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Postprint: Macroporous Resin Purification and Antioxidant Activity of Total Alkaloids from Mahonia fortunei Leaves

Authors: Jia Kai, Liu Jun, Geng Xiaotong, Zhang Yaozhou, Xiao Ying

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Abstract

To determine the optimal process conditions for the separation and purification of total alkaloids from Mahonia fortunei leaves using macroporous resin and to evaluate their antioxidant activity, six macroporous adsorption resins were compared for their static adsorption and desorption effects on total alkaloids, the optimal resin was selected, and its dynamic purification process conditions for total alkaloids were investigated. The DPPH method was employed to evaluate the antioxidant performance of total alkaloids before and after purification. The results demonstrated: (1) AB-8 macroporous adsorption resin exhibited the best purification effect, with optimal process conditions as follows: sample loading concentration of 50 mg · mL-1 (crude drug concentration), sample loading volume of 26 BV, and sample loading flow rate of 2 BV · h-1; after adsorption completion, washing with 3 BV of water followed by elution with 4 BV of 50% ethanol, under which conditions the total alkaloid content increased from 13.33% to 56.64%. (2) The DPPH radical scavenging capacity of each sample was in the order: reference standard Vc (IC50=10.39 g · mL-1) > purified total alkaloids (IC50=39.08 g · mL-1) > crude total alkaloids (IC50=55.28 g · mL-1). AB-8 macroporous adsorption resin can effectively enrich the active fraction of total alkaloids from Mahonia fortunei leaves, and the total alkaloids from Mahonia fortunei leaves possess certain antioxidant activity.

Full Text

Purification and Antioxidant Activity of Total Alkaloids from *Mahonia fortunei* Leaves by Macroporous Resin

JIA Kai, LIU Jun, GENG Xiaotong, ZHANG Yaozhou, XIAO Ying*

College of Pharmaceutical Engineering, Xinyang Agriculture and Forestry University, Xinyang 464000, Henan, China

Abstract

To determine the optimal purification conditions and antioxidant activity of total alkaloids from *Mahonia fortunei* leaves using macroporous resin, we compared the static adsorption and desorption capacities of six macroporous adsorption resins for total alkaloids, selected the optimal resin, and investigated its dynamic purification parameters. The antioxidant capacity before and after purification was evaluated using the DPPH method. The results showed: (1) AB-8 macroporous adsorption resin exhibited the best purification performance. The optimal conditions were: sample concentration of 50 $mg \cdot mL^{-1}$ (crude herbal dose), sample volume of 26 BV, and flow rate of 2 $BV \cdot h^{-1}$. After adsorption, impurities were removed with 3 BV of water, followed by elution with 4 BV of 50% ethanol. Under these conditions, the total alkaloid content increased from 13.33% to 56.64%. (2) The DPPH radical scavenging capacity followed the order: control Vc ($IC_{50} = 10.39$ $g \cdot mL^{-1}$) > purified total alkaloids ($IC_{50} = 39.08$ $g \cdot mL^{-1}$) > crude total alkaloids ($IC_{50} = 55.28$ $g \cdot mL^{-1}$). AB-8 macroporous adsorption resin can effectively enrich the active fraction of total alkaloids from *M. fortunei* leaves, which possess notable antioxidant activity.

Keywords: *Mahonia fortunei* leaf; total alkaloids; AB-8 macroporous resin; purification; antioxidant activity

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Introduction

Mahonia fortunei (Lindl.) Fedde, belonging to the family Berberidaceae and genus *Mahonia* Nutt., is an evergreen shrub widely used in traditional medicine for clearing heat, detoxifying, and nourishing yin (Flora of China Commission, 2001). It is commonly employed to treat dysentery, diarrhea, pharyngitis, and toothache. Modern pharmacological studies have demonstrated that plants in this genus exhibit antibacterial (Pu et al., 2016), anti-inflammatory (Hu et al., 2016), and antitumor activities (Wong et al., 2009), with clinical applications primarily in eczema (Donsky & Clarke, 2007) and psoriasis treatment (Janeczek et al., 2018). The *Pharmacopoeia of the People' s Republic of China* (2020 edition) lists the dried stem as the medicinal part, with alkaloids such as berberine, jatrorrhizine, and palmatine as the main active components (Chinese Pharmacopoeia Commission, 2020). However, studies have shown that the leaves also contain these alkaloids (Fan et al., 2011; Zhang et al., 2017).

