

Exploration of Purification Process for Total Phenolics from Whole Silkworm Bodies

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Abstract [Objectives] This study was conducted to determine macroporous resin purification process parameters for total phenolics from whole silkworm bodies. [Methods] The macroporous resin type was screened through static adsorption and desorption experiments. The process parameters, including sample solution concentration, pH, ethanol volume fraction of desorption solution, and flow rate, were optimized by static adsorption/desorption kinetics and single-factor experiments. [Results] The optimal resin was D101. The optimal purification conditions were as follows: sample solution pH of 2.0, sample concentration of 0.2 mg/mL, 60% ethanol as the desorption solution, sample-loading flow rate of 1.6 mL/min, and desorption flow rate of 1.6 mL/min. Under these conditions, the purity of total phenolics from whole silkworm bodies increased from (7.26% $\pm$ 0.12%) to (23.57% $\pm$ 1.65%), representing a 3.2-fold increase in purity. [Conclusions] This purification process significantly improved the purity of total phenolics from whole silkworm bodies with relatively low cost, providing a technical reference for the resource development of silkworm.

Key words Silkworm, Total phenolics, Purification process, Macroporous resin

0 Introduction

The silkworm (*Bombyx mori* Linnaeus) belongs to the genus *Bombyx* in Bombycidae of Lepidoptera, and is an economic insect that feeds exclusively on mulberry leaves and spins silk to form cocoons. In addition to silk production, the silkworm can absorb and utilize the components of ingested mulberry leaves. Its body is rich in alkaloids, flavonoids, phenols, lactones, organic acids, sugars, and glycosides^[1–2], and exhibits antioxidant, lipid-lowering, and hypoglycemic effects^[3–5]. The medicinal value of the silkworm has been documented in ancient works such as *Qimin Yaoshu*, *Compendium of Materia Medica*, and *Chinese Materia Medica*. As the saying goes, "it can strengthen the body when consumed and cure various diseases when used as medicine".

Previous research by our group has shown that phenolics are the main active components responsible for the antioxidant activity of whole silkworm bodies^[6]. Polyphenolic compounds exhibit excellent antioxidant capacity^[7] and have become a research hotspot in exogenous antioxidants in recent years. Phenolic compounds are aromatic compounds with phenol as the basic framework, characterized by the substitution of one or more phenolic hydroxyl groups on the benzene ring. Phenolic hydroxyl groups are readily oxidized, enabling them to scavenge free radicals and chelate metal ions to prevent the formation of hydroxyl radicals, thereby exerting antioxidant effects^[8–9].

Polyphenols obtained by general extraction are crude poly-

phenols, which still contain non-phenolic impurities such as polysaccharides and cellulose, resulting in relatively low purity. Therefore, it is necessary to adopt certain methods to remove impurities and improve the purity of polyphenols^[10–11]. Macroporous resins are widely used in polyphenol purification due to their advantages of high adsorption capacity, easy regeneration, and low cost^[12]. However, studies on the purification process of total polyphenols from whole silkworm bodies have not been reported yet. In this study, optimal macroporous resin was selected by measuring static adsorption and desorption conditions, and the purification process parameters of whole silkworm bodies were optimized, aiming to provide a reference for the industrial utilization of silkworm resources.

1 Materials and methods

1.1 Materials and reagents Silkworm (*Bombyx mori*, Jing-song $\times$ Haoyue) was obtained from Guangdong Guangtong Silkworm Eggs (Group) Co., Ltd. Macroporous resins D101, AB-8, and HPD100 were purchased from Shanghai Yuanye Biotechnology Co., Ltd. Folin-Ciocalteu reagent was obtained from Jincloone (Beijing) Biotechnology Co., Ltd. Gallic acid standard was purchased from Beijing Solarbio Science & Technology Co., Ltd.

1.2 Instruments and equipment Ultrasonic cleaner (Skymen Cleaning Equipment Shenzhen Co., Ltd.); peristaltic pump (Baoding Longer Precision Pump Co., Ltd.); UV-visible spectrophotometer (Beijing Purkinje General Instrument Co., Ltd.); electronic balance (Changzhou Ohaus Instrument Co., Ltd.).

