

Article

Optimization of the Purification Process for C-Glycosyl Flavones from *Phyllostachys edulis* Leaves

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Abstract

A preparative purification process was developed for the enrichment of four C-glycosyl flavones from leaves of *Phyllostachys edulis* (Carrière) J.Houz., namely orientin, isoorientin, vitexin, and isovitexin. The crude *P. edulis* leaf extract was used as the feed material, and HPLC was employed for quantitative analysis. Macroporous resins and polyamide resins were compared in terms of their adsorption and desorption performance toward the target compounds. The results showed that D101 macroporous resin provided the best overall process suitability among the tested resins. The optimal purification conditions were an initial sample concentration of 10 mg·mL⁻¹, a resin-to-sample loading ratio of 5:1 (g·g⁻¹), a sample-loading flow rate of 4 BV·h⁻¹, impurity removal with 15% ethanol, elution with 60% ethanol, an elution volume of 3 BV, and a 60% ethanol elution flow rate of 3 BV·h⁻¹. After D101 resin purification, the yield of the four C-glycosyl flavones was 11.712 mg·g⁻¹ relative to the crude extract, and their combined content in the D101-purified flavonoid-enriched powder reached 68.359 mg·g⁻¹. Further purification using polyamide resin with a particle-size range of 0.15–0.25 mm and 40% ethanol resulted in the recovery of 16.128 mg·g⁻¹ of the four C-glycosyl flavones relative to the D101-purified feed material, while their combined content in the polyamide-purified flavonoid-enriched powder reached 76.939 mg·g⁻¹. This process provides a basis for the preparative separation and utilization of C-glycosyl flavones from *P. edulis* leaves.

Keywords: C-glycosyl flavones; *Phyllostachys edulis* leaves; macroporous resin; polyamide resin; adsorption–desorption; purification



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1. Introduction

Bamboo resources are important non-wood forest resources with considerable ecological value and economic development potential. *Phyllostachys edulis* (Carrière) J.Houz., commonly known as moso bamboo and called maozhu in China, is one of the most widely distributed and abundant bamboo species in the country [1,2]. Bamboo resources also have broad potential for value-added conversion and energy applications [3]. Current utilization of *P. edulis* mainly focuses on bamboo culm processing, bamboo shoot production, and the chemical conversion of bamboo materials, whereas its leaves are often discarded as processing by-products or directly burned, resulting in the loss of potentially valuable resources [3,4]. Therefore, the development of natural bioactive constituents from *P. edulis* leaves represents an important approach to improving the comprehensive utilization of bamboo resources [5–7].

More broadly, bamboo leaves have long been used as traditional herbal medicines and food materials in East Asia, including in traditional applications for conditions such as pyrexia and edema [8]. Bamboo leaves contain various chemical constituents, including flavonoids, phenolic acids, polysaccharides, and multiple trace elements [9–11]. Among these compounds, bamboo-leaf flavonoids have attracted increasing attention because of their potential biological activities, such as antioxidant, anti-inflammatory, lipid-regulating, and glucose-regulating effects [12–14]. The representative C-glycosyl flavones in bamboo leaves mainly include orientin, isoorientin, vitexin, and isovitexin [15–17]. These four compounds are C-glycoside isomers of luteolin and apigenin. Their sugar moieties are linked to the flavone aglycone through C–C bonds, which generally confers higher structural stability than ordinary O-glycosides [18]. Meanwhile, the presence of multiple phenolic hydroxyl groups gives these compounds a certain degree of hydrophilicity.

In existing bamboo-leaf flavonoid products and related studies, total flavonoid content is often used as a quality evaluation index. However, this index cannot accurately reflect the composition or enrichment degree of specific active constituents. In contrast, establishing quantitative analysis and purification processes based on defined monomeric or characteristic components is more beneficial for quality control and subsequent application development of bamboo-leaf flavonoid products [19–21]. As the major C-glycosyl flavones in bamboo leaves, orientin, isoorientin, vitexin, and isovitexin can serve as key indicators for evaluating the purification efficiency of bamboo-leaf flavonoid extracts [11,15,22].

Macroporous resin adsorption is widely used for the enrichment of plant flavonoids because of its good adsorption selectivity, mild desorption conditions, convenient regeneration, and suitability for preparative-scale processing [23]. Polyamide resin can adsorb flavonoids through hydrogen bonding between its amide groups and the phenolic hydroxyl groups of flavonoid compounds, thereby showing strong enrichment capacity toward flavonoids [24–27]. Because these two types of resins differ in adsorption mechanisms and selectivity, their rational combination may help remove sugars, proteins, pigments, and other impurities while increasing the content of the four target C-glycosyl flavones in bamboo-leaf flavonoid powder.

Based on these considerations, this study used the crude *P. edulis* leaf extract as the feed material, and the HPLC-determined contents of the four C-glycosyl flavones were used as evaluation indices. Suitable macroporous resins and polyamide resins were systematically screened, and the conditions for a two-stage tandem purification process were optimized. The aim of this study was not to re-evaluate the biological activities of bamboo-leaf flavonoids, but to establish a preparative enrichment process targeting four defined C-glycosyl flavones. This work may provide an experimental basis for the valorization of *P. edulis* leaves and the preparation of bamboo-leaf flavonoid intermediates.

2. Materials and Methods

2.1. Materials, Reagents, and Instruments

Reference standards of orientin, isoorientin, vitexin, and isovitexin, each with a purity of not less than 98%, were purchased from Shanghai Ronghe Pharmaceutical Technology Development Co., Ltd. (Shanghai, China). HPLC-grade methanol, acetonitrile, and formic acid were supplied by Fisher Scientific (Waltham, MA, USA). Analytical-grade absolute ethanol, petroleum ether, acetone, concentrated hydrochloric acid, and sodium hydroxide were obtained from Beijing Chemical Works (Beijing, China).

The AB-8, ADS-17, D101, DM130, NKA-9, and X-5 macroporous resins were purchased from Zhengzhou Qinshi Technology Co., Ltd. (Zhengzhou, China). Polyamide resins with particle-size ranges of 0.25–0.55 mm, 0.15–0.25 mm, and 0.12–0.15 mm were purchased from Shanghai Kunke Instrument Equipment Co., Ltd. (Shanghai, China).

HPLC analyses were performed using a Waters 2695 high-performance liquid chromatography system equipped with a Waters 2996 photodiode-array detector and a Waters XTerra MS C18 column (250 mm $\times$ 4.6 mm, 5 μ m; Waters Corporation, Milford, MA, USA). An R-220 rotary evaporator (Büchi, Flawil, Switzerland) was used during preparation of the crude leaf extract, whereas an N-1200B rotary evaporator (Tokyo Rikakikai Co., Ltd., Tokyo, Japan) was used during concentration of the resin eluates. The other main instruments included a vacuum freeze dryer (LABCONCO, Kansas City, MO, USA), a ZHWY-200B thermostatic shaker (Shanghai Zhicheng Analytical Instrument Manufacturing Co., Ltd., Shanghai, China), and glass chromatography columns (2 cm $\times$ 35 cm and 8 cm $\times$ 65 cm; Beijing Chemical Glass Station, Beijing, China).

