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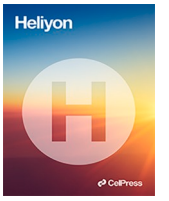

Production of Rhamnosyl Icariside II by snailase hydrolysis of *Epimedium wushanense* extracts

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ABSTRACT

Rhamnosyl Icariside II is a rare secondary flavonoid glycoside isolated from *Epimedium* L. plants. It has better stability and physiological activity than the primary flavonoid glycosides of *Epimedium* L., therefore, conversion of the primary flavonoid glycoside into Rhamnosyl Icariside II would be desirable. In this study, a method for the enzymatic production of Rhamnosyl Icariside II from the total flavonoids of *Epimedium wushanense* was established, and the conditions were optimized. Six commercial enzymes were screened, and the reaction conditions for the best enzyme were optimized. Snailase was the most effective hydrolase, and the highest yield was obtained under the optimized conditions. To facilitate industrial production of Rhamnosyl Icariside II, a scaled-up pilot test was performed. The reaction solution was extracted with n-butanol to obtain the Rhamnosyl Icariside II crude product, which was then subjected to silica gel column chromatography and preparative chromatography. Finally, a product of Rhamnosyl Icariside II with purity of 99.1 % was achieved, in a total yield of 46.8 %. Compared to direct extraction and acid hydrolysis, this method improves the product yield and purity, which is of great significance for the large-scale production of Rhamnosyl Icariside II. This study provides a basis for the physiological activity study of Rhamnosyl Icariside II, and offers possibilities for future applications in the healthcare sector.

1. Introduction

Epimedium is a traditional Chinese herbal medicine widely distributed in Asia, which has pharmacological effects such as tonifying kidney, strengthening bones and muscles, and expelling wind and dampness [1,2]. The main active components of Epimedium are flavonoids, lignans, alkaloids, polysaccharides, and volatile oils [3,4]. Epimedium flavonoids are 2-phenylchromone derivatives with an 8-isopentenyl substitution structure as the core and hydroxyl, alkoxy, and methoxy substituents. Epimedium flavonoids have demonstrated many pharmacological activities, such as, anti-osteoporosis [5], anti-inflammatory [6], and promotion of sexual function [7], anti-aging [8] and anticancer [9]. The flavonoids (Fig. 1) contained in Epimedium are mainly trisaccharide glycoside (e.g.: Epimedin A, B, C) and disaccharide glycoside (e.g.: Icariin, Rhamnosyl Icariside II). Owing to the strong hydrophilicity of the

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trisaccharide glycosides, it is difficult for the primary glycosides to be absorbed by human small intestine [10], which limits the beneficial effects provided by the flavonoids. Conversion of the primary flavonoid glycoside into a secondary glycoside improves its lipophilicity, and facilitates absorption into the blood to a greater extent and therefore exerts its maximum efficacy [11,12].

Rhamnosyl Icariside II, a rare secondary flavonoid glycoside, showed similar pharmacological activity to other Epimedium flavonoids [13,14]. Currently, the main methods for obtaining Rhamnosyl Icariside II are direct extraction from Herba Epimedii [15,16] or transformation from Epimedin C [17,18]. The direct extraction from Epimedium plants is complicated and expensive; furthermore, obtaining a large amount of high-purity Rhamnosyl Icariside II is remaining challenge. Rhamnosyl Icariside II can also be obtained by split off C-7 glucose from Epimedin C. The methods based on chemical hydrolysis form a number of by-products due to the poor selectivity in the course of hydrolysis [19]. Therefore, the mild enzymatic hydrolysis of primary flavonoid glycosides into secondary flavonoid glycosides is considered to be the most specific, efficient and eco-friendly method [20,21].

Snailase is a complex mixture of more than 20 enzymes, including cellulase, hemicellulases, *etc*, which is obtained by extraction from the digestive tracts or the ingluvies of mollusks from the genus *Limax* [22]. As reported, snailase can hydrolyze glucose glycosides, and could be a promising tool for the conversion of Epimedium flavonoids [23–25]. At present, there are few reports on the enzymatic production of Rhamnosyl Icariside II, and the conversion yield is too low to content the requirements of industrial production. In this study, high-purity Rhamnosyl Icariside II was obtained based on the snailase enzymatic hydrolysis of *Epimedium wushanense* extracts, which provided a feasible method for its large-scale production and possibility for its potential physiological activity research.