1.3 Experimental methods

1.3.1 Plotting of standard curve. First, 10 mg of gallic acid standard was dissolved in distilled water to prepare a gallic acid standard solution. Next, the standard solution was diluted to prepare a series of solutions with concentration gradients of 0.00, 0.01, 0.02, 0.03, 0.04, and 0.05 mg/mL, and the absorbance

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values were determined by the Folin-Ciocalteu method. A standard curve was plotted with the standard concentration as the abscissa and the absorbance value as the ordinate. The linear regression equation was obtained as $y = 14.34x + 0.0093$, with $R^2 = 0.9992$.

1.3.2 Pretreatment of macroporous resins. D101 and HPD100 macroporous resins were activated with anhydrous ethanol. After soaking for 4 h, the anhydrous ethanol was filtered off, and the resins were rinsed with deionized water and stored and soaked at room temperature. AB-8 macroporous resin was soaked in 3% – 5% sodium hydroxide solution for 24 h, then rinsed with deionized water until the pH was neutral. Subsequently, 95% ethanol solution was added for soaking for 4 h, followed by rinsing with deionized water, and finally stored and soaked at room temperature.

1.3.3 Screening of macroporous resins. First, 1 g of each pretreated resin was weighed and added to 20 mL of whole silkworm total phenolic extract at a certain concentration. Next, the mixture was placed in a constant temperature shaker and shaken at 25 °C and 120 r/min for 24 h for adsorption. Next, 0.2 mL of the supernatant was taken to determine the total phenolic concentration C_1 , and the adsorption rate of the macroporous resin was calculated according to the following formula.

The macroporous resin saturated with adsorption was washed with deionized water to remove impurities, filtered under vacuum, and then placed in a 100 mL conical flask. Next, 20 mL of 70% ethanol solution was added, and the mixture was placed in a constant temperature shaker at 25 °C and 120 r/min for desorption for 24 h. Subsequently, 0.2 mL of the supernatant was taken to determine the total phenolic concentration C_2 , and the desorption rate of the macroporous resin was calculated according to the following formula.

$$\text{Adsorption rate} = \frac{C_0 - C_1}{C_0} \times 100\% \quad (1)$$

$$\text{Desorption rate} = \frac{C_2 \times V_2}{(C_0 - C_1) \times V_1} \times 100\% \quad (2)$$

where C_0 : initial concentration of the total phenolic extract (mg/mL); C_1 : concentration of the total phenolic extract after adsorption (mg/mL); C_2 : concentration of total phenolics in the desorption solution (mg/mL); V_1 : volume of the total phenolic extract (mL); V_2 : volume of the desorption solution (mL).

1.3.4 Static adsorption and desorption kinetics of D101 macroporous resin. First, 1.0 g of D101 macroporous resin was added in a 100 mL conical flask, and 20 mL of the total phenolic extract at a certain concentration was added. The flask was placed in a constant temperature shaker and shaken at 25 °C and 120 r/min for adsorption for 8 h. Every 1 h, 0.2 mL of the supernatant was sampled to determine the total phenolic concentration until no significant fluctuation was observed, and a static adsorption curve was plotted. Next, the D101 macroporous resin saturated with adsorption was washed with deionized water to remove impurities, filtered under vacuum, and then added in a 100 mL conical flask. Subsequently, 20 mL of 70% ethanol solution was added, and the flask was placed in a constant temperature shaker at 25 °C and 120

r/min for desorption for 8 h. Every 1 h, 0.2 mL of the supernatant was taken to determine the total phenolic concentration until no significant fluctuation was observed, and a static desorption curve was plotted.