2.2. Preparation of the Crude *P. edulis* Leaf Extract

A 2.0 kg portion of shade-dried *P. edulis* leaf powder was extracted under reflux with 75% (*v/v*) aqueous ethanol at a solid-to-liquid ratio of 1:20 (g:mL) and 70 °C for 2 h per extraction cycle. The extraction was repeated four times. After each extraction, the mixture was filtered, and the resulting filtrates were combined. Ethanol was removed from the combined extract by evaporation under reduced pressure. The concentrated extract was then partitioned three times with an equal volume of petroleum ether to remove pigments and other lipophilic constituents. The aqueous phase was collected, further concentrated under reduced pressure, and vacuum-dried to obtain the crude *P. edulis* leaf extract. Because aqueous ethanol also co-extracted non-target constituents, the resulting material, which contained the four target C-glycosyl flavones together with other co-extracted compounds, was designated as the crude *P. edulis* leaf extract rather than a purified flavonoid fraction. The crude extract was used as the feed material for macroporous resin purification to enrich orientin, isoorientin, vitexin, and isovitexin while removing co-extracted non-target constituents.

2.3. HPLC Quantitative Analysis

HPLC was used to determine the contents of orientin, isoorientin, vitexin, and isovitexin in samples obtained at different purification stages. Chromatographic separation was performed on a Waters XTerra MS C18 column (250 mm $\times$ 4.6 mm, 5 μ m). The mobile phase consisted of acetonitrile and 0.5% formic acid in water at a ratio of 14:86 (*v/v*), and isocratic elution was carried out for 25 min. The flow rate was 1.0 mL $\cdot$ min^{−1}, the column temperature was maintained at 30 °C, the injection volume was 10 μ L, and detection was performed at 360 nm. The target compounds were qualitatively identified by comparing their retention times and UV absorption spectra with those of the reference standards, and quantification was performed using peak areas.

2.4. Resin Pretreatment and Regeneration

Macroporous resins were first washed with absolute ethanol to remove fine particles and impurities, and then soaked for 12 h to allow complete swelling. After wet packing into the column, the resins were washed with absolute ethanol until no white turbidity was observed in the effluent, and then washed with distilled water until no ethanol odor remained. The resin bed was subsequently treated sequentially with 3 BV of 5% NaOH solution and 3 BV of 5% HCl solution at a flow rate of 3 BV $\cdot$ h^{−1}, followed by washing with distilled water until neutral. The pretreated resins were stored in absolute ethanol and washed with distilled water before use until the ethanol concentration reached zero. Polyamide resins were pretreated using the same procedures, including ethanol swelling, wet column packing, and acid–alkali treatment. Acid–alkali or acetone treatment was used to remove strongly adsorbed impurities from the resins after use [23,24,28]. However, the adsorption

and desorption performance of the regenerated resins over repeated operational cycles was not systematically evaluated in the present study.

2.5. Macroporous Resin Purification

In the static adsorption–desorption tests, six pretreated macroporous resins, each equivalent to 1 g dry weight, were separately mixed with 55 mL of crude *P. edulis* leaf extract solution at a concentration of 10 mg·mL^{−1} and shaken at 200 rpm for 12 h. After adsorption equilibrium had been reached, the contents of the four C-glycosyl flavones in the filtrate were determined. An equal volume of 70% ethanol was then added for desorption for 12 h, and the contents of the target compounds in the desorption solution were measured. The adsorption capacity, desorption capacity, adsorption rate, and desorption rate were calculated as follows:

$$Q_e = \frac{W_0 - W_e}{W}, \quad (1)$$

$$Q_d = \frac{W_d}{W}, \quad (2)$$

$$S = \frac{W_0 - W_e}{W_0} \times 100\%, \quad (3)$$

$$D = \frac{W_d}{W_0 - W_e} \times 100\%. \quad (4)$$

In Equations (1)–(4), Q_e and Q_d represent the adsorption capacity and desorption capacity, respectively; S and D represent the adsorption rate and desorption rate, respectively; W_0 , W_e , and W_d represent the total amounts of the four C-glycosyl flavones in the initial sample solution, the solution after adsorption, and the desorption solution, respectively; and W represents the dry weight of the resin.

Based on the static screening results, ADS-17, DM130, and D101 resins were further selected for adsorption isotherm and adsorption kinetic analyses. In the adsorption isotherm experiments, crude *P. edulis* leaf extract solutions at concentrations of 3, 5, 8, 10, 12, and 15 mg·mL^{−1} were added, and the mixtures were shaken for 12 h at 15, 25, and 35 °C, respectively. In the adsorption kinetic experiments, the adsorption capacities were determined at 0.5, 1, 2, 4, 8, and 12 h, and the data were fitted using the Lagergren pseudo-first-order kinetic equation:

$$-\ln\left(1 - \frac{Q_t}{Q_e}\right) = k_1 t, \quad (5)$$

where Q_t is the adsorption capacity at time t , Q_e is the adsorption capacity at equilibrium, and k_1 is the pseudo-first-order adsorption rate constant (h^{−1}).

In the dynamic adsorption experiments, ADS-17 and D101 macroporous resins, each equivalent to 20 g dry weight, were wet-packed into a 2 cm × 35 cm chromatography column, with a packed bed height of 12.5 cm. A crude *P. edulis* leaf extract solution at a concentration of 10 mg·mL^{−1} was loaded onto the column at a flow rate of 3 BV·h^{−1}. The loading effluent was reloaded onto the column three times, after which distilled water was used to remove part of the sugars and water-soluble proteins. The target compounds were then eluted with 60% ethanol. In the dynamic experiments, the adsorption capacity, desorption capacity, adsorption rate, and desorption rate were calculated as follows:

$$Q_e = \frac{W_0 - W_r - W_{dw}}{W}, \quad (6)$$

$$Q_d = \frac{W_d}{W}, \quad (7)$$

$$S = \frac{W_0 - W_r - W_{dw}}{W_0} \times 100\%, \quad (8)$$

$$D = \frac{W_d}{W_0 - W_r - W_{dw}} \times 100\%. \quad (9)$$

In Equations (6)–(9), W_r and W_{dw} represent the total amounts of the four C-glycosyl flavones in the loading effluent and the water-washing fraction, respectively; the other symbols have the same meanings as those defined for Equations (1)–(4).

After D101 had been identified as the suitable resin, the dynamic breakthrough and elution behaviors, initial sample concentration, ethanol concentration for impurity removal, ethanol concentration for target-compound elution, and sample-loading flow rate on purification performance were further investigated. For the loading-flow-rate experiment, crude *P. edulis* leaf extract solutions were passed through the D101 resin column at sample-loading flow rates of 1, 2, 3, 4, and 5 BV·h^{−1}. After sample loading, the columns were washed with 3 BV of distilled water, and the target compounds were subsequently eluted with 3 BV of 60% ethanol at a fixed elution flow rate of 3 BV·h^{−1}. For process verification, 150 g of the crude *P. edulis* leaf extract was purified using the optimized D101 macroporous resin process. The 60% ethanol eluent was collected, concentrated, and freeze-dried, and the contents of the four C-glycosyl flavones were then determined.

2.6. Polyamide Resin Purification

The D101-purified flavonoid-enriched powder was used as the feed material to compare the purification performance of polyamide resins with three different particle-size ranges. Polyamide resin equivalent to 10 g dry weight was wet-packed into a column, and 200 mL of a solution prepared from the D101-purified powder at a concentration of 10 mg·mL^{−1} was loaded at a flow rate of 3 BV·h^{−1}. After the effluent had been reloaded onto the column three times, the column was first eluted with distilled water and then with 40% ethanol. The contents of the four C-glycosyl flavones in each fraction were determined.

Subsequently, the selected polyamide resin was used to compare the desorption effects of 10%, 20%, 30%, 40%, 50%, 60%, and 70% ethanol on the target compounds. For process verification, 20 g of the D101-purified flavonoid-enriched powder was loaded onto a polyamide resin column with a particle-size range of 0.15–0.25 mm. The 40% ethanol eluent was collected, concentrated, and freeze-dried, and the contents of the four C-glycosyl flavones were then determined.

