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Original article

Preparation of the particles of Acanthopanax senticosus

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ABSTRACT

The main objective of this study is to establish the best preparation technology of the particles of Acanthopanax senticosus. First, take the reflux extraction method extract of Acanthopanax senticosus coarse powder, optimized by orthogonal experimental method, to flavonoids flavonoids extraction extraction rate as the indexes to determine the effects of extraction temperature, ethanol concentration, extraction time on flavonoids content. Then by a wet granulation of thorn slender acanthopanax particles, taste with granules, forming rate, melting rate as index to investigate the influences of materials adding amount of granules effect. The results showed that the ethanol water heating reflux extraction method to extract the temperature of 70 deg, the percentage of ethanol 75%, extraction time 2.5 h, the highest content of total flavonoids in the extract. Join the 5 ml and 10 g in the extract of acacia honey, dextrin, starch, sugar ratio for 3:4:8, the best taste of Acanthopanax granules. In the end, the best preparation technology of the granules is established, and the process is simple, which is suitable for the large-scale production of the factory.

1. Introduction

Acanthopanax senticosus is the dry root, stem and rhizome of Acanthopanax senticosus Acanthopanax, senticosus Harms. Its sexual temperature, taste slightly bitter, pungent. Among them, the main chemical components are Acanthopanax senticosus, polysaccharides, flavonoids and some trace elements and amino acids. In recent years, with in-depth study of Acanthopanax medicinal composition pharmacology showed that Acanthopanax senticosus in addition to the treatment of cardiovascular and cerebrovascular diseases, diabetes, neurasthenia, also can enhance the immunity, antitumor, anti radiation, anti fatigue and other effects on the body. Wang & Hai (2009), through the establishment of the system, the particle model of lipid peroxidation of in vitro model, study the antioxidant effect of Acanthopanax senticosus extract on free radicals. The results show that Acanthopanax ethanol and water extract showed strong antioxidant effect. Acanthopanax senticosus is a total flavonoids, has a wide range of pharmacological activities, and has a good effect on cardiovascular and cerebrovascular diseases. Chen & Song (2002) analyzed the flavonoids in Acanthopanax senticosus by electrospray ionization tandem mass spectrometry, mainly quercetin and related flavonoid glycosides. Some scholars found 16 species of Acanthopanax senticosus glycosides A1, A2, A3, A4, C1, C2, C3, C4, D1, D2, D3, B2, E in Acanthopanax senticosus leaves (Shao, 1989; Li et al., 1989; Zhu & Li, 2013).

Acanthopanax senticosus, also known as Acanthopanax senticosus, which has the pharmacological effects of strengthening the body, nourishing the intellect and tranquilizing the mind, invigorating the kidney and invigorating the spleen (Zhang et al. (2018)). Acanthopanax senticosus has the functions of anti-fatigue, improving sleep, enhancing learning and memory (Chen et al., 2017; Wu et al., 2015; Wang et al., 2016). It can make the motor and sensory centers more stable and improve the efficiency of brain work. Diwu et al. (2013), Solomon et al. (2013) and Huang et al. (2013) have shown that changes in synaptic plasticity of hippocampal neurons are closely related to Alzheimer's disease.

Xu et al. (2018) explored the effects of Acanthopanax senticosus E on learning and memory ability and plasticity of hippocampal neurons in rats with fornix-hippocampal fimbria injury. Acanthopanax senticosus E can increase the activity of Na+-K+-ATPase in erythrocyte membrane and hippocampal CA3 region in a dose-

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dependent manner, suggesting that this component can reduce the apoptosis of hippocampal pyramidal cells, and its mechanism may be related to providing good energy support for neurons (Kobayashi et al., 2011).

2. Experimental part

2.1. Introduction

This experiment by orthogonal experimental design, the indexes with Acanthopanax senticosus extract flavonoids in ethanol water, reflux of Acanthopanax extract into coarse powder, extraction temperature, ethanol concentration, extraction time and other factors on flavonoid content influence, to provide the experimental basis for the development of extraction and preparation technology of preparation of Acanthopanax senticosus particle research and new drugs, for Acanthopanax senticosus granules preparation research has realistic significance.