1.3.5 Optimization of static adsorption and desorption conditions for D101 macroporous resin. First, 1.0 g of pretreated D101 macroporous resin was added in a 100 mL conical flask, and the total phenolic extract was added. The flask was placed in a constant temperature shaker and shaken at 25 °C and 120 r/min for adsorption for 5 h. Next, 0.2 mL of the supernatant was sampled to determine the total phenolic concentration, and the adsorption rate was calculated using the formula. The effects of different sample solution concentrations (0.2, 0.4, 0.6, 0.8, 1.0 mg/mL) and different pH values (2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0) on the adsorption rate were compared. Subsequently, the macroporous resin saturated with adsorption was washed to remove impurities, and then placed in a constant temperature shaker at 25 °C and 120 r/min for desorption for 4 h. The effect of different ethanol volume fractions (50%, 60%, 70%, 80%, 90%) in the desorption solution on the desorption rate was analyzed.

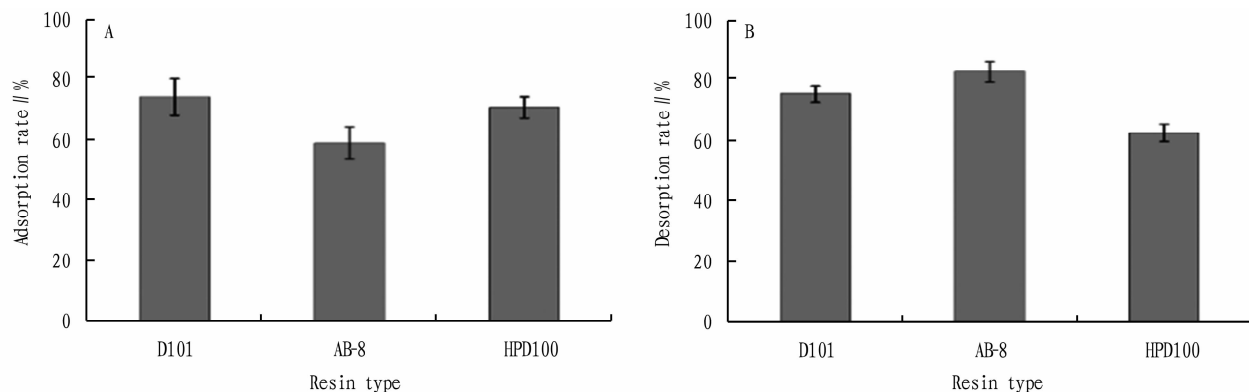
1.3.6 Optimization of dynamic adsorption and desorption conditions for D101 macroporous resin. A wet packing method was adopted. In specific, 5.0 g of pretreated D101 macroporous resin was packed into a glass chromatography column (Φ 3 cm $\times$ 30 cm). Five aliquots of 25 mL of the total phenolic extract at 0.2 mg/mL were adjusted to pH 2.0 and loaded at different flow rates (0.8, 1.6, 2.4, 3.2, 4.0 mL/min). Then, 0.2 mL of the effluent was sampled to determine the total phenolic concentration, and the adsorption rate was calculated using the formula.

Another five aliquots of 25 mL of the total phenolic extract at 0.2 mg/mL were adjusted to pH 2.0 and loaded at a constant flow rate of 1.6 mL/min. After complete adsorption, the resin column was rinsed with deionized water, and 25 mL of 60% ethanol solution was added for desorption at different flow rates (0.8, 1.6, 2.4, 3.2, 4.0 mL/min). Then, 0.2 mL of the desorption solution was sampled to determine the total phenolic concentration, and the desorption rate was calculated using the formula.

1.4 Data processing All experimental data are expressed as mean $\pm$ standard deviation of three biological replicates. Origin 2021 was employed for data visualization.

2 Results and analysis

2.1 Screening of macroporous resins As shown in Fig. 1A, the adsorption rates of different types of macroporous resins ranked as D101 (63.33 $\pm$ 1.01)% > AB-8 (59.26 $\pm$ 2.01)% > HPD100 (58.68 $\pm$ 1.74)%. As shown in Fig. 1B, the desorption rates of different types of macroporous resins were as follows; D101 (67.99 $\pm$ 2.75)% > AB-8 (63.84 $\pm$ 2.94)% > HPD100 (60.51 $\pm$ 1.72)%. Both the adsorption rate and desorption rate were highest for D101. Therefore, D101 was selected as the most suitable macroporous resin type for subsequent total phenolic purification experiments of whole silkworm bodies.