3. Results and Discussion

3.1. Method Validation

The analytical method was validated in terms of sensitivity, precision, repeatability, stability, and recovery, and the four target C-glycosyl flavones—orientin, isoorientin, vitexin, and isovitexin—were well separated within 22 min. Representative HPLC chromatograms are shown in Figure 1, in which peaks 1–4 correspond to orientin, isoorientin, vitexin, and isovitexin, respectively. The method showed low detection limits as well as good accuracy and was therefore suitable for the determination of the four C-glycosyl flavones in bamboo-leaf samples.

Using HPLC, the chromatograms of the bamboo-leaf flavonoid powder were obtained by comparing the retention times of the four C-glycosyl flavone reference standards with those of the corresponding components in the sample (Figure 1).

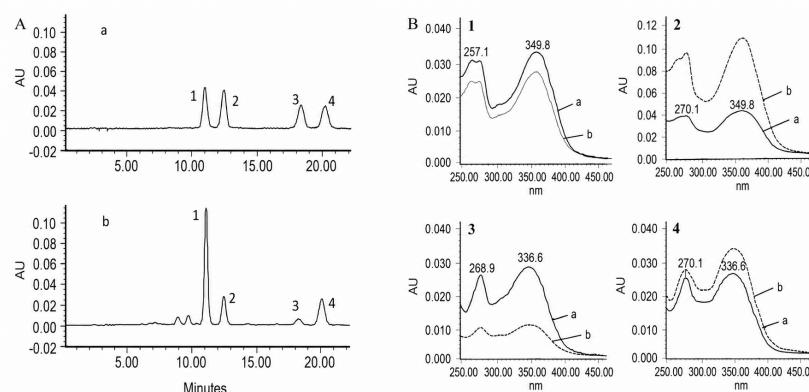


Figure 1. HPLC chromatograms and UV spectra of the four C-glycosyl flavones in mixed standards and bamboo-leaf flavonoid powder: (A) HPLC chromatograms of mixed standards of the four C-glycosyl flavones (a) and bamboo-leaf flavonoid powder (b); (B) UV spectra of the four C-glycosyl flavones in mixed standards and bamboo-leaf flavonoid powder.

3.2. Optimization of Macroporous Resin Purification

3.2.1. Static Adsorption–Desorption Screening of Macroporous Resins

In this study, six macroporous resins with different polarities were selected to evaluate their adsorption and desorption performance toward the four C-glycosyl flavones in bamboo leaves, in order to screen a suitable macroporous resin for subsequent purification. The physical properties of the six macroporous resins are shown in Table 1.

Table 1. Physical properties of six macroporous resins.

Resin	Polarity	Particle Diameter (mm)	Moisture Content (%)	BET Surface Area (m ² ·g ^{−1})
AB-8	Weakly polar	0.3–1.2	65–75	≥480
ADS-17	Nonpolar	0.3–1.25	60–70	90–150
D101	Nonpolar	0.3–1.25	65–75	≥550
DM130	Weakly polar	0.3–1.2	65–75	500–550
NKA-9	Polar	0.3–1.2	65–75	250–290
X-5	Nonpolar	0.3–1.2	56–66	500–600

3.2.2. Saturated Adsorption and Desorption Performance

The saturated adsorption capacity, desorption capacity, adsorption rate, and desorption rate were calculated according to the equations described above, and the results are shown in Table 2.

Table 2. Static adsorption and desorption results of the four C-glycosyl flavones on six macroporous resins.

Parameter	AB-8	ADS-17	D101	DM130	NKA-9	X-5
Q_e (mg·g ^{−1})	4.963 ± 0.030	4.946 ± 0.047	4.725 ± 0.016	5.012 ± 0.022	5.507 ± 0.042	3.673 ± 0.004
S (%)	64.07 ± 0.39	65.06 ± 0.62	61.58 ± 0.21	65.31 ± 0.29	72.43 ± 0.55	48.31 ± 0.06
Q_d (mg·g ^{−1})	3.843 ± 0.051	4.046 ± 0.055	3.736 ± 0.030	4.054 ± 0.039	4.632 ± 0.021	2.712 ± 0.051
D (%)	77.42 ± 1.49	81.80 ± 0.34	79.07 ± 0.90	80.89 ± 0.49	84.12 ± 0.27	73.84 ± 1.30

The adsorption capacity (Q_e) reflects the actual adsorption ability of the resin and is an important parameter for resin selection and evaluation of resin regeneration performance. The desorption capacity is used to evaluate both the desorption ability of the resin and the elution ability of the desorbent. The adsorption rate and desorption rate express the adsorption and desorption performance of the resin as percentages and are also important parameters for screening macroporous resins [28,29].

As shown in Table 2, X-5 resin exhibited the lowest adsorption and desorption rates. This may be because the interactions between the functional groups of the four C-glycosyl flavones and X-5 resin, mainly through van der Waals forces, were relatively weak, resulting in insufficient adsorption onto the resin matrix. In addition, when 70% ethanol was used as the desorption solvent, the target compounds could not be completely desorbed, leading to substantial loss of the four C-glycosyl flavones. Therefore, X-5 resin was not suitable for the purification of these four C-glycosyl flavones.

NKA-9 resin showed very high adsorption and desorption rates. However, NKA-9 was not selected for subsequent experiments after considering its adsorption performance together with practical operational considerations. AB-8, ADS-17, DM130, and D101 resins each showed certain advantages and limitations in terms of adsorption and desorption performance. Considering their polarity, structural characteristics, and purification performance, ADS-17, DM130, and D101 were selected for further purification studies of the four C-glycosyl flavones.

3.2.3. Static Adsorption Isotherms

Two adsorption isotherm models are commonly used to describe adsorption behavior: the monolayer adsorption isotherm model, represented by the Langmuir equation, and the empirical exponential adsorption isotherm model, represented by the Freundlich equation [30,31]. The Langmuir model assumes that each adsorption site on the adsorbent surface can accommodate only one adsorbate molecule and is therefore commonly used to describe monolayer adsorption [31]. In contrast, the Freundlich model is widely used to describe both physical and chemical adsorption and can also be applied to multilayer adsorption processes. The equations are as follows:

Langmuir equation:

$$\frac{C_e}{Q_e} = \frac{C_e}{Q_m} + \frac{1}{K_L Q_m}. \quad (10)$$

Freundlich equation:

$$Q_e = K_f \cdot C_e^{1/n}. \quad (11)$$

The linearized Freundlich equation is expressed as:

$$\ln Q_e = \ln K_f + \frac{1}{n} \ln C_e. \quad (12)$$

where C_e is the equilibrium concentration after saturated adsorption by the resin ($\text{mg} \cdot \text{mL}^{-1}$), Q_m is the maximum saturated adsorption capacity ($\text{mg} \cdot \text{g}^{-1}$), K_L is the Langmuir constant ($\text{mL} \cdot \text{mg}^{-1}$), and K_f is the Freundlich constant, which is used to evaluate adsorption capacity. The parameter n is an empirical constant, generally greater than 1, and is related to the heterogeneity of the surface energy of the solid adsorbent.

In this study, the Langmuir and Freundlich adsorption models were used to fit the adsorption isotherm data, and the corresponding regression equations and parameters are shown in Tables 3 and 4. The correlation coefficients indicated that the adsorption of the four C-glycosyl flavones on ADS-17, DM130, and D101 macroporous resins generally fitted both the Langmuir and Freundlich models, with correlation coefficients greater than 0.93. At different temperatures, the correlation coefficients of the Langmuir model were closer to 0.99, suggesting that the Langmuir model provided a better description of the adsorption process [31].

