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Rugao area Pterocarya stenoptera leaves tannin extraction technology research

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Abstract. Department of Pterocarya stenoptera dicotyledonous juglandales juglandaceae Pterocarya plants, the whole body can be used as a medicine. This article use the continuous reflux method to studying the extraction process of Pterocarya stenoptera leaves of tannins in the area of Jiangsu Rugao. Through single factor experiment and orthogonal experiment to obtain continuous reflux method was used to extract Pterocarya stenoptera leaves of tannins the optimum technological conditions. Through to the extract of ultraviolet and GC-MS analysis results show that, different extraction agents, tannin content of the extractive and chemical composition are different. Deionized water and ethanol aqueous extract of difference for Pterocarya stenoptera leaves decoction and alcohol mechanism of two kinds of different uses for the treatment of disease research to provide the reference.

Introduction

Pterocarya stenoptera alias ghost maple, Pterocarya stenoptera willow, gold trees ect[1](Figure1),department head dicotyledoneae juglandaceae Pterocarya stenoptera walnut genus, mainly in plenty of rain the temperate and subtropical regions[2]. In China, north from Hebei, Shaanxi, south to Guangdong, Guangxi, east from Jiangsu, Zhejiang, Shanghai, west to Sichuan, Yunnan, Guizhou and other provinces have the presence of the tree, Among them Jiangsu and Zhejiang are our maple foster breeding base. Foreign maple mainly in Japan of the southern half of Honshu, northern Iran, southern part of the Korean Peninsula and other regions.

Fig. 1 Pterocarya stenoptera C.DC

According to the "Compendium of Materia Medica" records, Pterocarya stenoptera leaves, bark and roots can be used as medicine. Bark and roots indications rheumatism numb, cold-damp bone ache, odontia, head pain, hemorrhoids, itch, empyrosis , ulcer ect[3]; leaves indications asthma, chronic bronchitis, joint ache, ulcer furuncle swollen, itch pruritus cutis, oidium dermatitidis eczema, burn as well as all kinds of lichen planus circumscriptus[4].

The author's childhood had body roseola, itchiness, Under scratching the body fester, Western medicine has no effect after treatment, On the recommendation of the practitioner of Chinese medicine, picking Pterocarya stenoptera leaves to bath, after a single use have good curative effect,the second use will be able to cure. Until now is still fresh in my memory.

A lot of literature suggests, the major medicinal ingredients of Pterocarya stenoptera are tannin, naphthoquinone, terpene , steroid and their chemical compound[5]. Among them, tannin of Pterocarya stenoptera is the medicinal ingredients of the treatment of skin diseases and all kinds of ulcer, trauma. Therefore, this paper use Pterocarya stenoptera leaves of Rugao city, Jiangsu province for the study object, research tannin extraction process, in order to obtain the tannin extract optimum process of Pterocarya stenoptera leaves.

Rugao area Pterocarya stenoptera leaves tannin extraction experiment

Tannin extraction method have impregnation method, decocting method, continuous reflux method and ultrasonic extraction method and so on. Among them, in a Soxhlet extractor conducted continuous reflux method is domestic and foreign scholars recognized a classic method and generally accepted and widely used in the extraction of herbal medicine[6], and the method is also used in this study.

The overall experimental procedures and technical route shown in Figure 2.

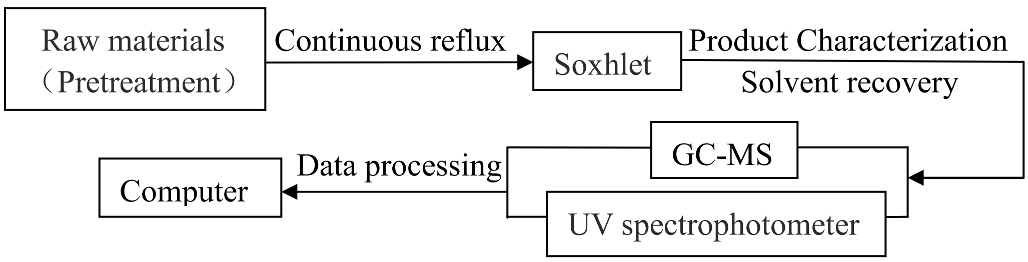

Fig.2 The technical route of experiments

After Pterocarya stenoptera leaves washing to dry, using traditional Chinese medicine pulverizer crush, respectively 20 mesh, 40 mesh, 60 mesh, 80 mesh, 100 mesh and 120 mesh sieve, pressing 10 g / parts sealed refrigeration spare.