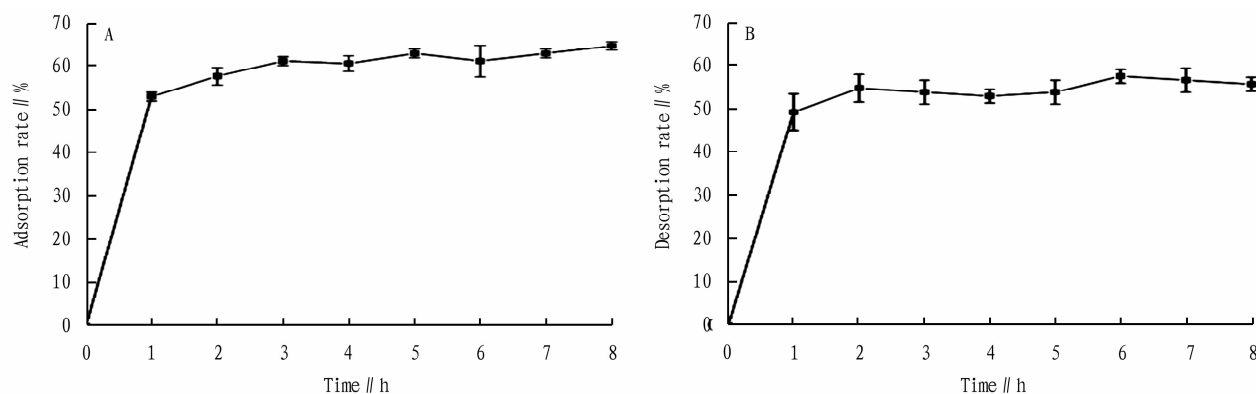
NOTE A. Adsorption rate; B. Desorption rate.

Fig. 1 Adsorption and desorption rates of different types of resins for total phenolics from silkworm

2.2 Static adsorption-desorption kinetics curve of D101 macroporous resin

Through static adsorption-desorption kinetics experiments, the adsorption and desorption rates of D101 macroporous resin for total phenolics from whole silkworm bodies at different time points were investigated. As shown in Fig. 2, both the adsorption and desorption rates of D101 resin increased with time. As shown in Fig. 2A, D101 macroporous resin exhibi-

ted rapid adsorption of total phenolics within 1 h, and adsorption equilibrium was reached at 5 h. As illustrated in Fig. 2B, total phenolics were rapidly eluted within 1 h. Thereafter, the desorption rate gradually stabilized and desorption equilibrium was achieved at 4 h. Accordingly, 5 h was selected as the static adsorption time and 4 h as the static desorption time for subsequent experiments.



NOTE A. Adsorption kinetic curve; B. Desorption kinetic curve.

Fig. 2 Adsorption-desorption kinetic curves of total phenolics from silkworm on resin

2.3 Optimization of static adsorption and desorption conditions for D101 macroporous resin

As shown in Fig. 3A, the adsorption rate gradually decreased with the total phenolic concentration increasing. When the concentration was 0.2 mg/mL, the adsorption rate reached its maximum value of $(62.17\% \pm 1.74\%)$. It may be attributed to the fact that higher concentrations lead to more impurities in the solution, which block the resin pores and reduce the adsorption rate. Therefore, 0.2 mg/mL was selected as the sample loading concentration for subsequent experiments on total phenolics from whole silkworm bodies.

Fig. 3B illustrates that the adsorption rate of the macroporous resin decreased with increasing pH. When the pH was 2, the adsorption rate reached its maximum value of $(63.68\% \pm 1.45\%)$. It indicates that the adsorption rate of total phenolics is higher under acidic conditions, because total phenolics exist in molecular form under acidic conditions, which is more favorable for adsorp-

tion by macroporous resins.