According to the assumptions of the Langmuir isotherm model, it can be preliminarily inferred that the adsorption of the four C-glycosyl flavones on ADS-17, DM130, and D101 resins occurred mainly as monolayer adsorption on relatively homogeneous adsorbent surfaces with similar adsorption capacities at different adsorption sites. Q_m

represents the maximum amount of the four C-glycosyl flavones that can be adsorbed per unit mass of resin. When the temperature increased to 35 °C, the Q_m values of all three macroporous resins increased, indicating that higher temperature was favorable for the adsorption process. However, considering that temperature control is not always convenient in macroporous resin purification, room temperature is usually preferred for practical purification operations.

For the Freundlich model, the adsorption characteristic constant n ranged from 2 to 10, indicating favorable adsorption of the four C-glycosyl flavones on ADS-17, DM130, and D101 resins. In general, n values greater than 1 suggest that adsorption can proceed readily under the tested conditions. However, the Freundlich parameter n mainly reflects adsorption intensity and surface heterogeneity, and it should not be used alone to infer adsorption rate or adsorption time. Therefore, the adsorption kinetics of the selected resins were further evaluated.

Table 3. Adsorption equation and model parameters of Langmuir on ADS-17, DM130 and D101 at different temperatures.

Temperature	Resin	Langmuir Equation	Correlation Coefficient	Q_m (mg·g ^{−1})	K_L (mL·mg ^{−1})
15 °C	ADS-17	$C_e/Q_e = 0.1908C_e + 0.0010$	0.9970	5.241 ± 0.012	190.800 ± 0.042
15 °C	DM130	$C_e/Q_e = 0.1845C_e + 0.0014$	0.9953	5.420 ± 0.020	131.786 ± 0.052
15 °C	D101	$C_e/Q_e = 0.1818C_e + 0.0009$	0.9987	5.501 ± 0.014	202.000 ± 0.035
25 °C	ADS-17	$C_e/Q_e = 0.1788C_e + 0.0015$	0.9842	5.593 ± 0.051	119.200 ± 0.129
25 °C	DM130	$C_e/Q_e = 0.1670C_e + 0.0015$	0.9880	5.988 ± 0.045	111.333 ± 0.330
25 °C	D101	$C_e/Q_e = 0.2101C_e + 0.0010$	0.9909	4.760 ± 0.010	210.100 ± 0.086
35 °C	ADS-17	$C_e/Q_e = 0.1200C_e + 0.0017$	0.9822	8.333 ± 0.044	70.588 ± 0.095
35 °C	DM130	$C_e/Q_e = 0.1383C_e + 0.0012$	0.9967	7.231 ± 0.018	115.250 ± 0.073
35 °C	D101	$C_e/Q_e = 0.1607C_e + 0.0020$	0.9794	6.223 ± 0.120	80.350 ± 0.139

Table 4. Adsorption equation and model parameters of Freundlich on ADS-17, DM130 and D101 at different temperatures.

Temperature	Resin	Freundlich Equation	Correlation Coefficient	n	K_f
15 °C	ADS-17	$\ln Q_e = 0.2682 \ln C_e + 2.3373$	0.9643	3.729 ± 0.153	10.353 ± 0.035
15 °C	DM130	$\ln Q_e = 0.3543 \ln C_e + 2.5443$	0.9329	2.823 ± 0.235	25.939 ± 0.217
15 °C	D101	$\ln Q_e = 0.2665 \ln C_e + 2.3770$	0.9605	3.752 ± 0.400	10.773 ± 0.026
25 °C	ADS-17	$\ln Q_e = 0.2603 \ln C_e + 2.2959$	0.9497	3.842 ± 0.134	9.933 ± 0.087
25 °C	DM130	$\ln Q_e = 0.3065 \ln C_e + 2.5047$	0.9494	3.263 ± 0.220	12.240 ± 0.069
25 °C	D101	$\ln Q_e = 0.2323 \ln C_e + 2.1341$	0.9315	4.305 ± 0.174	8.449 ± 0.160
35 °C	ADS-17	$\ln Q_e = 0.4094 \ln C_e + 3.0582$	0.9894	2.443 ± 0.028	21.289 ± 0.017
35 °C	DM130	$\ln Q_e = 0.3712 \ln C_e + 2.9666$	0.9546	2.694 ± 0.032	19.426 ± 0.073
35 °C	D101	$\ln Q_e = 0.3065 \ln C_e + 2.4619$	0.9930	3.263 ± 0.011	11.727 ± 0.015

3.2.4. Static Adsorption Kinetics

Adsorption kinetic analysis is mainly used to describe the rate at which an adsorbent adsorbs solutes. The adsorption rate reflects the retention time of the adsorbates on the resin surface during their interaction with the macroporous resin. In this study, the adsorption capacity of the resin at time t was determined, and Q_t was fitted against t using the Lagergren pseudo-first-order kinetic equation [32]. The pseudo-first-order kinetic fits are shown in Figure 2, and the corresponding fitting equations and k_1 values are listed in Table 5.

Table 5. Pseudo-first-order kinetic equations and fitted parameters.

Resin	Fitting Equation	Correlation Coefficient	k_1 (h ^{−1})
ADS-17	$-\ln(1 - Q_t/Q_e) = 0.6710t$	0.9900	0.6710 ± 0.0230
DM130	$-\ln(1 - Q_t/Q_e) = 0.4082t$	0.9902	0.4082 ± 0.0109
D101	$-\ln(1 - Q_t/Q_e) = 0.4793t$	0.9902	0.4793 ± 0.0195

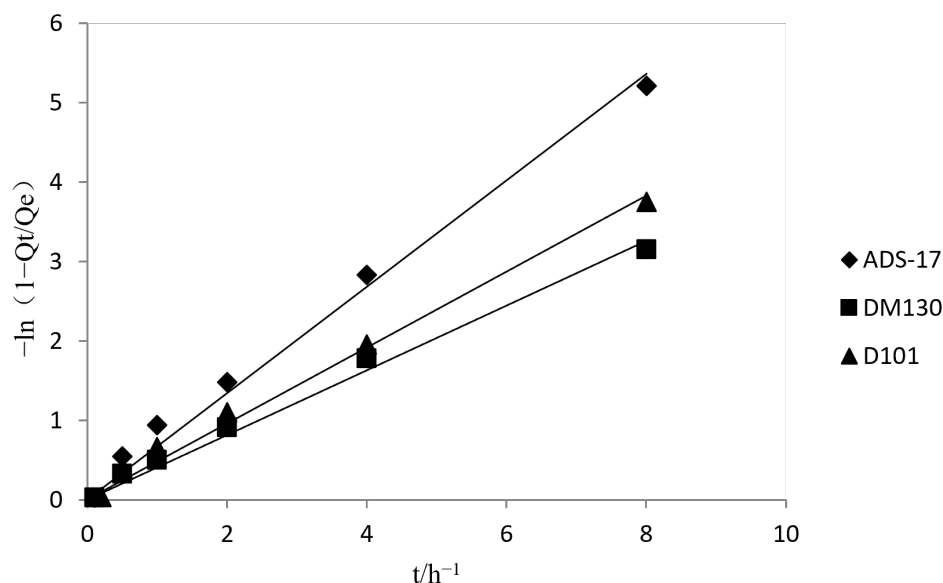


Figure 2. Pseudo-first-order kinetic fits for adsorption on ADS-17, DM130 and D101 macroporous resins.