2.2. Experimental materials, reagents and instruments

2.2.1. Experimental materials

Acanthopanax senticosus was purchased from Tongrentang pharmacy (by the professor of Harbin University of Commerce, he was identified as the root of Acanthopanax senticosus.)

2.2.2. Experimental drugs

Standard of rutin: purchased from China Pharmaceutical and biological products inspection office

Sodium nitrite: analysis of pure, purchased from Tianjin Tianli Chemical Reagent Co., Ltd.

Sodium hydroxide: analysis pure, purchased from Tianjin continental chemical reagent factory

Aluminum nitrate: analysis pure, purchased from Tianjin chemical reagent three factory

95% alcohol: Medical specifications, purchased from Dezhou Le Kang disinfection products Co., Ltd.

2.3. Experiment content and method

2.3.1. Study on extraction process of senticosus

2.3.1.1. Extraction process of senticosus. Although the water extraction and alcohol precipitation method can extract the effective components, such as alkaloid salt, glycosides, organic acids, amino acids, polysaccharides and so on (Leem et al., 2014), but at the same time, some water soluble impurities, such as starch, protein, phlegmatic, tannin, pigment, inorganic salt. Therefore, this experiment uses the method of alcohol extraction and water precipitation. The effective ingredients of Acanthopanax senticosus are extracted by the method of reflux of ethanol at certain concentration.

The dried acanthopanax root crushing, sieving, respectively weighing 5 g acanthopanax root powder with round bottom flask, the solid-liquid ratio was 1:20 (g:ml) with ethanol water solution, mixing in the constant temperature water bath extraction, the extraction liquid is filtered three times, collecting into liquid reserve.

2.3.1.2. Optimization of extraction process by orthogonal experiment. According to the single factor level Table 1, each factor was selected at three levels. The effect of extraction temperature, percentage of ethanol and extraction time on the extraction rate of flavonoids was investigated by L9 (3^4) orthogonal experimental table. The optimum process conditions for extraction of total flavonoids from Acanthopanax senticosus by ethanol water reflux were obtained.

Table 1
Factor level table.

Factor	Extraction temperature (Times) A	Ethanol concentration (%) B	Extraction time (H) C
1	60	65	1.5
2	70	75	2.0
3	80	85	2.5

2.3.2. Study on the preparation technology ofAcanthopanax senticosus granules

2.3.2.1. Optimization of the preparation process of Acanthopanax senticosus granules. Mix dextrin, starch and sugar powder into tray according to the amount of excipients in Table 2. Add Acanthopanax senticosus extract and stir thoroughly. Add 1 ml 75% concentration of ethanol, granulation, drying into the tray, and dried in an oven at 60 12 h. The solubility and molding rate of the granules were investigated. The three indexes were comprehensively evaluated. The total score was 100, and the solubility and molding rate were 50. The synthetic score of each prescription was calculated to optimize the preparation process.

2.3.2.2. Determination of total flavonoids in Acanthopanax senticosus granules.

(1) Preparation of standard solution: the precise standard of rutin standard 13.90 mg, dissolved in 75% ethanol, moved into 50 ml volumetric flask, added 75% ethanol to 50 ml, and got a standard solution of 0.278 mg/ml concentration (Liu and Liu, 2011).
(2) The preparation of the sample solution for the trial: precise determination of Acanthopanax senticosus crude powder 2 g, adding 75% ethanol 20 ml, heating reflux extraction 3 times, respectively 1.5 h, 2.0 h, 2.5 h. The filter residue is washed with 75% ethanol, and the filtrate is combined. The filtrate is placed in a small beaker. The water bath is heated to remove the ethanol solvent, cool down, then dissolved in deionized water, the volume is fixed to 25 ml, and shaken, and then the sample solution is obtained.
(3) Content determination method: take the suitable amount of test solution, take deionized water as blank control, place in ultraviolet spectrophotometer, and determine absorbance at 500 nm.
(4) Drawing the standard curve of rutin: precision of rutin standard solution, 1 from 2, 3, 4, 5 and 6.0 ml, respectively. The 25 ml volumetric flask, add 6 ml deionized water and 5% sodium nitrite solution 1 ml, shake, static 6 min; adding

Table 2
Compatibility of extracts and excipients.

