Enrichment and purification of phenols from *Callicarpa nudiflora* Hook. et Arn by HP-20 macroporous resin

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Abstract: Objective To study the technological parameters of the purification process of phenols from *Callicarpa nudiflora* (*C. nudiflora*) with HP-20 macroporous resin.**Methods** The kinetic adsorption and desorption experiments were carried out on HP-20 macroporous resin (HP-20) to optimize the separation process of phenols. Additionally, the effects of four parameters including adsorption flow rate, elute flow rate, volume of 10% ethanol and 50% ethanol solution for elution were explored by a L4/3 orthogonal experiment. Finally, content of phenols in samples before and after being treated by HP-20 were compared. **Results** The results showed that a good separation and purification of crude total phenols extract from *C. nudiflora* was achieved using HP-20. The optimum conditions were found to be: initial concentration of 50 mg·mL^{-1}, absorb flow rate of 1 mL·min^{-1}, elute flow rate of 3 mL·min^{-1}, 5 BV of 10% ethanol solution for prewash and 10 BV of 50% ethanol solution for desorption solvent. **Conclusion** The content of phenols is above 40% in *C. nudiflora* after being treated by HP-20, indicating that HP-20 could be successfully applied to enrich and purify phenols in *C. nudiflora*.

Introduction

Luo Hua zi zhu (*C. nudiflora*) is belong to Verbenaceae family, which is also called zizhu, ganfengchai, baihuacha, fantangye, dayebanjiu, xiyebanjiuhua and so on. It is widely distributed in the south of China, such as Hainan, Guangxi and Guangdong province, especially in Hainan province. In addition, it has also been widely planted in the country of India, Vietnam and Malaysia[1-3]. It tastes bitter, slightly acrid and moderate. Owing to the effects on anti-inflammatory detoxification and convergence bleeding, it is commonly used to treat purulent inflammation, acute infectious hepatitis, burns, scald, traumatic hemorrhage and other diseases. The chemical compositions of *C. nudiflora* are complicated, mainly including flavonoids, tannins, phenolic acid, polysaccharide and other compounds. The phenolic acids significantly shorten the part activated clotting time live enzymes activity of pharmacological effects, so the composition may be the *C. nudiflora* hemostatic function of main active ingredient[4-7].

Experiments

Experimental materials

C. nudiflora dry extract powder, hereinafter referred to as the dry extract, provided by the jiu zhi tang Hainan Pharmaceutical Co., Ltd. Its batch number was 110500. All chemicals and regents used in the experiments were of analytical grade.

HP-20 was purchased from Mitsubishi chemical corporation (Japan). The resin was soaked with 95% ethanol for 24 h to swell adequately. Subsequently the resin was eluted by 95% ethanol until white casse disappeared when the eluting reagent was mixed with pure water (1:5, V/V), and then the resin was washed with pure water until the liquor had no alcoholic odor[8].

Experimental methods

Determination of phenols content by UV spectrophotometry

Gallic acid was employed as a standard compound to determine the content of phenols by UV spectrophotometry in this study. The working curve of gallic acid was: y=30.223x+0.011 (n=7, r= 0.9992, 0.0000～0.0240 mg·mL^{-1}, detective wavelength of 760 nm), where y is absorbance of the tested sample and x is concentration of the tested sample.

Kinetic absorption and desorption tests

The Kinetic-Tandem assay was employed to investigate kinetic adsorption capacity of HP-20. The assay was performed as follows: The experiment was carried out in a glass column (25 mm × 500 mm) wet packed with the HP-20. The bed volume (BV) of the resin was 20 mL. 500 mL sample solution (the initial concentration was 10 mg·mL^{-1}) flowed through the column at the flow rate of 2 mL·min^{-1}. The content of phenols (C_1) in elution (E_1) was detected by UV analysis when the sample solution drained away through the column. Subsequently the elution flowed through the column again at the same flow rate. Concentration of phenols (C_2) in elution (E_2) was analyzed. Repeated the above steps until C_{n+1} changed little comprised with C_n. At this moment the kinetic absorption can be seen reaching the equilibrium point. Then kinetic absorption mass of HP-20 was calculated[8].

Five sample solutions with different initial concentrations (10, 20, 30, 40 and 50 mg·mL^{-1}) were employed to investigate effect of the initial concentration of sample solution on the enrichment and purification process. The seven samples were dealt with same parameters including sample quality, absorb flow rate, elute flow rate and so on. These experiments were carried out in seven pillars of glass (20 mm × 500 mm) wet packed with the HP-20. The BV of the resins were 15 mL. The kinetic absorption capacities were compared when the absorb processes were finished[8].

The experiment was carried out in a glass column (25 mm × 500 mm) wet packed with the HP-20. The BV of the resin was 20 mL. The resin column that reached absorb equilibrium was used to do kinetic desorption tests based on a gradient elution program. The column was eluted by pure water, 10% ethanol, 30% ethanol, 50% ethanol, 70% ethanol, 80% ethanol for 5 BV, respectively, at a flow rate of 2 mL·min^{-1}.The concentration of phenols in desorption solution collected at 1 BV internals was monitored[8].