2. Materials and methods

2.1. Materials and chemicals

Epimedins A, B, and Epimedin C (Batch NO: 190,926, 191,101, 191,021; purity ≥98.0 % by HPLC) were purchased from Shaanxi Lebo Biochemical Technology Co. Ltd. (Shaanxi, China). Icariin (Batch NO: 220,428; purity ≥98.0 % by HPLC) was purchased from Chengdu Herb Substance Co., Ltd. (Sichuan, China). Rhamnosyl Icariside II and Baohuoside I (Batch NO: DST210414-090, DST200518-092; purity ≥98.0 % by HPLC) were purchased from Chengdu DeSiTe Biological Technology Co., Ltd. (Sichuan, China). HPLC-grade acetonitrile was obtained from Fisher Scientific (Waltham, MA, United States) and other analytical grade reagents were purchased from Tianjin Fuyu Fine Chemical Co., Ltd. (Tianjin, China). Diastase (Batch NO: D1115) and Naringinase (Batch NO: T410441) were purchased from Beijing InnoChem Science and Technology Co., Ltd. (Beijing, China). Cellulase (Batch NO: 68042578) was purchased from Qingdao Haiweisen Biotechnology Co. Ltd (Qingdao, China). Pectinase (Batch NO: 20230024) was purchased from Shenzhen Hengsheng Biotechnology Co. Ltd (Shenzhen, China). Snailase (Batch NO: 20230228) was purchased from Biorigin (Beijing) Inc (Beijing, China). β-glucosidase (Batch NO: J14HS182613) was obtained from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China).

2.2. Preparation of E. wushanense extracts

E. wushanense was obtained from Shaanxi Jinhuifang Traditional Chinese Medicine Technology Company, and identified by Professor Yong Wang of Shaanxi University of Technology. The leaves of *E. wushanense* were dried in a blast drying oven at 55 °C for 2 h and then crushed to 40 meshes. The raw material was extracted twice by 20 times volume of 45 % ethanol under ultrasound assisted

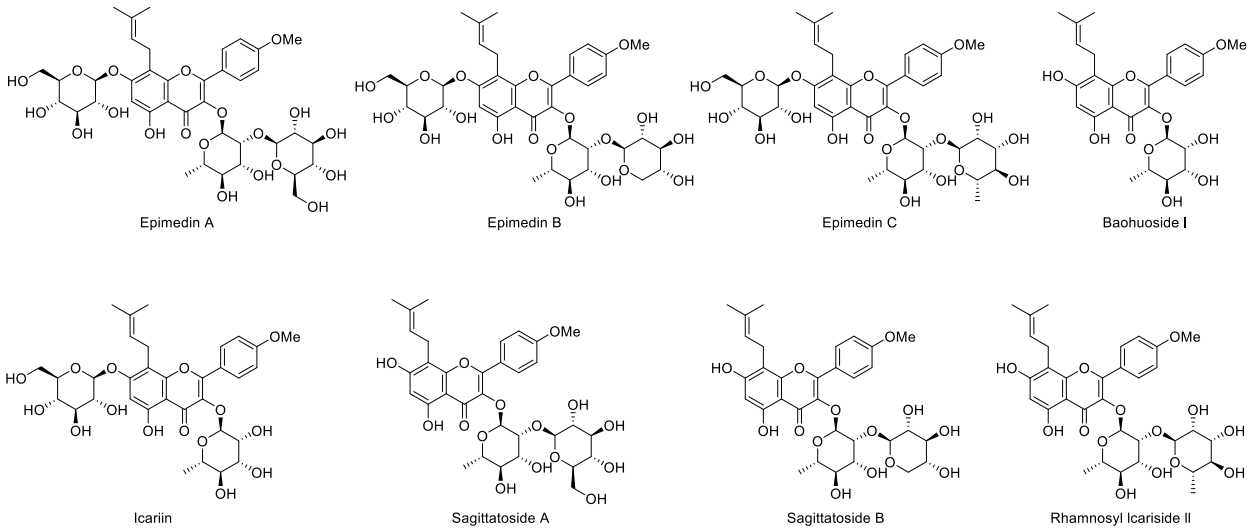

Fig. 1. Chemical structure of major flavonoids from Epimedium.

extraction at 50 °C (500 W) for 1 h. The extracts were filtrated and the filtrates were concentrated under reduced pressure at 65 °C for 1 h to obtain the total flavonoids extracts of *E. wushanense*.