According to the results in Fig. 3C, the desorption rate of macroporous resin rose first and then declined with the ethanol volume fraction increasing, peaking at $(68.81\% \pm 2.60\%)$ under 60% ethanol. This phenomenon may be attributed to the high polarity of total phenolics from whole silkworm bodies; high-concentration ethanol disrupts hydrogen-bond interactions between total phenolics and the resin, facilitating their desorption.

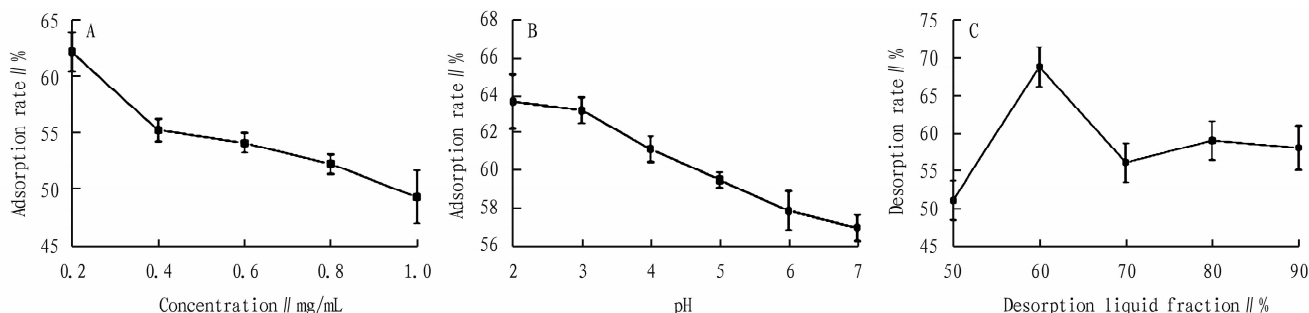
2.4 Optimization of dynamic adsorption and desorption conditions for D101 macroporous resin

As shown in Fig. 4A, with the flow rate of the sample solution increasing, the adsorption rate first increased and then decreased. When the flow rate of the sample solution was 1.6 mL/min, the adsorption rate reached its maximum value of $(80.22\% \pm 1.29\%)$. At higher flow rates, the contact time between the total phenolic solution and the resin was shorter, and the phenolics flowed out with the solution before be-

ing adsorbed.

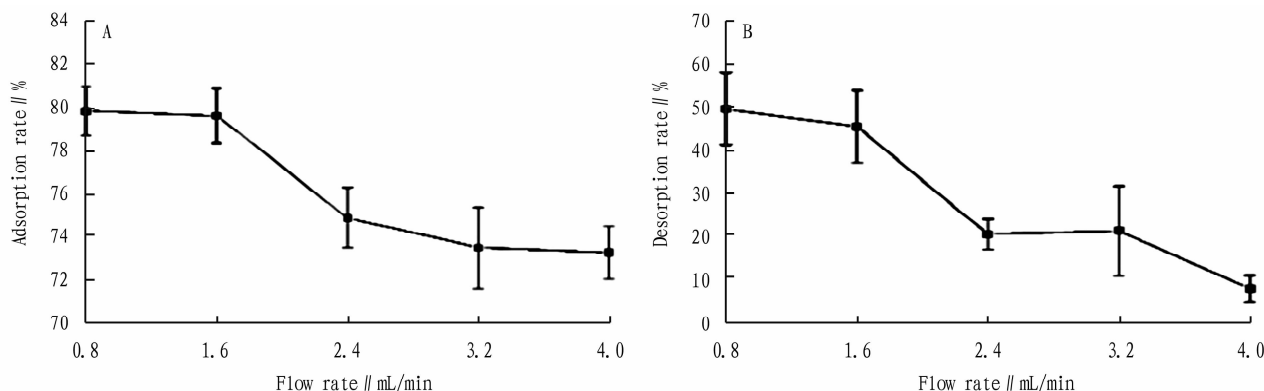
According to the results in Fig. 4B, the desorption rate showed a decreasing trend with the flow rate of the desorption solution increasing. At higher desorption flow rates, the contact time between the ethanol solution and the resin was shorter, and the interaction forces between the polyphenols and the resin could not be sufficiently disrupted, leading to a decrease in desorption rate. When the flow rate was 0.8 mL/min, the desorption rate reached