L4/3 orthogonal experiment

In order to fully study the best parameters in process of purification of phenols by HP-20, L4/3 orthogonal experiment were performed in nine pillars of glass (20 mm × 500 mm). The BV of the resins were 15 mL. The parameters to be tested and their values are listed in Table 1. After each test was finished, the yield mass of extract, yield mass of phenols, yield ratio and content of phenols were investigated[8].

Table 1 The parameters tested in the orthogonal experiment

Absorb flow rate (mL·min^{-1})	Elute flow rate (mL·min^{-1})	Volume of 10% ethanol for prewash （BV）	Volume of 50% ethanol solution for desorption solvent （BV）
1	2	3	3
2	3	5	5
4	4	7	10

Verification tests

In order to validation the best parameters in process of purification of phenols by HP-20. Three verification tests were performed in three pillars of glass (20 mm × 500 mm). The BV of the resins were 15 mL. After each test was finished, the yield mass of extract, yield mass of phenols, yield ratio, content of phenols, average of phenols content and RSD were investigated.

Results and Discussion

Absorption and desorption kinetics

In our study, the kinetic-Tandem absorption test was carried out to determine the maximum absorption capacity when the HP-20 resin reached the equilibrium point in kinetic process. The capacity of adsorption is calculated as follows. Adsorption capacity: $Q=(C_0-C_e)*V_0/V$. where Q is the adsorption capacity, which represents the mass of adsorbate adsorbed on 1 mL wet resins at adsorption equilibrium; C_0 and C_e is the initial and equilibrium concentration of phenols in the sample solutions, respectively; V_0 is the initial volume of sample solution, and V is the volume of the wet resins. According to the dates shown in Table 2, after thirdly tandem absorption process, the resins reached its equilibrium point in kinetic absorption process. The maximum absorption capacity was calculated as 30.6058mg/ (1 mL wet resins), which can be used to determine the volume of HP-20 on the basis of the mass of sample.

Table 2 The result of absorption capacity in the Kinetic-Tandem assay

Count of tandom	Reaction volume (mL)	A (760nm)	The total phenol content in the reaction (mg)	The total phenol concentration ($mg \cdot mL^{-1}$)	Absorption mass($mg \cdot mL^{-1}$)
0	0.005	0.249	0.0079	1.5750	--
1	0.05	0.480	0.0155	0.3104	31.6150
2	0.05	0.560	0.0182	0.3633	30.2915
3	0.05	0.541	0.0175	0.3507	30.6058
4	0.05	0.541	0.0175	0.3507	30.6058
5	0.05	0.541	0.0175	0.3507	30.6058

In order to investigate the initial concentration of loading sample solution, five concentrations (10, 20, 30, 40 and 50 $mg \cdot mL^{-1}$) of sample solution were selected. Shown in Fig. 1, when initial concentration has changed from 10 to 50 $mg \cdot mL^{-1}$, the absorption capacities did not increase significantly. At the same time，taking into account factors such as sample on solubility and time, 50 $mg \cdot mL^{-1}$ was chosen to be initial concentration of the sample solution.

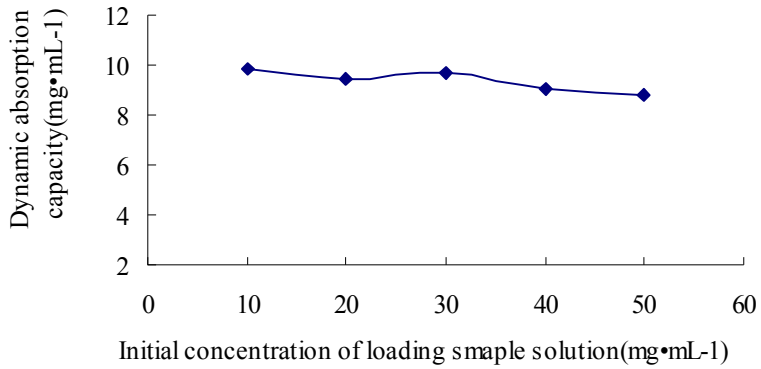

Fig.1 The effect of initial concentration of loading sample solution on kinetic absorption capacity

The kinetic desorption curve on HP-20 was obtained based on a gradient elution program. As depicted in fig. 2, the phenols absorbed by HP-20 were desorbed almost completely after eluted by 30% and 50% ethanol. Hence, the appropriate and economic ethanol concentration of elution solution can be optimized as 50%.