In a Soxhlet extractor, respectively with deionized water, ethanol - water, methanol, acetone, etc. as extraction agent extraction experiment, after completion of the extraction the extract obtained by solvent recovery operations.

The extract was measured by using the GC-MS and an ultraviolet spectrophotometer, by computer-aided calculation to obtain its chemical composition and tannin content.

On the basis of the single factor impact analysis to design of orthogonal test table, Through orthogonal test and the validation to determine the optimum process conditions of the tannin extract in Pterocarya stenoptera leaves.

Univariate Results and discussion

2.1 Extractant screening

Take 80 mesh sample respectively with deionized water, ethanol - water, methanol, acetone, etc. as extraction agent[7]. In the soxhlet extractor, controlled the temperature below the boiling point of the solvent, extraction for 6 h. After the extract was pretreated, by UV scanning to obtain the UV spectrum ,Figure 3.

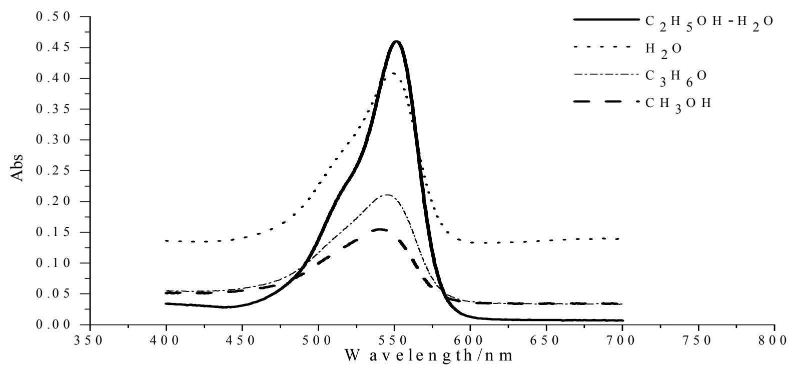

Fig.3 The relationship between Absorbance and Different Solvent categories

Figure 3 shows that, ethanol-water solution extracted Pterocarya stenoptera tannin content highest, followed is deionized water. By GC-MS spectra against the database analysis shows, using deionized water as the extraction agent, in the extract the content of phenolic compounds accounted for 59.83% of extrac total weight, mainly comprising 2-methoxy-4-methyl phenol, cis-1,2-dihydro-catec, 2-methoxy-4-methylphenol, eugenol, etc. Using ethanol-water the extraction agent, in the extract phenolic compounds total weight is 62.38%, mainly comprising guaiacol, 2-methoxy-4-methyl phenol, cresol, eugenol, etc. Using acetone the extraction agent, in the extract phenolic compounds weight is 54.63%, mainly comprising o-cresol, D- gluconic acid, resorcinol, cis-1,2-dihydro-catechol, etc. But using methanol the extraction agent, in the extract phenolic compounds total weight is only 9.02%. So the best extraction solvent is ethanol -water solvent[8].

2.2 Influence of extractant concentration

Take 80 mesh sample with different concentrations of ethanol - water solution extraction 6h in a Soxhlet extractor. By measuring the extract tannin content showed: Within a certain range, tannin extraction rate and ethanol-water solution the concentration of the aqueous solution is proportional, optimum solvent concentration determined by orthogonal experiments needed.

2.3 Influence of extraction time

Take 80 mesh sample with the concentration of 80% ethanol-water solution as the extraction agent, in soxhlet extractor extraction different time length. The results showed: extraction time within 2h to 6h in the tannin content of the extract is increased linearly, and at 6h reached the highest, the extraction time for fell slightly in 7h and 8h, and at the bottom of the wall with black paste carbide. Therefore, the optimum extraction time is 6h.

2.4 Influence of sample particle size

Take samples of different particle size with 80% ethanol-water solution as the extraction agent, in soxhlet extractor extraction 6h. The results showed: when the sample particle size is 20 mesh to 80 mesh, with the increase of grain size decrease tannin extraction yield, with 80 mesh sample extraction yield is the highest; 120 mesh particle diameter is about 125μm, close to the diameter of the plant cell, in the pretreatment will cause the loss of part of the tannins, led to tannin extraction rate is no increase in lower instead. Comprehensive above factors, with over 80 mesh sieve to extract particle size sample is the best.