Previous studies have proposed mass-transfer rate models for adsorption processes. In general, adsorption is considered to involve three stages: liquid film diffusion, intraparticle diffusion, and adsorption reaction, and the overall adsorption process may be controlled by one or more of these steps. Because the adsorption reaction is usually rapid, it is often not regarded as the rate-controlling step. Therefore, the effects of liquid film diffusion and intraparticle diffusion on the overall adsorption process are mainly considered, and the rate-controlling step can be evaluated based on the linear relationship with time.

In this study, the adsorption kinetic data were fitted using the Lagergren pseudo-first-order kinetic equation. As shown in Figure 2 and Table 5, the coefficients of determination (R^2) of the three resins were all above 0.99, indicating that the pseudo-first-order kinetic model adequately described the adsorption behavior of the selected resins. However, this fitting result alone was insufficient to identify a single rate-controlling step. The adsorption process may have involved both liquid film diffusion and intraparticle diffusion, and further diffusion-model analysis would be required to distinguish their relative contributions. Therefore, this aspect was not further investigated in the present study. According to Table 5, the pseudo-first-order adsorption rate constants (k_1 values) of ADS-17 and D101 were higher than that of DM130, indicating relatively faster adsorption rates. Therefore, ADS-17 and D101 resins were selected for subsequent dynamic adsorption experiments.

3.2.5. Dynamic Screening of Macroporous Resins

Based on the results of the static adsorption experiments, ADS-17 and D101 resins were selected for dynamic adsorption experiments. The adsorption capacity, desorption

capacity, adsorption rate, and desorption rate were calculated and compared to further screen a suitable macroporous resin [11,15]. The experimental results are shown in Table 6.

Table 6. Results of dynamic adsorption and screening experiment on ADS-17 and D101 macroporous resins.

Resin	Compound	W_0/mg	$Q_e/(\text{mg} \cdot \text{g}^{-1})$	$Q_d/(\text{mg} \cdot \text{g}^{-1})$	$S/\%$	$D/\%$
ADS-17	Orientin	47.583 ± 0.800	1.716 ± 0.019	1.669 ± 0.043	72.12 ± 0.43	97.28 ± 1.47
	Isoorientin	60.910 ± 0.377	2.349 ± 0.013	1.969 ± 0.022	77.13 ± 0.04	83.83 ± 1.41
	Vitexin	14.034 ± 0.346	0.586 ± 0.016	0.439 ± 0.002	83.46 ± 0.25	74.92 ± 1.81
	Isovitexin	13.292 ± 0.362	0.629 ± 0.020	0.503 ± 0.001	94.70 ± 0.38	79.84 ± 2.61
	Sum of the four C-glycosyl flavones	135.819 ± 1.161	5.280 ± 0.028	4.580 ± 0.024	77.75 ± 0.25	86.74 ± 0.02
D101	Orientin	44.776 ± 1.071	1.698 ± 0.034	1.406 ± 0.001	75.85 ± 0.32	82.79 ± 1.59
	Isoorientin	56.398 ± 0.433	2.275 ± 0.009	1.851 ± 0.033	80.68 ± 0.31	81.34 ± 1.12
	Vitexin	13.612 ± 0.180	0.566 ± 0.008	0.531 ± 0.001	83.12 ± 0.08	93.87 ± 1.17
	Isovitexin	14.543 ± 0.122	0.679 ± 0.002	0.593 ± 0.002	93.36 ± 0.19	87.39 ± 1.16
	Sum of the four C-glycosyl flavones	129.329 ± 1.562	5.218 ± 0.043	4.381 ± 0.036	80.69 ± 0.31	83.96 ± 0.01

As shown in Table 6, both ADS-17 and D101 resins exhibited good adsorption and desorption performance toward the four C-glycosyl flavones. D101 showed a higher overall adsorption rate for the sum of the four target compounds than ADS-17, although the relative performance differed among the individual compounds. Both D101 and ADS-17 are nonpolar macroporous resins; therefore, the difference in their purification performance cannot be attributed to resin polarity alone. D101 has a substantially larger BET surface area than ADS-17, providing a greater accessible interfacial area for adsorption. The four target C-glycosyl flavones contain aromatic flavone skeletons together with hydrophilic glucose and hydroxyl groups. Their aromatic moieties may interact with the nonpolar resin matrix mainly through hydrophobic and dispersion interactions, whereas the glucose and hydroxyl groups maintain sufficient solubility in the aqueous loading solution. These combined properties may favor reversible adsorption of the target compounds on D101 and their subsequent desorption with aqueous ethanol [33]. Although the overall desorption rate of D101 was slightly lower than that of ADS-17, D101 showed the best overall process suitability among the tested resins and was selected on the basis of its overall adsorption performance and operational considerations rather than because it showed the highest value for every individual parameter. Because the pore-size distributions and detailed surface chemistries of the resins were not experimentally characterized in the present study, the individual contributions of pore matching and specific surface interactions could not be conclusively distinguished.

3.2.6. Mass Balance During Dynamic D101 Purification

A stage-specific mass balance was calculated from the mean values obtained in the dynamic D101 screening experiment using 20 g of dry resin. The initial loading, adsorbed amount, combined loss in the loading effluent and water-washing fraction, amount recovered in the 60% ethanol eluate, and unrecovered amount are summarized in Table 7.

Table 7. Stage-specific mass balance of the four C-glycosyl flavones during dynamic D101 purification.

Compound	Initial Amount (mg)	Adsorbed (mg)	Effluent + Wash Loss (mg)	60% Ethanol Recovery (mg)	Unrecovered (mg)	Overall Recovery (%)
Orientin	44.776	33.960	10.816	28.120	5.840	62.80
Isoorientin	56.398	45.500	10.898	37.020	8.480	65.64
Vitexin	13.612	11.320	2.292	10.620	0.700	78.02
Isovitexin	14.543	13.580	0.963	11.860	1.720	81.55
Total	129.329	104.360	24.969	87.620	16.740	67.75

Based on these data, 104.360 mg of the four target compounds was adsorbed from an initial loading of 129.329 mg. A total of 87.620 mg was subsequently recovered in the 60% ethanol eluate, corresponding to an overall recovery of 67.75% relative to the initial loading. The combined loss in the loading effluent and water-washing fraction was 24.969 mg, whereas 16.740 mg of the adsorbed compounds was not recovered in the target ethanol fraction. The available records did not retain the amounts of the target compounds in the loading effluent and water-washing fraction separately; therefore, these two losses are reported as a combined value. The unrecovered amount represents the difference between the adsorbed amount and the amount recovered in the 60% ethanol eluate and may include compounds remaining on the resin or other unquantified process losses. These calculations provide a mass balance only for the dynamic D101 stage. A continuous mass balance across the complete D101–polyamide sequence could not be reconstructed because the two process-verification experiments could not be confirmed as one continuous material batch. In addition, the static screening data were retained as calculated adsorption and desorption parameters and were not sufficient for an independent fraction-by-fraction mass balance.

3.2.7. Breakthrough and Dynamic Elution Curves of D101 Resin

After sample loading, the effluent was collected in fractions of 40 mL each. The contents of the four C-glycosyl flavones in each fraction were determined, and the breakthrough curve of the macroporous resin was plotted, as shown in Figure 3A.