Current research on *Mahonia* alkaloids has focused primarily on content determination (Zhang et al., 2017; Zhong, 2019) and extraction processes (Zhou et al., 2015; Yan et al., 2015), while purification studies have mostly involved chromatographic separation of individual compounds (Cui et al., 2018). Notably, no studies have reported on the purification of total alkaloid fractions. According to statistics, 18 Class 5 traditional Chinese medicine (TCM) new drugs were approved for production in China between 2009 and 2018 (Zhang et al., 2021), indicating that the development of active fractions has become a crucial direc-

tion for innovative TCM drug research. To meet the requirement that active fractions in Class 5 TCM new drugs must exceed 50% purity, establishing an industrially scalable purification process is critical for successful drug development. Among approved Class 5 TCM new drugs, 50% employ macroporous resin purification (Zhang et al., 2021), demonstrating that this technology has become a common and effective method for purifying active fractions.

Additionally, studies have reported that aqueous extracts from *M. bealei* leaves exhibit strong antioxidant activity (Hu et al., 2011), though the specific antioxidant components were not investigated. Alkaloids from *Mahonia* stems have demonstrated antioxidant capacity, with total alkaloids showing stronger activity than individual compounds (Zhu et al., 2016). To provide a reference for industrially obtaining high-purity, high-activity total alkaloids from *M. fortunei* leaves and to promote the utilization of leaf resources, this study investigated the purification of total alkaloids using macroporous resin technology and evaluated antioxidant activity using the DPPH method. By comparing the adsorption and desorption characteristics of six resins with different polarities and assessing antioxidant activity changes before and after purification, we aimed to: (1) optimize the macroporous resin purification parameters for total alkaloids from *M. fortunei* leaves, and (2) preliminarily evaluate the in vitro antioxidant activity of these alkaloids.

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1. Materials and Methods

1.1 Instruments and Reagents Instruments: TU-1810 UV-Vis spectrophotometer (Beijing Purkinje General Instrument Co., Ltd.); AB135-S electronic balance (Mettler-Toledo Instruments (Shanghai) Co., Ltd.); DKZ-1 constant temperature oscillating water bath (Shanghai Yiheng Scientific Instruments Co., Ltd.); RE-52A rotary evaporator (Shanghai Yarong Biochemical Instrument Factory).

Reagents: D101, AB-8, ADS-3, DA201, NKA-9, and LD605 macroporous resins (Tianjin Haoju Resin Technology Co., Ltd.); berberine hydrochloride reference standard (batch No. HB03313, purity $ $98%, for content determination, Shaanxi Huayu Biotechnology Co., Ltd.); 1,1-diphenyl-2-picrylhydrazyl (DPPH, analytical grade, Shanghai Macklin Biochemical Technology Co., Ltd.); Vc (analytical grade, Sinopharm Chemical Reagent Co., Ltd.); ethanol (analytical grade, Tianjin Damao Chemical Reagent Factory); water was purified.

Plant Material: Fresh *M. fortunei* leaves were collected from the campus of Xinyang Agriculture and Forestry University in late December and identified by Associate Professor Liang Lixiang of the Department of Traditional Chinese Medicine Resources and Development. The leaves were washed, shade-dried, destemmed, pulverized, and passed through a 40-mesh sieve.

1.2 Experimental Methods

1.2.1 Determination of Total Alkaloid Content Total alkaloid content was determined according to literature methods (Zhang et al., 2017). Using berberine hydrochloride as the reference standard at a detection wavelength of 345 nm, the linear regression equation was A = 0.06373C - 0.00681 (R^2 = 0.9999).

1.2.2 Preparation of Sample Solution and Crude Total Alkaloids Approximately 100 g of *M. fortunei* leaf powder (40-mesh) was accurately weighed and extracted three times with 8 volumes (v/w) of 75% ethanol under reflux for 2 h each time. After cooling to 40 °C, the mixture was filtered, and the filtrates were combined and concentrated under reduced pressure until ethanol-free. The concentrate was left to stand overnight, then filtered to remove precipitated black colloidal material. The filtrate was diluted with water to 25 times the original herb volume (equivalent to 40 $mg \cdot mL^{-1}$ crude herbal concentration) to obtain the sample solution. Other concentrations were prepared similarly. Following the same extraction method, the filtrate was concentrated and vacuum-dried to constant weight to obtain crude total alkaloids (purity 13.33%), which were stored in a desiccator.

