with the extension of ultrasonic time, the extracting amount of juglone would increase firstly, when the ultrasonic time exceeded 30min, the extracting amount of juglone decreased gradually. Juglone was a small molecule of naphthone, it was sensitive to temperature, it was easy to sublimate at 45℃, and the long time of ultrasonic treatment lead to the solution temperature rised, so the stability of juglone was destroyed by high temperature.

According to figure 3, the effects of different solid-liquid ratio on juglone were shown.

Figure 1. Standard Curve of Juglone.

Figure 2. Effects of Different Time on Juglone.

Figure 3. Effects of Different Solid-liquid Ratio on Juglone.
We could learn from figure 3 that with the rise of solid-liquid ratio, the extracting amount of juglone would increase. But such increase was not observed when solid-liquid ratio was increased from 1:35 to 1:45. Considered the saving of extracting solvent, it was more appropriate to select the ratio of 1:35.

Table 2. Orthogonal Experimental Results of Extracting Technology of Juglone from Walnut Green Husk.

| No. | A  | B  | The content of Juglone mg/g |
|-----|----|----|-----------------------------|
| 1   | 1  | 1  | 2.5091                      |
| 2   | 1  | 2  | 2.5549                      |
| 3   | 1  | 3  | 2.6425                      |
| 4   | 2  | 1  | 2.4833                      |
| 5   | 2  | 2  | 2.5446                      |
| 6   | 2  | 3  | 2.5748                      |
| 7   | 3  | 1  | 2.4821                      |
| 8   | 3  | 2  | 2.5427                      |
| 9   | 3  | 3  | 2.5732                      |
| K1  | 7.7065 | 7.4745 |
| K2  | 7.6027 | 7.6422 |
| K3  | 7.598  | 7.7905 |
| K1  | 2.5688 | 2.4915 |
| K2  | 2.5342 | 2.5474 |
| K3  | 2.5326 | 2.5968 |
| R   | 0.0361 | 0.1053 |

Table 3. Analysis of Variance of Extracting Technology.

| Source            | SS     | df | F     | P     |
|-------------------|--------|----|-------|-------|
| Time              | 0.003  | 2  | 4.274 | >0.05 |
| Solid-liquid Ratio| 0.017  | 2  | 28.404| <0.05 |
| Error             | 0.001  | 4  |       |       |

It could be seen from table 2 and table 3 that the order of influence of each factor on the extracting technology was B>A, the sample to solvent ratio had great influence, followed by extracting time. Analysis of variance showed that factor B had significant effect on extracting technology. We could determine that the optimal combination was A1B3, this demonstrated that extractions were performed at 25min, sample to solvent ratio was 1:40. Three validation experiments were carried out according to the optimal technology, and the results showed that the extracting amounts of juglone were 2.5833, 2.5620, 2.5270 mg/g, RSD was 1.11%. Optimal extracting technology was stable.

Table 4. Dyeing Effects of Different Metal Ions.

| Different Metal Ions    | Different Colours          |
|-------------------------|----------------------------|
| ferrisulphas            | yellow                     |
| magnesium acetate       | yellow                     |
| sodium sulfate          | wine-colored               |
| copper sulfate          | blackish green             |
| potassium chloride      | Yellow                     |
Table 5. Influence of Different Metal Ions on Dyeing Effects.

| Different Metal Ions       | L*   | a*   | b*   | ∆E  |
|----------------------------|------|------|------|-----|
| standard sample            | 65.79| 5.99 | 19.88|     |
| errisulphas                | 36.26| 6.49 | 22.24| 29.64|
| potassium chloride         | 32.14| 5.64 | 21.81| 33.72|
| magnesium acetate          | 29.93| 6.56 | 17.86| 35.93|
| copper sulfate             | 28.27| 3.34 | 14.30| 38.03|
| sodium sulfate             | 26.86| 8.23 | 11.35| 39.92|

It could be seen from table 4 and table 5 that copper ion, sodion were mordants with good dyeing effects.

Table 6. Influence of Different Dyeing Time on Dyeing Effects.

| Time      | L*   | a*   | b*   | ∆E  |
|-----------|------|------|------|-----|
| standard sample | 65.79| 5.99 | 19.88|     |
| 20 min    | 37.24| 6.55 | 14.37| 29.08|
| 40 min    | 33.06| 7.89 | 14.01| 33.31|
| 60 min    | 25.87| 8.45 | 13.76| 40.75|
| 80 min    | 23.76| 8.98 | 12.77| 42.73|
| 100 min   | 21.45| 9.47 | 11.36| 45.29|
| 120 min   | 18.13| 9.87 | 11.23| 48.59|
| 140 min   | 17.22| 10.42| 10.01| 49.76|
| 160 min   | 17.01| 10.49| 9.78 | 50.02|

It could be seen from table 6, the staining lasted from 120 to 160 min, and there was no difference between 120 to 160 min, so in order to save time, we could selecte for 120 min.