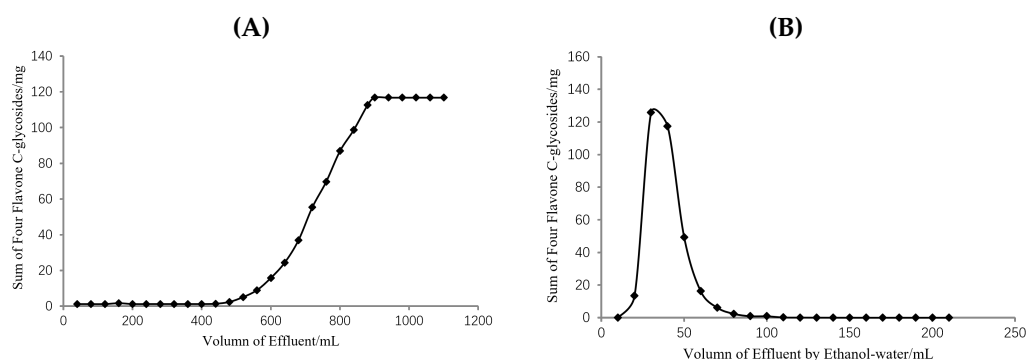


Figure 3. Dynamic adsorption behavior of the four C-glycosyl flavones on D101 macroporous resin: (A) breakthrough curve; (B) elution curve.

As shown in Figure 3A, when the sample loading volume was low, sufficient binding sites were available on the resin bed, and the breakthrough amount of the four C-glycosyl flavones was very low and nearly undetectable. When the loading volume reached 400 mL, the contents of the four C-glycosyl flavones in the effluent began to increase. This may be because, as the loading volume gradually increased, the amount of impurities also increased. These impurities competed with the four C-glycosyl flavones for binding sites on the resin bed and partially displaced the target compounds from the resin, resulting in a rapid increase in the four C-glycosyl flavones in the effluent, which could then be detected by HPLC. When the loading volume was further increased to 900 mL, the adsorption

capacity of the resin bed reached saturation. At this stage, the adsorption and desorption of the four C-glycosyl flavones on the resin bed approached dynamic equilibrium, and the contents of the C-glycosyl flavones in the effluent showed no obvious further change [23,34].

After the resin reached adsorption saturation, the target compounds were eluted with 60% ethanol, and the eluent was collected in fractions of 10 mL each. The contents of the four C-glycosyl flavones in each fraction were determined, and the dynamic elution curve was plotted, as shown in Figure 3B. The total amount of the four C-glycosyl flavones in the first collected fraction was 0.09 mg. With continued elution, the total amount of the four C-glycosyl flavones reached the highest level in the third and fourth fractions, corresponding to the concentrated elution stage of the target compounds. At this stage, the total amount of the four C-glycosyl flavones in the eluent reached 125.841 mg. With further elution, the collected amount of the four C-glycosyl flavones gradually decreased, indicating that the target compounds adsorbed on the column bed had been progressively eluted. When the ethanol elution volume reached 120 mL, corresponding to approximately 3 BV, only a very small amount of the four C-glycosyl flavones remained in the eluent and could be considered negligible. Therefore, an ethanol elution volume of 3 BV was sufficient to fully elute the four C-glycosyl flavones adsorbed on the column bed [11,35,36].

3.2.8. Effect of Initial Sample Concentration on D101 Resin

In this experiment, crude *P. edulis* leaf extract solutions with initial concentrations of 6, 8, 10, and 12 mg·mL^{−1} were used to investigate the effect of sample concentration on the adsorption performance of D101 resin. The related parameters are shown in Table 8.

Table 8. Effect of initial concentration of crude extract solution on adsorption capacity on D101 macroporous resins.

Initial Concentration of Crude Extract Solution (mg · mL ^{−1})	W ₀ /mg	Q _e / (mg · g ^{−1})	Q _d / (mg · g ^{−1})	S/%	D/%
6	42.342 ± 0.037	2.117 ± 0.105	1.835 ± 0.047	99.99 ± 0.36	86.69 ± 0.09
8	46.701 ± 0.080	2.305 ± 0.096	2.032 ± 0.018	98.70 ± 0.41	88.15 ± 0.25
10	57.197 ± 0.035	2.802 ± 0.035	2.730 ± 0.008	97.98 ± 0.44	97.42 ± 0.13
12	74.240 ± 0.067	3.382 ± 0.040	3.286 ± 0.010	91.11 ± 0.68	97.15 ± 0.37

As shown in Table 8, the initial sample concentration affected the adsorption and desorption performance of D101 resin toward the four C-glycosyl flavones. When the initial concentration was below 10 mg·mL^{−1}, the adsorption rates showed little difference and were all above 95%, indicating that most of the orientin, isoorientin, vitexin, and isovitexin in the crude extract solution had been adsorbed onto the macroporous resin. As the sample concentration further increased, the resin gradually approached adsorption saturation, and the adsorption rate decreased to some extent. During ethanol elution, the desorption rates also showed obvious differences. For resin beds loaded with higher sample amounts, ethanol showed higher desorption efficiency toward the target compounds, and most of the four C-glycosyl flavones were eluted, with desorption rates exceeding 97%. In contrast, resin beds loaded with lower sample concentrations showed relatively lower desorption rates. Based on these results, 10 mg·mL^{−1} was selected as the initial sample concentration.

3.2.9. Effect of Ethanol Concentration on D101 Resin

Previous studies have shown that macroporous resins can adsorb a wide range of substances, including sugars, proteins, phenolic acids, and flavonoids [29,37]. Ethanol can be used as the elution solvent for gradient elution, and crude fractionation of bamboo-leaf flavonoids can be achieved based on the different van der Waals interactions between different components and the macroporous resin. In this study, low- and high-concentration

ethanol solutions were used for elution, and the final desorption percentage of the four C-glycosyl flavones was used as the evaluation index to select the appropriate ethanol concentration for elution.

Low-concentration ethanol for impurity removal. Five low-concentration ethanol solutions, namely 10%, 15%, 20%, 25%, and 30% ethanol, were used to elute the resin bed, and the results are shown in Figure 4A. When the ethanol concentration was below 15%, the desorption rate of the four C-glycosyl flavones was lower than 4%, indicating that only a small amount of the target compounds was eluted, while the relative proportion of impurities in the eluent was high. As the ethanol concentration further increased, the desorption rate of the four C-glycosyl flavones increased rapidly. When the ethanol concentration reached 30%, the desorption rate reached 46.66%, indicating that nearly half of the target compounds adsorbed on the resin bed had been eluted.

Although increasing the ethanol concentration could promote the elution of impurities, it also increased the elution loss of the target compounds, resulting in substantial loss of the four C-glycosyl flavones. Therefore, 15% ethanol was selected as the low-concentration elution solvent for impurity removal. Under this condition, the loss of the four C-glycosyl flavones was limited, while the relative proportion of impurities in the eluent remained relatively high.

High-concentration ethanol for target-compound elution. Five high-concentration ethanol solutions, namely 40%, 50%, 60%, 70%, and 80% ethanol, were used to elute the resin bed, and the results are shown in Figure 4B. When the ethanol concentration increased from 40% to 60%, the desorption rate of the four C-glycosyl flavones increased significantly. This may be because ethanol competed with the macroporous resin for the target compounds and increased their solubility in the ethanol solution, allowing the target compounds to gradually desorb from the chromatography column. However, when the ethanol concentration exceeded 60%, the desorption rate showed no obvious further change. At this stage, the ethanol solution may have eluted the target compounds from the resin column to the maximum extent, while the remaining components may have been irreversibly or strongly adsorbed on the resin bed. Therefore, 60% ethanol was selected as the high-concentration elution solvent. Under this condition, the four C-glycosyl flavones showed relatively high desorption rates, while the amount of organic solvent used was reduced.