1.2.3 Selection of Macroporous Resin Type 1.2.3.1 Resin Pretreatment: Different resin types (D101, AB-8, ADS-3, DA201, NKA-9, LD605) were soaked in 95% ethanol for 12 h to fully swell, then packed into columns using the wet method. The resins were washed with 95% ethanol until the eluate showed no white turbidity upon water addition, then washed with purified water until ethanol-free.

1.2.3.2 Static Adsorption and Desorption Tests: Accurately weighed 1.0 g portions of pretreated resin were placed in 100 mL conical flasks with 20 mL sample solution (40 $mg \cdot mL^{-1}$ crude herbal concentration). The flasks were oscillated in a constant temperature water bath for 2 h (30 °C, 120 rpm), then left to stand for 24 h. After filtration, the filtrate was appropriately diluted and absorbance measured to calculate total alkaloid concentration, from which saturation adsorption capacity and static adsorption rate were determined. The filtered resin was surface-dried and transferred to 100 mL conical flasks with 30 mL of 95% ethanol, then oscillated for desorption under the same conditions. After filtration and dilution, the total alkaloid concentration was measured to calculate desorption capacity and rate.

1.2.3.3 Dynamic Adsorption and Elution Tests: Pretreated D101, AB-8, and LD605 resins were packed into columns (1.0 cm × 20 cm, 10 mL resin volume). Sample solution (100 mL, 40 $mg \cdot mL^{-1}$ crude herbal concentration) was loaded at 2 $BV \cdot h^{-1}$, and the effluent was collected. Each resin column was then washed successively with 50 mL purified water and 100 mL 80% ethanol at 2 $BV \cdot h^{-1}$, with effluents collected. After appropriate dilution, total alkaloid concentrations were measured to calculate dynamic adsorption and desorption rates.

1.2.4 Optimization of AB-8 Resin Purification Process **1.2.4.1 Determination of Sample Concentration:** Pretreated AB-8 resin was packed into columns (1.0 cm × 20 cm, 10 mL). Sample solutions with different crude herbal concentrations were loaded at 2 BV·h^{-1}, collecting 2 BV fractions. Each fraction was diluted and analyzed for total alkaloid concentration to evaluate the effect of sample concentration on resin saturation adsorption capacity.

1.2.4.2 Determination of Flow Rate and Sample Volume: Pretreated AB-8 resin was packed into columns (1.0 cm × 20 cm, 10 mL). Sample solution (50 mg·mL^{-1} crude herbal concentration) was loaded at different flow rates to evaluate their effect on saturation adsorption capacity.

1.2.4.3 Determination of Water Volume for Impurity Removal: Pretreated AB-8 resin was packed into columns (1.0 cm × 20 cm, 10 mL). After loading 260 mL sample solution (50 mg·mL^{-1}) at 2 BV·h^{-1}, the column was washed with water, collecting 1 BV fractions. Total alkaloid concentration and solid matter mass were measured for each fraction to determine the optimal water volume based on total alkaloid content in the solids.

1.2.4.4 Determination of Ethanol Eluent Concentration and Volume: Four equal portions of pretreated AB-8 resin were packed into columns (1.0 cm × 20 cm, 10 mL each). After loading 260 mL sample solution (50 mg·mL^{-1}) at 2 BV·h^{-1} and washing with 3 BV water, elution was performed with 30%, 40%, 50%, and 60% ethanol, respectively. Eluates were collected in 1 BV fractions to generate elution curves.

1.2.4.5 Validation Test: Pretreated AB-8 resin was packed into a larger column (2.6 cm × 30 cm, 100 mL). After loading 2.6 L sample solution (50 mg·mL^{-1}) at 2 BV·h^{-1}, washing with 3 BV water, and eluting with 4 BV of 50% ethanol, the ethanol eluate was collected, concentrated, and dried. Total alkaloid content was measured, and the validation was repeated three times.