Prescription number	1	2	3	4	5	6	7	8	9
Medicinal extract (g)	10	10	10	10	10	10	10	10	10
Dextrin (g)	2	3	4	2	3	4	2	3	4
Sugar (g)	2	2	2	3	3	3	4	4	4
Starch (g)	8	10	12	10	12	8	12	8	10

10% aluminum nitrate solution 1 ml, and static 6 min 10 ml; add 5% sodium hydroxide solution, 75% ethanol fixed volume, shake, static 15 min color, with deionized water as blank, at 500 nm on the determination of the absorption (National Pharmacopoeia Commission, 2015).

(5) Stability test: take the same test solution 2 ml respectively into 25 ml volumetric flask, add 6 ml deionized water and 5% sodium nitrite solution 1 ml, and static 6 min; aluminum nitrate solution 1 ml, adding 10% shake, static 6 min; sodium hydroxide solution was added and 5% 10 ml, 75% B alcohol volume, shake, static 15 min color, absorbance was measured at 0, 0.5, 1, 1.5 and 2.0 h respectively, were measured 5 times, calculating the relative standard deviation (RSD).

(6) Precision test: a certain concentration of standard solution 2 ml, respectively into 25 ml volumetric flask, add 6 ml deionized water and 5% sodium nitrite solution 1 ml, and static 6 min; aluminum nitrate solution 1 ml, adding 10% shake, static 6 min; sodium hydroxide solution was added and 5% 10 ml, 75% alcohol volume, shake, static 15 min color, the determination of the absorption, 6 consecutive times, calculate the relative standard deviation (RSD).

(7) Repeatability test: take 6 batches of the same sample accurately, each about 2G, add 75% ethanol 20 ml, heat reflux extraction 3 times, respectively 1.5 h, 2.0 h, 2.5 h. The filter residue is washed with 75% ethanol, and the filtrate is combined. The filtrate is placed in a small beaker. The water bath is heated to remove the ethanol solvent, cool down, dissolved in deionized water, fixed volume to 25 ml, shaken, and obtained the test sample solution. Each 2 ml, respectively into 25 ml volumetric flask, add 6 ml deionized water, 5% sodium nitrite solution 1 ml, shake, static 6 min; adding 10% aluminum nitrate solution 1 ml, shake, static 6 min; adding 5% 10 ml of sodium hydroxide, with 75% ethanol fixed volume, shake, static 15 min color, determination of absorbance. The calculation of relative standard deviation (RSD).

(8) Recovery test: weigh accurately known content of acanthopanax powder of 6 copies, each about 0.5 g, rutin standard were added with a certain amount of 20 ml, adding 75% ethanol, refluxing and extracting for 3 times, respectively 1.5 h, 2.0 h, 2.5 h. Filter, filter residue washed with 75% ethanol with the filtrate; filtrate in a small beaker, water bath heating to volatile solvent, cooling, dissolved in deionized water and set the volume to 25 ml strain; each 2 ml, respectively into 25 ml volumetric flask, add 6 ml deionized water and 5% sodium nitrite solution 1 ml. Static 6 min; aluminum nitrate solution 1 ml, adding 10% shake, static 6 min; sodium hydroxide solution was added and 5% 10 ml, with 75% ethanol fixed volume, shake, static 15 min color, determination of absorbance of different samples, calculate the relative standard deviation (RSD).

2.4. Experimental results and discussion

2.4.1. Orthogonal experiment to optimize the extraction process

The result of orthogonal experiment is shown by Table 3. The result of variance analysis is shown in Table 4.