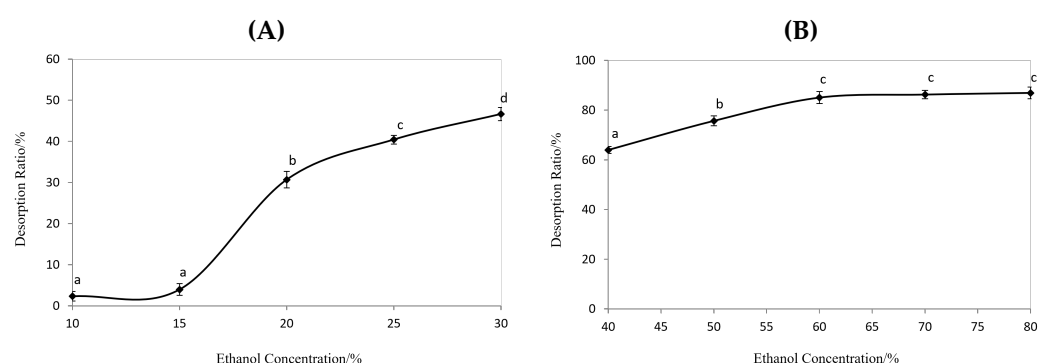


Figure 4. Effects of low and high ethanol concentrations on the desorption ratio of the four C-glycosyl flavones: (A) low-concentration ethanol elution; (B) high-concentration ethanol elution.

3.2.10. Effect of Sample-Loading Flow Rate on D101 Resin

Crude *P. edulis* leaf extract solutions were passed through the D101 resin column at sample-loading flow rates of 1, 2, 3, 4, and 5 BV·h^{−1}. After sample loading, each column was washed with 3 BV of distilled water, and the target compounds were subsequently eluted with 3 BV of 60% ethanol at a fixed elution flow rate of 3 BV·h^{−1}. The desorption

ratio measured after each loading treatment was used as the evaluation index, and the results are shown in Figure 5.

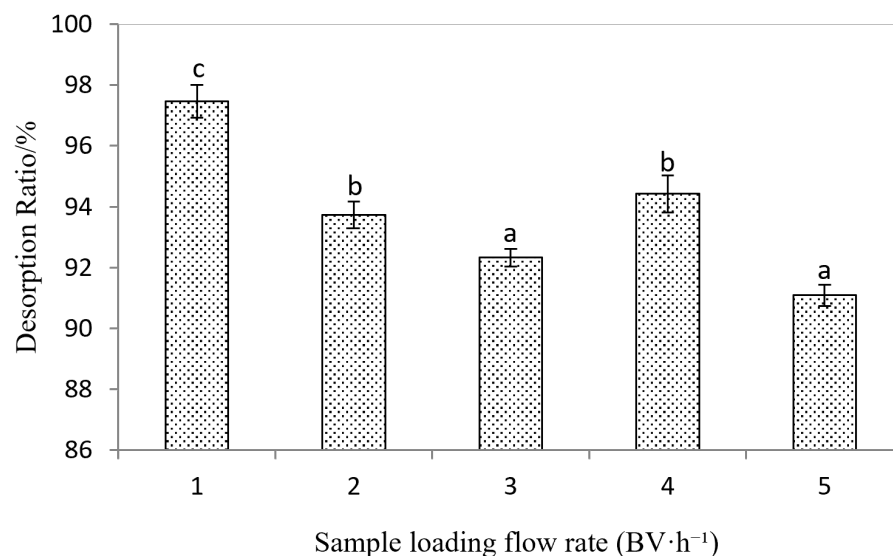


Figure 5. Effect of sample-loading flow rate on the subsequent desorption ratio of the four C-glycosyl flavones.

As shown in Figure 5, the subsequent desorption ratio of the four C-glycosyl flavones was highest when the sample-loading flow rate was 1 BV·h⁻¹. A lower loading flow rate allowed sufficient contact between the target compounds and the resin bed; however, it also substantially prolonged the sample-loading step. As the loading flow rate increased, the subsequent desorption ratio showed no marked further change. Considering both purification performance and operational efficiency, 4 BV·h⁻¹ was selected as the appropriate sample-loading flow rate. The flow rate of the subsequent 60% ethanol elution was maintained at 3 BV·h⁻¹.

3.2.11. Verification of the D101 Resin Purification Process

Using the optimized macroporous resin purification process, 150 g of the crude *P. edulis* leaf extract was purified, concentrated, and dried to obtain 25.7 g of D101-purified flavonoid-enriched powder. The amounts of orientin, isoorientin, vitexin, and isovitexin were 487.147 mg, 928.844 mg, 88.400 mg, and 252.431 mg, respectively, giving a total of 1756.822 mg for the four C-glycosyl flavones. Accordingly, the yield of the four C-glycosyl flavones relative to the crude extract was 11.712 mg·g⁻¹, and their combined content in the D101-purified flavonoid-enriched powder reached 68.359 mg·g⁻¹.

The present study focused on resin screening and single-cycle process optimization. Although regeneration procedures were established, adsorption and desorption performance over 5–10 consecutive operational cycles was not quantified. Therefore, the present results demonstrate single-cycle purification performance but do not establish resin service life or long-term process economics. Multi-cycle regeneration tests remain necessary before process scale-up.

3.3. Optimization of Polyamide Resin Purification

3.3.1. Effect of Polyamide Resin Particle Size

Polyamide resin adsorbs flavonoids mainly through hydrogen bonding between its amide groups and the hydroxyl groups of flavonoid molecules. Its adsorption capacity is mainly related to the number and position of hydroxyl groups in the flavonoid molecules,

as well as their ability to form hydrogen-bonding associations. Previous studies have shown that polyamide resin has a significant enrichment effect on flavonoid compounds [24,25,27].

In this study, three commonly used polyamide resins with different particle-size ranges were selected to evaluate their adsorption and desorption performance toward the four C-glycosyl flavones in bamboo leaves, and to screen a suitable polyamide resin for further purification. The experimental results are shown in Table 9.

Table 9. Results of the screening experiment using polyamide resins with different particle-size ranges.

Particle-Size Range	Compound	W_0/mg	$Q_e/(\text{mg} \cdot \text{g}^{-1})$	$Q_d/(\text{mg} \cdot \text{g}^{-1})$	$S/\%$	$D/\%$
0.25–0.55 mm	Orientin	7.022 ± 0.083	0.5410 ± 0.0120	0.5060 ± 0.0050	77.07 ± 0.75	93.46 ± 2.90
	Isoorientin	21.383 ± 0.080	1.5930 ± 0.0100	1.3480 ± 0.0050	74.52 ± 0.20	84.61 ± 0.26
	Vitexin	2.669 ± 0.016	0.2070 ± 0.0020	0.1790 ± 0.0001	77.57 ± 0.26	86.45 ± 0.84
	Isovitexin	9.770 ± 0.025	0.8690 ± 0.0040	0.6470 ± 0.0070	88.92 ± 0.12	74.45 ± 1.14
	Sum of the four C-glycosyl flavones	40.844 ± 0.007	3.2100 ± 0.0002	2.6800 ± 0.0020	78.60 ± 0.01	83.47 ± 0.06
0.15–0.25 mm	Orientin	7.022 ± 0.083	0.6840 ± 0.0080	0.6760 ± 0.0030	97.38 ± 0.04	98.81 ± 1.70
	Isoorientin	21.383 ± 0.080	1.9840 ± 0.0080	1.8080 ± 0.0070	92.79 ± 0.01	91.14 ± 0.69
	Vitexin	2.669 ± 0.016	0.2420 ± 0.0010	0.2370 ± 0.0004	90.65 ± 0.07	98.06 ± 0.36
	Isovitexin	9.770 ± 0.025	0.9170 ± 0.0020	0.8350 ± 0.0060	93.86 ± 0.03	91.04 ± 0.82
	Sum of the four C-glycosyl flavones	40.844 ± 0.007	3.8270 ± 0.0001	3.5560 ± 0.0090	93.70 ± 0.02	92.92 ± 0.25
0.12–0.15 mm	Orientin	7.022 ± 0.083	0.6930 ± 0.0080	0.6640 ± 0.0007	98.72 ± 0.02	95.73 ± 1.25
	Isoorientin	21.383 ± 0.080	2.0410 ± 0.0070	1.9150 ± 0.0050	95.44 ± 0.01	93.85 ± 0.08
	Vitexin	2.669 ± 0.016	0.2440 ± 0.0020	0.2120 ± 0.0020	91.23 ± 0.09	86.92 ± 1.53
	Isovitexin	9.770 ± 0.025	0.9390 ± 0.0020	0.8630 ± 0.0005	96.15 ± 0.03	91.88 ± 0.16
	Sum of the four C-glycosyl flavones	40.844 ± 0.007	3.9170 ± 0.0004	3.6540 ± 0.0090	95.90 ± 0.03	93.28 ± 0.23

