Purification and Antioxidant Activity of Flavonoids from Chromolaena Odorata

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Abstract. To purify the flavonoids from Chromolaena odorata (COF) and evaluate its antioxidant activities in vitro. Methods: The COF were purified by macroporous adsorption resin with optimal purification process, and its antioxidant activities were evaluated by scavenging rate of DPPH free radical, ABTS free radical and superoxide anion radical. Results: The COF were obtained using with AB-8 macroporous adsorption resin washed by 60% ethanol at the flow rate of 2.0 mL/min, and the purity was 74.22%. After purification, the scavenging activities of COF on DPPH free radical, ABTS free radical and superoxide anion radical, were 91.36%, 90.13% and 93.17%, respectively. The purified COF also showed a certain reducing power. Conclusions: The COF showed strong antioxidant activity in vitro.

Keywords: Chromolaena Odorata Flavonoids, Purification, Macroporous Adsorption Resin, Antioxidant Activity

1. Introduction
Chromolaena odorata is the compositae zellanium plant, whole herbs was used as medicine. Originally from America, it invaded guangdong, guangxi, hainan, yunnan and guizhou [1]. The main chemical constituents in Chromolaena odorata included volatile oil, terpenoids, phytosterols and flavonoids, among which flavonoids have the largest variety [2, 3]. There were 37 kinds of flavonoids isolated and identified from Chromolaena odorata and the main flavonoids were kaempfer-3-methoxy, rhamnose, tamarix, quercetin, kaempferol, frankinin and luteolin [4, 5]. COF have antioxidant, antibacterial, immunity enhancement, insect control, allelopathy and other effects [6].

At present, there are many researches on the extraction process optimization, component structure identification and pharmacological function of flavonoids from Chromolaena odorata [7, 8], but few reports on the purification and antioxidant activity [9]. Therefore, the whole plantgrass of Chromolaena odorata was used as raw material to extract flavonoids, and the purification process was optimized by using macroporous resin, and the antioxidant activity of flavonoids in Chromolaena odorata was evaluated in vitro and in vivo, providing an important theoretical basis for the comprehensive utilization of Chromolaena odorata.

2. Materials and methods
2.1. Materials and reagents
2.1.1. Material
Chromolaena odorata was mined, dried in cool place, after crushing, pass 60 target quasi-screens, standby.
2.1.2. Reagent and apparatus
WFJ2100-Visible spectrophotometer; Cs-700y multifunctional pulverizer; yongkang tianqi shengshi industry and trade co.LTD. AL104 analytical balance: tianjin meilong pharmaceutical machinery co., LTD. Fd-1a-50 cryogenic freeze dryer: jiangsu tianling instrument co.LTD. Type 1-16 centrifuge: SIGMA; Bk-400 BIOBASE series vertical automatic biochemical analyzer: jinan xinbeishi biotechnology co.LTD. WFJ2100 - Visible spectrophotometer
SFP kunming mice [Certificate no. : SCXK (liao) 2015-003]; Liaoning changsheng biotechnology co. LTD; DPPH: SIGMA-ALDRICH ; NKA-9、 D101、 AB-8、 H1020: Tianjin yunkai resin technology co.LTD.: Tris, ferric chloride, catechol and potassium ferricyanide were all pure.

2.2. The extraction technology COF
50% (v/v) ethanol was used as the extraction solvent. The flavonoids were extracted at 70°C for 4h at the ratio of 1:20 feed-to-liquid. After filtration, the flavonoids were reduced and concentrated.

2.3. Purification of COF
2.3.1. Calculation of adsorption rate and desorption rate
The method of calculating the content of COF was referred to Wang [10], the calculation formula is as follows:
\[
\text{Adsorption rate } \% = \frac{C_0 - C_1}{C_0} \times 100 \quad (1)
\]
\[
\text{Desorption rate } \% = \frac{C_2}{C_0 - C_1} \times 100 \quad (2)
\]
\(C_0\) The content of COF in the stock solution
\(C_1\) The content of COF in the solution after adsorption
\(C_2\) The content of COF in the solution after elution

2.3.2. Static adsorption
COF producing 5g, dissolve in 500 mL of deionized water, preparation of COF crude extract. Then weigh 5 g each of NKA-9, D101, AB-8 and H1020 macroporous adsorbent resin into a triangular bottle, add 50 mL of crude extract of COF to each, and oscillate at room temperature for 24h, measure the content of COF in the solution, and calculate the adsorption rate. The adsorbed and saturated macroporous resin was added with 50mL 60% ethanol solution, which was shaken at room temperature for 12h, filtered, and the content of COF in the filtrate was determined, and the elution rate was calculated.

2.3.3. Static adsorption curve
Weigh 5g AB-8 macroporous resin and place in triangular bottle, 50 mL crude extract of aircraft oxalanone was added, the room temperature shock, the content of flavonoids in aircraft was measured every 20 min, and the adsorption rate-time curve was drawn.

2.3.4. Effect of adsorbed liquid deposition
Weigh 5g AB-8 macroporous resin, put it in a triangular bottle, add 25, 50, 75, 100, 125 and 150 mL of crude extract of COF, shake at room temperature for 12 h, filter, measure the content of COF in filtrate, and calculate the adsorption rate.