The orthogonal Table 3 test results from the analysis of intuitive, with extraction of total flavonoids from Acanthopanax senticosus as index range value showed that the influencing factors of the main factors for the role of $R_1 > R_3 > R_2$, three levels each influence factor on the extraction of total flavonoids from Acanthopanax senticosus influence rate were: $A_2 > A_3 > A_1$, $B_3 > B_2 > B_1$, $C_3 > C_1 > C_2$. The optimum extraction rate of total flavonoids was $A_2B_3C_3$. From the analysis of variance (Table 4), we can see that the three factors have no significant effect on the yield of total flavonoids. In order to make the extraction rate of the 3 index components as large as possible, the number of extraction is selected 3 times. To sum up, determine the best process for $A_2B_3C_3$, that is, extraction temperature of 70 deg, ethanol percentage of 85%, extraction time 2.5 h.

2.4.2. Optimization of preparation process of Acanthopanax senticosus granules

Comprehensive evaluation results show that the best prescription for No. 8 prescription, namely 10 g 3 g 4 g extract, dextrin, powdered sugar, starch 8 g (see Table 5).

Table 3
Results of orthogonal experiment.

Factor	A	B	C	D	Flavone extraction rate (mg/g)
Experiment 1	1	1	1	1	8.372
Experiment 2	1	2	2	2	8.566
Experiment 3	1	3	3	3	9.457
Experiment 4	2	1	2	3	9.779
Experiment 5	2	2	3	1	10.964
Experiment 6	2	3	1	2	10.703
Experiment 7	3	1	3	2	9.669
Experiment 8	3	2	1	3	9.446
Experiment 9	3	3	2	1	9.203
Mean 1	8.798	9.273	9.507	9.513	
Mean 2	10.482	9.659	9.183	9.646	
Mean 3	9.439	9.788	10.030	9.561	
Range	1.684	0.515	0.847	0.133	

Table 4
Results of variance analysis.

Factor	Sum of squares of deviations	Freedom	F	F critical value	Saliency
Extraction temperature	4.333	2	2.218	5.140	
Ethanol concentration	0.430	2	0.220	5.140	
Extraction time	1.097	2	0.562	5.140	
Error	5.86	6			

Table 5
Comprehensive evaluation.

Prescription number	1	2	3	4	5	6	7	8	9
Dissolubility	13.38	16.84	21.5	24.56	33.52	39.48	42.46	47.92	49.08
Formability	45.88	46.72	47.54	48.16	48.42	48.58	48.54	48.20	46.10
The total score	59.27	63.56	69.04	72.72	81.94	88.06	91.00	96.12	95.18

2.4.3. Determination of total flavonoids in Acanthopanax senticosus granules

(1) Rutin standard curve

Linear regression was performed with absorbance (A) and concentration (C). The regression equation of the curve is: Y = 9.9957X − 0.004, R2 = 0.9999, indicating that the method has good internal relations in 0.018–0.97 mg/ml range, and can be used as a method for the determination of total flavonoids in Acanthopanax senticosus.

(2) Stability test

The concentration of 0.08 g/ml was 2 ml, the concentration was 0.08 g/ml, the absorbance was measured 7 times, and the relative standard deviation (RSD) of the result was 0.60%, indicating that the sample solution was basically stable in 2 h.

(3) Precision test

The absorbance of was measured 6 times for a certain concentration of standard solution 2 ml, and the relative standard deviation (RSD) was 0.18%, indicating good precision.

(4) Reproducibility test

6 batches of the same sample were weighed accurately, the concentration was 0.08 g/ml, and the absorbance was measured. Results the relative standard deviation (RSD) was 1.66%, and the results showed that the method was reproducible.

(5) Sample recovery test

6 doses of acanthopanax powder, which are known to be known, are taken into consideration, each of which is about 0.05 g, and a certain amount of rutin standard is added. The concentration is 0.65 mg/ml. The relative standard deviation (RSD) of the absorbance in each sample is 1.91%.

3. Conclusion

The optimum technological conditions for the preparation of Acanthopanax senticosus granules were studied. First of all, the extraction rate of total flavonoids from Acanthopanax senticosus was used as an index. The effects of extraction temperature, ethanol concentration and extraction time on the extraction rate of flavonoids were discussed. Finally, the optimum process conditions for extraction of total flavonoids from Acanthopanax senticosus by refluxing with ethanol water were obtained. After comprehensive consideration, the extraction of total flavonoids from Acanthopanax senticosus by ethanol water reflux is more suitable for large-scale production. The influence of the amount of auxiliary material on the granules was investigated. The granules of Acanthopanax senticosus were prepared by wet granulation. The optimum process was determined by the taste, molding rate and dissolution rate of granules.