its maximum value of $(61.87\% \pm 1.69\%)$, and when the flow rate was 1.6 mL/min, the desorption rate was $(61.04\% \pm 1.71\%)$, showing little difference between the two. However, when the flow rate exceeded 1.6 mL/min, the desorption rate decreased rapidly. A slower desorption flow rate is favorable for the dissociation of total phenolics from the resin, but reduces time efficiency. Therefore, 1.6 mL/min was selected as the flow rate of the desorption solution for subsequent experiments.



NOTE A. Sample solution concentration; B. Sample solution pH; C. Ethanol volume fraction of desorption solution.

Fig. 3 Effects of sample solution concentration, pH, and desorption solution volume fraction on static adsorption/desorption of macroporous resin



NOTE A. Sample-loading flow rate; B. Desorption flow rate.

Fig. 4 Effects of sample-loading flow rate and desorption flow rate on adsorption/desorption rates of macroporous resin

2.5 Verification test Using the wet packing method, purification was carried out under optimal conditions: total phenolics extract from whole silkworm bodies at 0.2 mg/mL, pH 2.0, sample-loading flow rate of 1.6 mL/min, 60% ethanol as the desorption solution, and desorption flow rate of 1.6 mL/min. After calculation, the purity of total phenolics from whole silkworm bodies increased from $(7.26\% \pm 0.12\%)$ to $(23.57\% \pm 1.65\%)$, representing a 3.2-fold increase in purity, indicating a significant purification effect. The results demonstrate that D101 macroporous resin has good purification efficiency for total phenolics from whole silkworm bodies.

3 Conclusions and discussion

In this study, three different types of macroporous resins (D101, AB-8, and HPD100) were compared for their adsorption and desorption rates of total phenolics from whole silkworm bodies, and D101 was selected as the optimal macroporous resin type. Using D101 macroporous resin for adsorption and desorption

of total phenolics from whole silkworm bodies, the equilibrium times for adsorption and desorption were determined to be 5 and 4 h, respectively. The optimal process parameters were as follows: sample solution concentration at 0.2 mg/mL, pH 2.0, ethanol volume fraction of desorption solution of 60%, sample-loading flow rate of 1.6 mL/min, and desorption solution flow rate of 1.6 mL/min. After purification under these parameters, the purity of total phenolics from whole silkworm bodies was increased by 3.2 fold, indicating a significant purification effect.

Under acidic conditions, phenolic compounds predominantly exist in molecular form, with reduced polarity and enhanced interaction with the non-polar D101 resin^[10,12-13]. Therefore, the adsorption capacity of total phenolics from whole silkworm bodies is stronger in acidic environments. Compared with plant-derived mulberry leaves, the optimal sample loading concentration for purification of total phenolics from whole silkworm bodies is lower, the ethanol concentration of the desorption solution is lower, and the sample loading flow rate is slower^[14]. These differences may

be attributed to the different complexity of their extract compositions. As a living organism, silkworms contain phenolic compounds that may exist in the form of metabolic conjugates (such as glycosides and sulfate conjugates) or interact with macromolecules such as proteins, resulting in physicochemical properties that differ from those of free phenolics in mulberry leaves. A lower ethanol concentration is sufficient for effective desorption, which may indicate that the adsorption force between total phenolics from whole silkworm bodies and the resin is relatively weak. The slower adsorption flow rate may be related to its mass transfer rate, requiring longer contact time to reach saturated adsorption^[15].

In this study, a macroporous resin purification process for total phenolics from whole silkworm bodies was preliminarily established, providing a technical reference for the deep development and comprehensive utilization of silkworm resources. Further studies can systematically evaluate the composition and bioactivity of the purified total phenolics.