As shown in Table 9, the adsorption and desorption rates of the polyamide resin with a particle-size range of 0.25–0.55 mm were obviously lower than those of the other two resins with smaller particle sizes. This may be because larger polyamide resin particles provided less effective contact with the target compounds at the same sample concentration, causing excessive target molecules to remain around the resin particles locally. As a result, some target compounds flowed out before being adsorbed, leading to substantial loss during sample loading. Consequently, the amount of target compounds actually adsorbed onto the resin bed decreased, and the amount eluted was also reduced.

When the particle-size ranges of the polyamide resin were 0.15–0.25 mm and 0.12–0.15 mm, the overall adsorption and desorption rates showed no obvious difference. Considering that larger particles generally allow a faster elution flow rate and can improve production efficiency, polyamide resin with a particle-size range of 0.15–0.25 mm was selected for the further purification of the four C-glycosyl flavones.

3.3.2. Effect of Ethanol Concentration on Polyamide Resin

In the polyamide resin purification experiment, ethanol–water solutions at different ratios were used as eluents. Through competitive interactions between the eluent and the resin bed for the target compounds, the target compounds were eluted from the resin column. The effect of different ethanol concentrations on the desorption performance of the resin toward the adsorbed flavonoids was investigated, with the desorption rate used as the evaluation index to select the appropriate ethanol concentration for elution [26]. The results are shown in Figure 6.

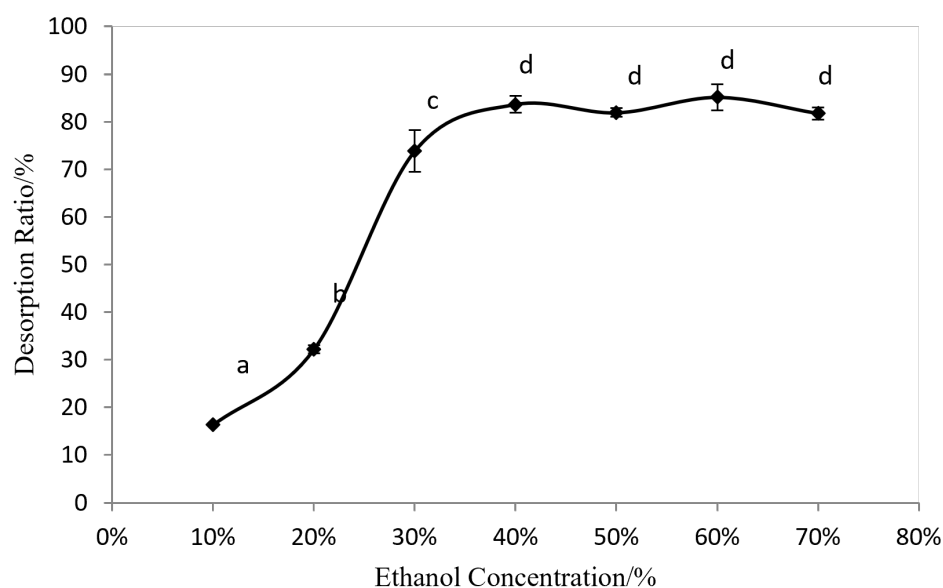


Figure 6. Effect of ethanol concentration on the desorption ratio of the four C-glycosyl flavones.

Seven ethanol concentrations were selected to elute the resin bed. When the ethanol concentration was 10%, part of the four C-glycosyl flavones had already been desorbed from the resin bed, with a desorption rate of 16.34%. This indicated that the four C-glycosyl flavones could be eluted relatively easily even at a low ethanol concentration. As the ethanol concentration increased, the desorption rate of the target compounds also increased accordingly. This may be attributed to the increased solubility of the target compounds in the ethanol–water solution, which promoted their desorption from the resin bed. However, when the ethanol concentration exceeded 40%, the desorption rate showed no obvious further change. At this stage, the ethanol solution may have eluted the target compounds from the resin bed to the greatest possible extent, while some target compounds and impurities still remained strongly adsorbed on the resin bed [36]. Therefore, 40% ethanol was selected as the elution solvent. Under this condition, the four C-glycosyl flavones showed a relatively high desorption rate, while the amount of ethanol used was greatly reduced.

3.3.3. Verification of the Polyamide Resin Purification Process

According to the above purification process, 20 g of the D101-purified flavonoid-enriched powder was further purified by polyamide resin, concentrated, and dried to obtain 4.26 g of the polyamide-purified flavonoid-enriched powder. The amounts of orientin, isoorientin, vitexin, and isovitexin were 37.260 mg, 182.556 mg, 23.506 mg, and 79.243 mg, respectively, giving a total of 322.565 mg for the four C-glycosyl flavones. Accordingly, the amount of the four C-glycosyl flavones recovered per gram of the D101-purified feed material was $16.128 \text{ mg} \cdot \text{g}^{-1}$, and their combined content in the polyamide-purified flavonoid-enriched powder reached $76.939 \text{ mg} \cdot \text{g}^{-1}$.

4. Conclusions

This study established a two-stage resin-based process for the preparative enrichment of orientin, isoorientin, vitexin, and isovitexin from the crude *P. edulis* leaf extract. Among the tested macroporous resins, D101 provided the most suitable overall performance when its adsorption and desorption behavior and operational considerations were evaluated together. Subsequent purification using polyamide resin with a particle-size range of 0.15–0.25 mm and elution with 40% ethanol further increased the enrichment of

the four target C-glycosyl flavones. The sequential use of D101 macroporous resin and polyamide resin therefore provided an effective approach for removing co-extracted non-target constituents and preparing a flavonoid-enriched powder characterized by four defined C-glycosyl flavones rather than by total flavonoid content alone. This target-oriented strategy provides a more specific basis for process control and quality standardization of *P. edulis* leaf-derived natural product intermediates. However, the current evidence is limited to single-cycle purification performance and a stage-specific mass balance for dynamic D101 purification. Multi-cycle resin-regeneration studies and a continuous mass balance across the complete purification sequence remain necessary before process scale-up.

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Abbreviations

The following abbreviations are used in this manuscript:

BET	Brunauer–Emmett–Teller
BV	Bed volume
HPLC	High-performance liquid chromatography
rpm	Revolutions per minute
UV	Ultraviolet

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