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References

Chen, B., Tan, M.C., Huang, J.L., 2017. Preliminary establishment of tree shrew model with senile dementia-blood stasis syndrome and evaluation of Panax notoginseng saponins intervention. Chinese J. Tradit. Chinese Med. 42 (6), 1175–1182.
Chen, M.L., Song, F.R., 2002. Analysis of flavonoids in leaves of Acanthopanax senticosus. Anal. Chem. 30 (6), 690–694.
Diwu, Y., Tian, J.Z., Shi, J., 2013. Effect of Xixin decoction on glycosylation modification of tau protein O-Glc NAc in brain of sporadic Alzheimer's disease rats. Chinese Patent Medicine. 35 (8), 1620–1625.
Huang, D.B., Hu, Z.H., Yu, Z.F., 2013. Eleutheroside B or E enhances learning and memory in experimentally aged rats. Neural Regen. Res. 8 (12), 1103–1112.
Kobayashi, D., Yoshimura, T., Johno, A., Sasaki, K., Wada, K., 2011. Toxicity of 4'-Omethylpyridoxine-5'-glucoside in Ginkgo biloba seeds. Food Chem. 126 (3), 1198–1202.
Leem, K.H., Lee, S., Kim, H.K., 2014. Extrusion processenhances the anti-inflammatory effect of acanthopanaxsenticosus leaves. J. Ex. Physiol. 23 (3), 911–916.
Li, Z.F., Wu, Z.H., Chen, G., Zhang, Q.H., Pei, Y.H., 1989. Two new com-pounds from Acanthopanax senticosus Harms. J. Asian Nat. Prod. Res. 11 (8), 715–718.
Liu, W.C., Liu, C.M., 2011. Isolation, extraction and antioxidant activity of total flavonoids from Acanthopanax senticosus leaves. Liaon. J. Tradit. Chinese Med. 38 (8), 1622–1625.
National Pharmacopoeia Commission, 2015. Pharmacopoeia of the People's Republic of China. Chemical Industry Press, Beijing, pp. 101–105.
Shao, C.J., 1989. Saponins from leaves of Acanthopanax senticous Harms. Chem. Pharm. Bull. 37 (1), 42.
Solomon, A., Kivipelto, M., Soininen, H., 2013. Prevention of Alzheimer's disease:moving backward through the lifespan. J. Alzheimers Dis. 33 (Suppl 1), S465–S469.
Wang, H.Z., Sun, Y., Li, L., Wang, S.J., 2016. Therapeutic effect of Acanthopanax senticosus injection combined with fluoxetine in the treatment of senile dementia with depression. Int. J. Psychiat. Med. 43 (1), 116–118.
Wang, X., Hai, C.X., 2009. The activity of Acanthopanax senticosus extract in free radical model. Carcinogenesis Distortion Mutation 21 (20), 127–131.
Wu, J.H., Du, Y.X., Huang, Q., 2015. Effects of Acanthopanax senticosus B/E on proliferation and expression of TGF-beta 1 and PPAR gamma in HBZY-1 cells under high glucose environment. Chinese J. Pathophysiol. 31 (3), 511–517.
Xu, Y.L., Du, W.X., Huang, D.B., 2018. Effects of Acanthopanax senticosus E on learning and memory ability and plasticity of hippocampal neurons in rats with fornix-hippocampal fimbria injury. Chinese Patent Med. 40 (11), 2368–2373.
Zhang, Y., Qin, H.Z., Wang, T., Niu, Y., Ge, Y., Qiao, L.X., Du, A.L., 2018. Effects of Acanthopanax senticosus injection on learning and memory ability and fibrous actin in hippocampus of rats with cerebral hypoxia. Chinese J. Diagn. 6 (04), 272–276.
Zhu, L.G., Li, Z.F., 2013. Chemical Constituents of Acanthopanax senticosus. Heilongjiang Tradit. Chinese Med. 42 (5), 852–855.