2.3. Enzymatic hydrolysis

2.3.1. HPLC-UV analysis of epimedium flavonoids

The flavonoids were determined using an Agilent 1260 HPLC instrument equipped with a G1311B 1260 Quat Pump, G7129B 1290 Vial sampler, G1316A 1260 column compartment, and G1315D 1260 DAD VL. The data and chromatograms were collected for processing using Agilent OpenLab Control Panel Software (Rev. 1.6.0.655). All sample solutions were separated on a ZORBAX TC-C18 (2) column (150 mm × 4.6 mm, 5 μm). The column temperature was kept at 30 °C throughout analysis, and the mobile phases consisted of acetonitrile (A) and 0.1 % phosphoric acid water (B). Gradient elution was performed at a flow rate of 1.0 mL/min, and the following program: 0–10 min, A (29 %) and B (71 %); 20–23 min, A (95 %) and B (5 %); and 25–27 min, A (29 %) and B (71 %). The UV detection wavelength was set at 270 nm and the injection volume was 10 μL.

2.3.2. Screening of enzymes

Six commercially available enzymes, naringinase, cellulase, snailase, pectinase, diastase, and β-glucosidase were investigated for their ability to convert Epimedin C in *E. wushanense* extracts to Rhamnosyl Icariside II. In detail, the 200 μL extraction (Epimedin C = 20.3 mg/mL) and 4 mg each of these enzymes were dissolved in 1.8 mL of 0.20 M HOAc-NaOAc buffer (pH 5.0) and reacted at 50 °C. Then, 100 μL of the reaction solution was diluted with 900 μL methanol at 1, 2, 4, 8, 12, 24, 36, 48 and 72 h respectively. The resulting solutions were subjected to filtration through a 0.45 μm polytetrafluoroethylene membrane syringe before subsequent HPLC-UV analysis. The conversion rate of Epimedin C and the yield of Rhamnosyl Icariside II were set as indicators for the selection of the enzymes with the best catalytic performance.

2.3.3. Optimization of enzymatic hydrolysis conditions

The effects of six factors, including the enzyme/Epimedium flavonoids ratio, type of buffer, pH of the buffer, reaction temperature, substrate concentration, and hydrolysis duration, on the conversion rate of Epimedin C and the yield of Rhamnosyl Icariside II, were investigated by a single factor experiment.

In detail, 200 μL of *E. wushanense* extracts (4 mg/mL) and snailase (snailase/Epimedium flavonoids = 0.2:1, 0.5:1, 1:1, 1.5:1, 2:1; *w/w*) were dissolved in 1.8 mL 0.2 M HOAc-NaOAc buffer (pH = 5.0) and reacted at 50 °C for 8 h. In addition, the hydrolysis efficiency in 0.2 M of different buffers (HOAc-NaOAc, CPBS, PBS; pH = 5.0) and pH (4.0–7.0) was compared. In addition, the conversion efficiency was studied at different reaction temperatures (35–70 °C) and substrate concentrations (0.1, 0.2 0.5, 1, 2, 3, 4, 5, 6, 8, 10 mg/mL), and the reaction time (10 min-10 h) was shown to be different under the optimized conditions. All experiments were performed in triplicate, the mean and standard deviation (SD) were calculated, with the data being presented as mean ± SD.

2.4. Production of Rhamnosyl Icariside II

2.4.1. Enzymatic hydrolysis of E. wushanense extracts

To further investigate the feasibility of the enzymatic hydrolysis system for industrial applications, a scaled-up pilot test was performed, which could be used to hydrolyze Epimedin C in extracts to produce Rhamnosyl Icariside II. Specifically, 500 mL of *E. wushanense* extract (Epimedin C = 20.3 mg/mL; Rhamnosyl Icariside II = 2.5 mg/mL) was measured in a triangular flask. The pH was adjusted to 5.5 by diluted hydrochloric acid, and 10 g of snailase (enzyme/Epimedium flavonoids = 1:1, *w/w*) was added. The flask was placed in a water bath shaker at 55 °C for 4 h, and the product was further separated and purified.

2.4.2. Purification and identification of Rhamnosyl Icariside II

After completion of the reaction, the mixture was boiled to inactivate the enzyme. The reaction solution was then centrifuged at 3040 rpm for 5 min and the precipitate was collected. The precipitate was dissolved in n-butanol for solid-liquid extraction, and the n-butanol phase was collected and evaporated to dryness under reduced pressure to obtain the crude product. The crude product was purified using silica gel column chromatography, eluted through ethyl acetate: methanol: water (7:2:0.2, *v/v/v*). After thin-layer chromatography analysis, the target components were combined and concentrated to constant weight under reduced pressure.