5. conclusion

Most scholars employed high performance liquid chromatography (HPLC) to study the extracting technology of juglone, but this method was complex and the cost was high. Therefore, spectrophotometry was adopted to study the extracting technology of juglone from walnut green husk in this paper, this method was simple and rapid, and showed satisfactory selectivity and repeatability. We determined the best extracting technology of juglone from walnut green husk: extractions were performed at 25°C for 25min, solvent to sample ratio was 40:1. In this condition, the maximum amount of juglone could reach to 2.5574 mg/g. Sodion was found to have better dyeing effect through the study of dyeing effects.

References

[1] Y. Lu, Quinones Chemical [M]. Beijing, Chem. Ind. Press.(2009):56
[2] B. Wen, S.P Liu, Research progress of juglone in qinglongyi [J]. J. Hebei. Univ. Technol. (Nat. Sci. Ed.) 33, 1 (2011) :141-144
[3] J. Li, Progress of research on anti-tumor effects of juglone [A]. Chin. Pharmacol. Soc.(2010):2
[4] L.H. Cheng, X.W. Huang, Extraction method and technology study of juglone in bark of juglan juglans [J]. Subtrop. Plant. Sci. 39, 4 (2010):33-35
[5] M.L. Sun, Z.Q. Song, G.Z. Fang, S.J. Li, H.J. Yuan, Extraction of juglone from the bark of jianju by vacuum distillation [J]. Chem. Ind. Forest. Prod, 6 (2007):113-115
[6] S.H. Xu, Z. Xu, toxic components of walnut and their application [J]. J. Shenyang. Agric. Univ. 21, 2 (1990):167-170
[7] X.B. Suo, K.B. Gao, Y.L. Zhang, Determination of juglone in qinglong garment by HPLC [J]. Chin Med Mat 26, 11 (2003):793
[8] C. Li, M.X. Zhang, W. Chen, *Synthesis research progress of juglone* [J]. Fine. Chem. Intermediate 40, 6 (2010):11-15

[9] T.C. Fischer, C. Gosch, B. Mirbeth B, J Agricic Food Chem 60, 49 (2012): 12074-12081

[10] M.T. Paulsen, M. Ljungman, Toxicol. Appl. Pharmacol 209, 1 (2005): 1-9

[11] C.L. Wang, J.H. Huo, W.M. Wang, *Research progress on anti-tumor effect and mechanism of juglone* [J]. Heilongjiang. J. Trad. Chin. Med. 47, 2 (2008):122-123

[12] H.L. Xu, X.F. Yu, S.C. Qu, Eur JPharmacol 645, 1-3 (2010):14-22

[13] L. Chen, J. Zhang, S.Y. Wang, D.W. Huang dewu, W.W. Gu, Acta. Lab. Anim. Sci. Sinc. 19, 4 (2011):339-344+382

[14] Y. Li, J.P.Zhao, Y.N. Wang, Z.P. Fu, N. Guo, J.R. Dong, Y. Xia, *Experimental study on antitumor activity in vitro of serum containing juglone* [J]. Trans. B. Inst. Technol. 33, 5 (2013):545-550

[15] X. Zhang, *Study on the effects of combined application of juglone and juglone and 5-fu on ct-26 cells in mouse colon cancer* [D] (BJUT, 2016)

[16] Y.L. Zhang, S.J. Yuan, X.J. Wang, B.K. Feng, R.G. Zhang, J.Q. Han, Trans. Chin. Soc. Agric. Eng. 31, 21 (2015):1-8

[17] J.H. Jiang, J.H. Huo, W.M. Wang, *Extraction technology optimization of total tannins in walnut green peel* [J]. Chin. J. Exp. Trad. Med. Form. 19, 2 (2013):14-16

[18] Q .Li, H. Tan, X. Yuan, J.L. He, H.N. Wei, Z.B. Li, *Determination of juglone in walnut green peel by HPLC* [J]. Jiangsu. Agri. Sci. 42, 1 (2014):259-261