References

[1] LIU J, LIAO ST, ZOU YX, *et al.* Extraction methods and process optimization of nutritional active components from male silkworm moths[J]. *Acta Sericologica Sinica*, 2013, 39(1): 146–151. (in Chinese).

[2] LIN YX, LUO XJ, FAN LJ, *et al.* Study on the nutritional value of male silkworm moths and the production technology of health wine[J]. *Guangxi Journal of Light Industry*, 2015, 31(4): 20–21. (in Chinese).

[3] WAN HQ, QI HY, XU GH, *et al.* Silkworm extract ameliorates type 2 diabetes mellitus and protects pancreatic β -cell functions in rats[J]. *Digital Chinese Medicine*, 2020, 3(4): 275–282.

[4] WANG ML, HUANG YQ, CAO Y, *et al.* Curative effect observation of silkworm powder on the rats with type 2 diabetes[J]. *Strait Pharmaceutical Journal*, 2015, 27(5): 38–40. (in Chinese).

[5] BIGANEH H, KABIRI M, ZEYNALPOURFATTAHI Y, *et al.* *Bombyx mori* cocoon as a promising pharmacological agent: A review of ethnopharmacology, chemistry, and biological activities [J]. *Heliyon*, 2022, 8(9): e10496.

[6] XIE Y, SUN ZC, GAO YX, *et al.* Screening of antioxidant active components from the larval bodies of *Bombyx mori* and analysis of non-targeted metabolomics[J]. *Acta Entomologica Sinica*, 2024, 67(11): 1461–1474. (in Chinese).

[7] CHEN YS, FU X, ZHOU L, *et al.* Antioxidant activity of the phenolics from *Adinandra nitida* leaves and their antiproliferative effect on HepG2 cells[J]. *Modern Food Science & Technology*, 2016, 32(8): 14–20. (in Chinese).

[8] KUREK-GÓRECKA A, RZEPECKA-STOJKO A, GÓRECKI M, *et al.* Structure and antioxidant activity of polyphenols derived from propolis [J]. *Molecules*, 2013, 19(1): 78–101.

[9] KIM SS, LIM J, BANG Y, *et al.* Licochalcone E activates Nrf2/antioxidant response element signaling pathway in both neuronal and microglial cells; Therapeutic relevance to neurodegenerative disease[J]. *J Nutr Biochem*, 2012, 23(10): 1314–1323.

[10] NONG JM, YE YM, LIAO ZD, *et al.* Purification of tea polyphenols by macroporous resin and its application properties[J]. *China Food Additives*, 2023, 34: 60–67. (in Chinese).

[11] LIU D, WU YH, LI WH, *et al.* Application of macroporous adsorptive resins in separation and purification of natural products [J]. *Chinese Traditional and Herbal Drugs*, 2016, 47(15): 2764–2770. (in Chinese).

[12] ZHANG GC, HUANG CH, LI S. Purification of total flavonoids from *Cirsium japonicum* DC. with macroporous resin and comparison of antioxidant activity of crude and purified flavonoids [J]. *Chemical Engineer*, 2024, 38: 99–104. (in Chinese).

[13] WANG ZY, XU LJ, MA KY, *et al.* Purification optimization of mung bean skin polyphenols by macroporous adsorption resin and its *in vitro* hypoglycemic activity [J]. *Science and Technology of Food Industry*, 2025, 46: 253–262. (in Chinese).

[14] WANG C, ZOU YX, LI Q, *et al.* An Investigation on composition and enrichment process of phenolic compounds in mulberry leaf[J]. *Acta Sericologica Sinica*, 2018, 44: 729–737. (in Chinese).

[15] GUO MX, SHEN Y, FENG YF, *et al.* Purification technology of polyphenol from *Curcuma longa* L. by macroporous resin[J]. *Chinese Journal of Ethnomedicine and Ethnopharmacy*, 2023, 32: 33–37. (in Chinese).

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