1.2.5 Determination of Antioxidant Activity Antioxidant activity was evaluated using the DPPH radical scavenging method, modified from literature (Li et al., 2018). DPPH (10 mg) was dissolved in 95% ethanol and diluted to 250 mL to prepare a 1$×10^{-4}$ mol·L^{-1} (40 g·mL^{-1}) working solution. Crude total alkaloids (black-green solid from Section 1.2.2), purified total alkaloids (yellow-green solid from Section 1.2.4.5), and Vc (positive control) were each accurately weighed (10 mg), dissolved in 95% ethanol, and diluted to 100 mL to obtain 100 g·mL^{-1} test solutions, which were then serially diluted to five concentrations. For the experimental group, 2 mL of each test solution was mixed with 2 mL DPPH working solution in a 10 mL stoppered test tube, shaken, and kept in the dark at room temperature for 30 min. The control group consisted of test solution mixed with 95% ethanol, while the blank group contained 95% ethanol and DPPH working solution. Absorbance was measured at 517 nm using 95% ethanol as the zero reference. Each concentration was tested in triplicate. The DPPH scavenging rate was calculated as:

Scavenging Rate (%) = [1 - (A_1 - A_2)/A_0] × 100

where A_0 is the blank group absorbance, A_1 is the experimental group absorbance, and A_2 is the control group absorbance.

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2. Results and Analysis

2.1 Total Alkaloid Content Determination The total alkaloid content in *M. fortunei* leaves was 4.16% (calculated as berberine hydrochloride). Based on the standard curve, the total alkaloid concentrations in sample solutions were 1.43 mg·mL^{-1} (at 40 mg·mL^{-1} crude herbal concentration) and 1.76 mg·mL^{-1} (at 50 mg·mL^{-1} crude herbal concentration).

2.2 Resin Selection Results

2.2.1 Static Adsorption and Desorption Test Results Six resins with different polarities were evaluated based on the properties of *Mahonia* alkaloids and resin characteristics (Table 1). The results showed that D101, AB-8, and LD605 resins exhibited superior adsorption and desorption performance compared to ADS-3, DA201, and NKA-9, warranting further investigation.

2.2.2 Dynamic Adsorption and Elution Test Results Dynamic adsorption and elution tests were conducted on D101, AB-8, and LD605 resins (Table 2). While all three resins showed similar elution capabilities (approximately 95% of total alkaloids were eluted after water and ethanol washing), AB-8 resin demonstrated superior dynamic adsorption capacity compared to LD605 and D101. Considering both adsorption and elution performance, AB-8 macroporous resin was selected for purifying *M. fortunei* total alkaloids.

2.3 Optimization of AB-8 Resin Purification Process

2.3.1 Determination of Sample Concentration Fractions were collected according to Section 1.2.4.1, and breakthrough curves were plotted as the ratio of effluent to initial total alkaloid concentration versus effluent volume. Saturation adsorption capacities at different concentrations were calculated (Figure 1 [Figure 1: see original paper] and Figure 2 [Figure 2: see original paper]). Within a certain range, increasing sample concentration advanced breakthrough time and increased saturation adsorption capacity. However, beyond 50 mg·mL^{-1} crude herbal concentration, adsorption capacity slightly decreased, likely due to competitive adsorption from impurities. Additionally, high-concentration solutions tended to precipitate upon standing. Therefore, 50 mg·mL^{-1} was selected as the optimal sample concentration.

2.3.2 Determination of Flow Rate and Sample Volume Using sample solution at 50 $mg \cdot mL^{-1}$ crude herbal concentration, saturation adsorption capacities at different flow rates were: 44.82 $mg \cdot mL^{-1}$ (1 $BV \cdot h^{-1}$), 43.05 $mg \cdot mL^{-1}$ (2 $BV \cdot h^{-1}$), 40.83 $mg \cdot mL^{-1}$ (3 $BV \cdot h^{-1}$), and 38.58 $mg \cdot mL^{-1}$ (4 $BV \cdot h^{-1}$). Lower flow rates increased saturation adsorption capacity and favored alkaloid adsorption, but excessively low rates reduced efficiency. Considering the modest differences in adsorption capacity and the need for operational efficiency, 2 $BV \cdot h^{-1}$ was selected. At this flow rate, when the sample volume reached 26 BV, the effluent concentration approached 50% of the initial concentration, with resin adsorption at 86.69% of saturation capacity. Thus, 26 BV was chosen as the optimal sample volume.