The resulting crude product was further purified through preparative chromatography. In detail, the crude product was dissolved in MeOH to a concentration of 100 g/L and submitted into dynamic axial compression column chromatography (DAC-HB50) using a column (5 × 25 cm) packed with Megres C18 (10 μm). The mixture was eluted with acetonitrile: water (60:40, *v/v*) at a flow rate of 65 mL/min. After segmented collection, evaporation and drying, Rhamnosyl Icariside II (purity >99 %) was obtained as a yellow powder and analyzed using HRMS (UPLC-Q-Orbitrap, Thermo Fisher Scientific), ^{1}H NMR, and ^{13}C NMR (AVANCE III HD 600 MHz, Bruker) for structure elucidation and identification.

2.5. Calculations

The concentrations of the residual epimedium flavonoids (Epimedin C as the index) and generated Rhamnosyl Icariside II in the reaction system were calculated. The conversion rate of Epimedin C and the generation rate of Rhamnosyl Icariside II were calculated using Formula (1) and (2):

$$I_1(\%) = \frac{C_{C0} - C_{C1}}{C_{C0}} \times 100\% \quad (1)$$

$$I_2(\%) = \frac{\frac{(C_{II1} - C_{II0}) \times V}{M_{II}}}{\frac{(C_{C0} - C_{C1}) \times V}{M_C}} \times 100\% \quad (2)$$

Where I_1 (Formula 1) is the conversion rate of Epimedin C; and I_2 (Formula 2) is the yield of Rhamnosyl Icariside II. C_{C0} and C_{C1} are the concentrations of Epimedin C before and after enzyme transformation, respectively, in mg/mL; C_{II0} and C_{II1} are the concentrations of Rhamnosyl Icariside II before and after enzyme transformation, respectively, in mg/mL; V is the volume of the reaction solution (mL); and M_C and M_{II} are the molar masses of Epimedin C and Rhamnosyl Icariside II, respectively, in g/mol.

3. Results and discussion

3.1. HPLC-UV chromatograms and standard curves

The HPLC chromatograms of the mixed standard reference substance and total flavonoids extracts of *E. wushanense* are shown in Fig. 2. The standard curves of Epimedin C and Rhamnosyl Icariside II were y = 1542.3x - 11.499 ($r^2 = 0.998$) and y = 2144.3x + 7.7833 ($r^2 = 0.997$), respectively, indicating that there was a good linear relationship between the concentrations of 0.4–4 mg/mL (Fig. 2A). The epimedium extracts (Fig. 2B) contains Epimedin A, Epimedin B, Epimedin C, Icariin, Rhamnosyl Icariside II, and Baohuoside I. Approximately 50 % of the flavonoids were found to be Epimedin C, followed by 10 % being Rhamnosyl Icariside II. According to the corresponding standard curve, the Epimedin C content in *E. wushanense* extracts was 20.3 mg/mL, and the Rhamnosyl Icariside II content was 2.5 mg/mL.

3.2. Screening of enzymes

As the hydrolysis of Epimedin C to Rhamnosyl Icariside II depends on breaking the β-glucosidic bond, we screened six commercial enzymes containing β-glucosidase, naringinase, cellulase, snailase, pectinase, and diastase. The HPLC spectrums of the Epimedium extracts following hydrolysis under same conditions and substrate concentration is shown in Fig. 3A.

When the concentration of Epimedin C and the enzyme in the reaction solution was 2 mg/mL, HPLC results after 24 h of the reaction showed that cellulase, snailase, and β-glucosidase entirely hydrolyzed the original flavonoids in Epimedium (Fig. 3B). Among them, the yield of Rhamnosyl Icariside II hydrolyzed by snailase was the highest, and the product was relatively stable in 8 h (Fig. 3C). Therefore, snailase was selected for the following experiment. In addition, we observed that the production of Rhamnosyl Icariside II was the highest when Epimedin C was completely hydrolyzed, and it decreased with increasing reaction time. Therefore, we believe that the hydrolysis of Epimedin C should be used as an indicator of the end of the reaction, with subsequent experiments being based on this principle.