2.3.5. Desorption solvent effect
Prepare 50 mL of ethanol solution with concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 95%, add 5g of adsorbed and saturated AB-8 resin, shake for 12h at room temperature, filter, determine the content of COF in filtrate, and calculate the elution rate.

2.3.6. Effect of desorption velocity
The macroporous resin was pretreated and placed in a column (Φ6.0×80 cm), balanced with deionized water for 12h, add 20 mL crude extract of COF, after the crude extract has all entered the resin layer, desorption with 60% (v/v) ethanol, adjust the desorption flow rates to 0.5, 1, 1.5, 2, 2.5 and 3 mL/min, after the desorption was completed, the eluent was combined to determine the content of flavonoids and calculate the elution rate.

2.4. Antioxidant activity of COF
For the scavenging effect of DPPH·, ABTS·+, total reducing capacity and scavenging effect of O2·-, refer to the determination method of Liu [11].

2.5. The data processing
The test data were expressed means ±s, SPSS 19.0 software was used for significant difference analysis, and Origin 8.5 software was used for drawing.

3. Results and analysis

3.1 Optimization of Purification Process of COF
3.1.1. Static adsorption resin is preferred
Fig. 1 shows the adsorption rate and elution rate of different macroporous adsorbents on COF, weak polarity AB-8 resin for COF the rate of adsorption and elution was the highest, 84.26% and 86.35%, respectively, was better than the other three kinds of macroporous resin, weak polarity of COF ketone resin in phenolic hydroxyl and glucoside keys ability is stronger, the combination of elution rate is higher at the same time, this paper selects the AB-8 resin purify COF.

![Fig.1](image)

**Fig.1** The adsorption and desorption of microporous adsorbent resins on COF

3.1.2. Static adsorption curve
The adsorption process of AB-8 resin on COF includes physical adsorption and chemical adsorption, the adsorption process is slow, the relationship between adsorption rate and time is shown in figure 2 .As can be seen from figure 2, with the extension of adsorption time, the adsorption rate of COF showed a trend of first increasing and then flattening. AB-8 resin belongs to the slow adsorption resin and belongs to the single-molecule adsorption so it is more conducive to the elution of COF and improve adsorption rate.
3.1.4. Optimization of desorption solvent
As can be seen in Fig. 4, the elution rate was as high as 81.38% when the ethanol concentration was 60% (v/v), the content of COF in the eluent was the highest, and most of it adsorbed on the resin could be eluted.
3.1.5. Optimization of desorption velocity

As can be seen in fig. 5, when the desorption flow rate ranged from 0.5 to 2.0 mL/min, the elution rate increased linearly, and at the flow rate of 2.0 mL/min, the maximum value reached 82.34%, after that, the elution rate decreased with the increase of desorption velocity. Therefore, desorption velocity was determined to be 2.0 mL/min.

3.2. Antioxidant activity of COF

The purity of COF was up to 74.72% after purification with AB-8 macroporous resin. In vitro antioxidant activity was evaluated, and the experimental results were shown in fig.6. The scavenging rate of DPPH· free radical increased linearly when the concentration of COF was 6.25 ~ 200 μg/mL. When more than 400 g/mL, the clearance rate changes tend to be flat and reach 91.36% at 800μg/mL. The clearance rate of DPPH· was higher than that of flavonoids in black fruit gland (clearance rate is about 10% at 1000μg/mL), and total flavonoids of grape peel residue [7] (clearance rate was about 70% at 400μg/mL). The ABTS·⁺ clearance rate increased when the concentration of flavonoids was 6.25 ~ 400μg/mL. However, the clearance rate did not change significantly when more than 400μg/mL, and the maximum value was 90.13%, which was comparable to that of flavonoids in rattan pods (the clearance rate was about 84.26% at 200 μg/mL). Within 6.25 ~ 200 μg/mL of flavonoid concentration, the O₂·⁻ clearance rate increased linearly, and reached the maximum value of 93.17% at the flavonoid concentration of 800 g/mL. Its scavenging ability was better than that of total flavonoids in jujube fruit (the scavenging rate was about 80.1% at 1000 g/mL), at the concentration of 600μg/mL, the total reduction capacity was the highest. Not only did the flavonoids of COF show good scavenging effects on DPPH·, ABTS⁺⁻ and O₂·⁻, but also had a dose-effect relationship. Although the antioxidant capacity was weaker than that of the VC group, as a natural antioxidant, it also showed strong antioxidant activity.

Fig. 5 Effect of flow velocity on desorption rate

![Graph showing the effect of flow velocity on desorption rate](image)

**a. DPPH· scavenging activities**

**b. ABTS⁺⁻ scavenging activities**
In this paper, macroporous resin was used to purify COF and evaluate their antioxidant activity in vitro. AB-8 macroporous adsorbent resin is the best resin for purifying COF, adsorption was performed with 10 BV loading sample, 60% ethanol was desorbed at a rate of 2 mL/min, and the purification effect was the best, the purity of COF can reach 74.22%; after purification, COF have strong scavenging effects on DPPH•, ABTS•− and O2•−. COF has strong antioxidant activity; the mechanism of antioxidant activity of COF will be discussed in the follow-up study.

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4. Conclusion

Fig. 6 DPPH, ABTS•−, O2•− radical scavenging activities and reducing power of COF

c. O2•− scavenging activities
d. The total reducing power
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