Orthogonal test

To determine the best material of Pterocarya stenoptera leaves tannin solid-liquid ratio and the pH and concentration of solvent. Therefore, for the above three factors, each factor set three levels orthogonal experiment design table, and to the orthogonal experiment result analysis of variance and poor (Shown in table 1, table 2, table 3).

Table1 The Factors and Levels Selected

Factor	A Material liquid ratio	B The solution pH	C Ethanol concentration (%)
Level	1:5	4	40
	1:10	6	80
	1:20	8	100

Table2 The Orthogonal table of tannins in pterocarya stenoptera leaves

Experiment No.	A	B	C	Tannin content (%)
1	1	1	1	3.510
2	1	2	2	6.605
3	2	1	2	7.861
…	…	…	…	…
K_1	225.63	228.75	186.18	
K_2	208.99	224.59	234.56	
K_3	214.19	195.47	228.07	
R	5.55	11.09	16.13	

Table3 Result of Analysis Variance

Source of variance	Average sum	DOF	Mean square
A	148.31	2	84.16
B	219.20	2	109.60
C	459.72	2	229.86

Table 3 the analysis of variance table show, hot reflux extraction of Pterocarya stenoptera leaves for the influence factors of total tannin the order is C>B>A, namely ethanol -water solution concentration the maximum influence of the extraction result, followed by the pH of the solution and solid-liquid ratio.

Orthogonal test results show, 40% of ethanol - water solution of hydrophobic tannic acid dissolving capacity is low, the anhydrous ethanol due to excessive concentration can block water soluble tannin seeped, So choose 80% ethanol - water solution as the best extracting agent.

Under the conditions of pH = 4, as the water-soluble proteins will precipitate resulting in some cell membrane is blocked, tannin extraction rate is lower; extraction yield the highest is pH = 6; when the pH value increase to 8, since tannins and phenolic compounds under alkaline conditions decreased sensitivity to oxygen and light, lead extraction rate decline. Therefore, pH = 6 is the optimum extraction conditions.

When the liquid ratio of 1:5, Pterocarya stenoptera leaves tannin can not be completely dissolved. When the liquid ratio of 1:20, although tannins can all be dissolved, but the late solvent recovery process is longer, cause the tannins oxidation. Therefore, Choose liquid ratio of 1:10.

According to the parameters of the above optimum for verification.Verification results show, average content of tannins accounted for 7.97% of the total sample, they were higher than the single factor experimental results. Explain according to single factor experiment combined with orthogonal experiment screening the best extraction technology conditions for feasible, it has operable and reproducibility.

Conclusions

Department of Pterocarya stenoptera dicotyledonous juglandales juglandaceae Pterocarya plants, the whole body can be used as a medicine. According to use the continuous reflux method to studying the extraction process of Pterocarya stenoptera leaves of tannins in the area of Jiangsu Rugao, and obtain the following conclusions:

(1) Through the single factor affecting experiment combined with orthogonal test, obtained use the continuous reflux method was used to extract Pterocarya stenoptera leaves the best process conditions, Respectively: sample particle size over 80 mesh sieve, the extraction time is 6h, liquid ratio is 1:10, pH＝6, the extract is 80% ethanol-water solution. Under optimum extraction conditions, tannin extracted sample accounts for about 8% of the total weight.

(2) Through to the extract of ultraviolet and GC-MS analysis results show that, different extraction agents, extract tannin content and chemical composition is different. Ethanol-water solution extracted Pterocarya stenoptera tannin content highest, in the extract phenolic compounds total weight is 62.38%, mainly comprising guaiacol, 2-methoxy-4-methyl phenol, cresol, eugenol, etc. Followed is deionized water, the content of phenolic compounds accounted for 59.83% of extrac total weight, mainly comprising 2-methoxy-4-methyl phenol, cis-1,2-dihydro-catec, 2-methoxy-4-methylphenol, eugenol, etc. Using ethanol-water the extraction agent, in the extract phenolic compounds total weight is 59.83%, mainly comprising guaiacol, 2-methoxy-4-methyl phenol, cresol, eugenol, etc.

(3) Using deionized water and ethanol aqueous extract of difference for Pterocarya stenoptera leaves decoction and alcohol mechanism of two kinds of different uses for the treatment of disease research to provide the reference.

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