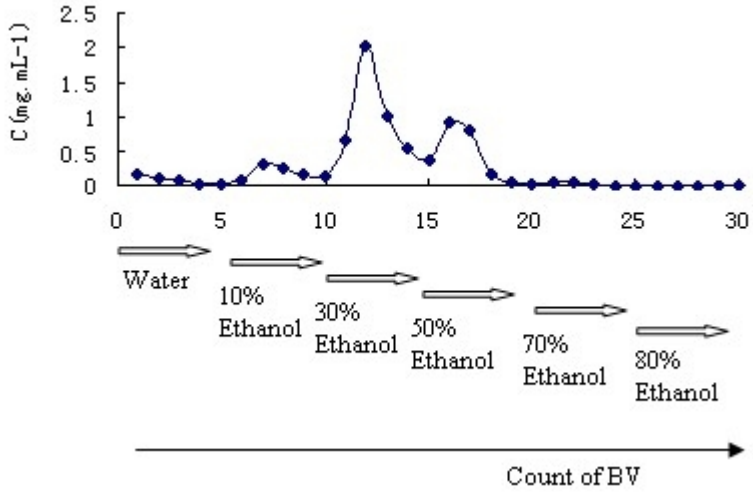

Fig.2 The kinetic desorption curve based on the gradient elution program

The optimization of parameters by orthogonal experiment

According table 3 and 4 the parameters of absorb flow rate, elute flow rate, volume of 10% ethanol for prewash and volume of 50% ethanol solution for desorption solvent were optimized as 1 $mL \cdot min^{-1}$, 3 $mL \cdot min^{-1}$, 5 BV and 10 BV, respectively. Furthermore, among these four parameters, volume of 50% ethanol solution for desorption solvent and volume of 10% ethanol solution for prewash were important factors in the enrichment and purification process of phenols in *C. nudiflora* according to Rj.

Table 3 Results of the orthogonal experiment

Order	Yield mass of extract (mg)	Yield mass of phenols (mg)	Content of phenols (%)
1	255.6	68.2	26.68
2	241.6	90.3	37.39
3	295.1	107.5	36.41
4	333.9	116.4	34.86
5	209.3	66.9	31.94
6	288.1	93.6	32.47
7	265.2	90.1	33.97
8	331.0	110.7	33.44
9	194.3	63.3	32.57

Table 4 Analysis of the orthogonal experiment on the basis of content of phenols in *C. nudiflora*

	Absorb flow rate ($mL \cdot min^{-1}$)	Elute flow rate ($mL \cdot min^{-1}$)	Volume of 10% ethanol for prewash (BV)	Volume of 50% ethanol solution for desorption solvent (BV)
Kj.1	33.49	31.84	30.86	30.40
Kj.2	33.09	34.26	34.94	34.61
Kj.3	33.33	30.72	34.49	34.90
Rj	0.4	3.54	4.08	4.50

The results of the Optimum conditions experiment

As listed in Table 5. There was good agreement among the three parallel experiments. After dealt by the optimal enrich and purify process on HP-20, the content of phenols in the sample is increased from 15.75% to 41.29%.

Table 5 Results of the Optimum conditions experiment

Order	Yield mass of extract (mg)	Yield mass of phenols (mg)	Content of phenols (%)	The average (%)	RSD(%)
1	310.6	129.1	41.56	41.29	3.70
2	317.5	125.9	39.65		
3	308.2	131.5	42.67		

Conclusion

In our study, the enrichment and purification process of phenols in *C. nudiflora* with HP-20 has been successfully developed. Our study showed that under the optimized conditions, *i.e*. initial concentration of 50 $mg \cdot mL^{-1}$, absorb flow rate of 1 $mL \cdot min^{-1}$, elute flow rate of 3 $mL \cdot min^{-1}$, 5 BV of 10% ethanol solution for prewash and 10 BV of 50% ethanol solution for desorption solvent, content of phenols in *C. nudiflora* was increased significantly after dealt by HP-20 according to UV spectrophotometry comparison. In conclusion, the result of our study suggested that macroporous resin adsorption method was applied successfully to enrich and purify phenols in *C. nudiflora*.

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References

[1] Yang Dan, Zhongzhi Qian, Yanze Liu, et al. J.Chin Herb Med. Vol. 2(2010), P.272～288
[2] Lin Dong, Mingsheng Liu, Jinhui Wang. J.Chin Med Chem. Vol. 19(2009), P.371(In Chinese)
[3] Feipeng Gao, Hao Wang, Wencai Ye, et al. J. Chin Pharm Univ. Vol. 41(2010), P.120～123 (In Chinese)
[4] William P Jones, A Douglas Kinghorn. J.Curr Bioact Compd. Vol. 4(2008), P.15～32
[5] WenLi Mei, Zhuang Han, HaiBin Cui, et al. J. Nat Prod Res. Vol. 24(2010), P.899～904
[6] Jijun Liang, Jinlong Qi, Li Li, et al.Chem Nat Comp. Vol. 47(2011), P.110
[7] Jie Zhang, Baoquan Li, Feng Feng, et al. J. Chin Mat Med. Vol. 35(2010), P.3297～3301 (In Chinese)
[8] Han Wei, Jinlan Ruan, Yongfang Lei, et al. J. Chin Nat Med. Vol. 10(2012), P.119～124