2.3.3 Determination of Water Volume for Impurity Removal Under conditions of 50 $mg \cdot mL^{-1}$ sample concentration, 26 BV sample volume, and 2 $BV \cdot h^{-1}$ flow rate, water washing results are shown in Table 3 . When the water volume reached 3 BV, the total alkaloid content in solids was 24.12%, indicating significant alkaloid loss. To balance impurity removal with minimal alkaloid loss, 3 BV was selected as the optimal water washing volume.

2.3.4 Determination of Ethanol Eluent Concentration and Volume Under the same loading conditions followed by 3 BV water washing, ethanol elution results are shown in Figure 3 [Figure 3: see original paper]. As ethanol concentration increased, elution efficiency improved, with alkaloids concentrated in 50% and 60% ethanol fractions, though 60% ethanol showed only marginal improvement over 50%. More than 90% of total alkaloids were eluted within 4 BV using 50% ethanol. Therefore, 50% ethanol was selected as the eluent at a volume of 4 BV.

2.3.5 Validation Test Results Under optimized conditions (50 $mg \cdot mL^{-1}$ sample concentration, 26 BV sample volume, 2 $BV \cdot h^{-1}$ flow rate, 3 BV water washing, and 4 BV 50% ethanol elution), validation results are shown in Table 4 .

2.3.6 Antioxidant Activity of Total Alkaloids Antioxidant activity results are presented in Figure 4 [Figure 4: see original paper]. Within the tested concentration range of 6.25–100 $g \cdot mL^{-1}$, DPPH radical scavenging rates for crude alkaloids, purified alkaloids, and Vc increased dose-dependently, with IC_{50} values of 55.28, 39.08, and 10.39 $g \cdot mL^{-1}$, respectively. Both crude and purified total alkaloids exhibited DPPH radical scavenging activity, with significantly enhanced antioxidant capacity after macroporous resin purification, though still lower than Vc at equivalent concentrations.

3. Discussion and Conclusion

During sample solution preparation, the concentrated extract must be left to stand until black colloidal material precipitates completely; otherwise, precipitation during storage would affect adsorption efficiency. Additionally, sample concentration should not be excessively high to avoid turbidity, likely due to the limited water solubility of *M. fortunei* alkaloids.

Different macroporous resins exhibit varying adsorption and desorption capacities, making proper resin selection crucial. Six resins with different polarities were compared for their static and dynamic performance. Weakly polar (AB-8, LD605) and non-polar (D101) resins showed higher adsorption capacities for *M. fortunei* total alkaloids. Considering both adsorption and elution capabilities, AB-8 resin was selected for purification, consistent with literature reports for alkaloid purification from *Phellodendron amurense* and *Coptis chinensis* containing berberine (Liu et al., 2010; Lin et al., 2013). The optimized AB-8 resin process parameters were: 50 $mg \cdot mL^{-1}$ sample concentration, 26 BV sample volume, 2 $BV \cdot h^{-1}$ flow rate, 3 BV water washing, and 4 BV 50% ethanol elution. The purified total alkaloid content exceeded 50%, meeting the requirement for Class 5 TCM new drug active fractions.

Similar to the stems, alkaloids are the main active components in *M. fortunei* leaves. These alkaloids are predominantly quaternary ammonium compounds, such as berberine and jatrorrhizine, with extensive pharmacological activities. Berberine can inhibit reactive oxygen species (ROS) generation, induce antioxidant defenses, and trigger oxidative stress in diseased cells, making it a promising antioxidant candidate (Zhao et al., 2019). Based on the pharmacological role of antioxidants in neurodegenerative disease prevention and treatment, research suggests that *Mahonia* stem rich in berberine and its derivatives holds significant potential as a therapeutic agent for neurodegenerative diseases (Liu, 2019). This study demonstrated that *M. fortunei* total alkaloids possess antioxidant capacity, which improves after purification. Therefore, the non-medicinal leaf portion warrants greater attention in resource utilization.

This study provides a feasible approach for extracting and separating total alkaloids from *M. fortunei* leaves, offering a pathway to utilize this non-medicinal plant resource. The AB-8 macroporous resin purification process is simple, effective, and stable, providing a reference for the development, preparation, and industrial production of *M. fortunei* total alkaloids.

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