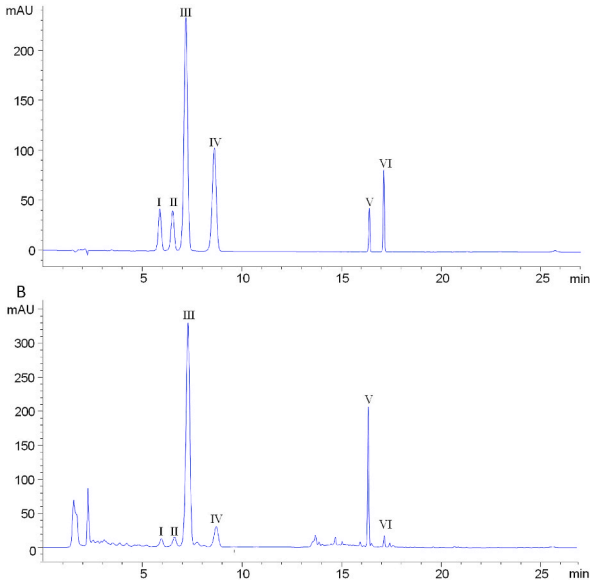

Fig. 2. HPLC chromatogram of the standard reference substances (A) and total flavonoids extracts of *E. wushanense* (B). I: Epimedins A, II: Epimedins B, III: Epimedin C, IV: Icariin, V: Rhamnosyl Icariside II and VI: Baohuoside I.

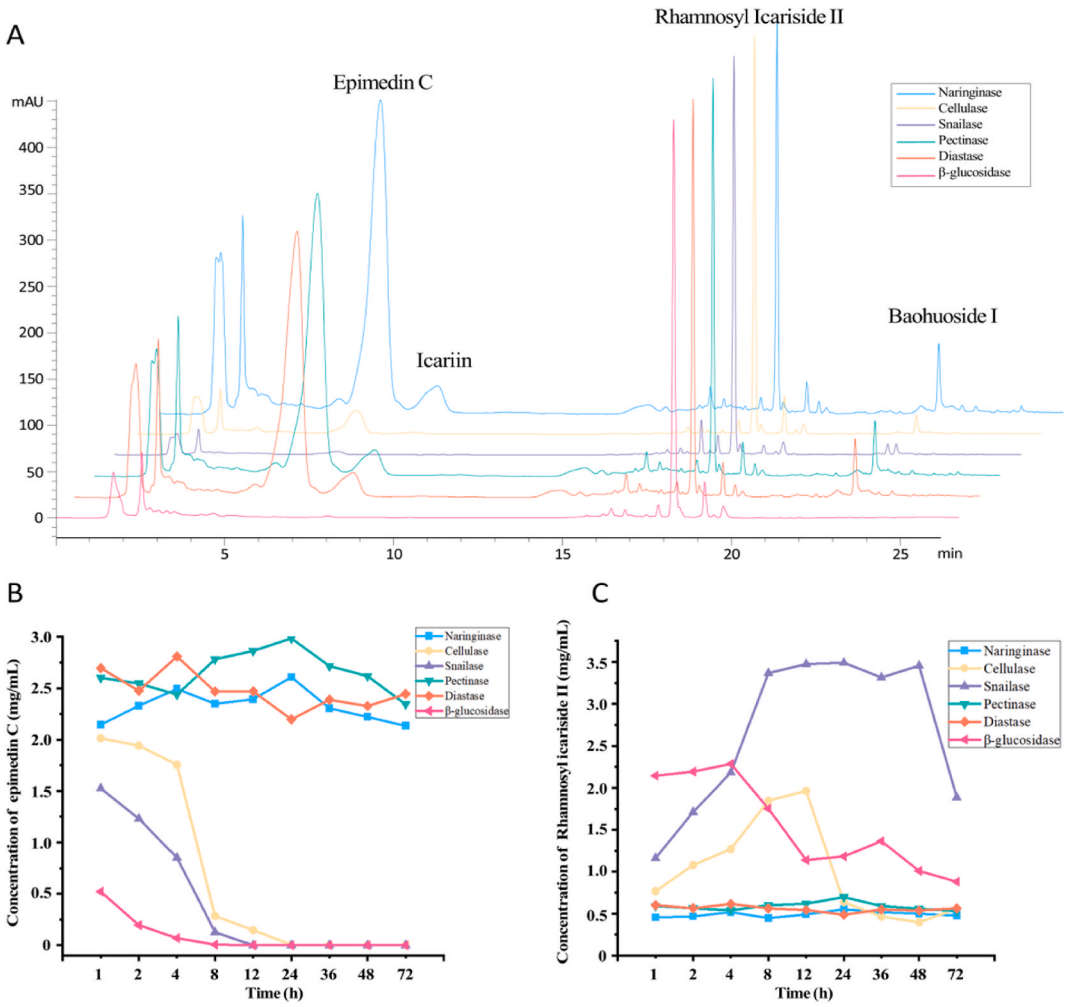

Fig. 3. Results of enzyme screening. (A) HPLC chromatograms of samples catalyzed by different enzymes at 50 °C for 72 h, (B) Concentration of Epimedin C following enzymatic hydrolysis, (C) Concentration of Rhamnosyl Icariside II after enzymatic hydrolysis.

3.3. Optimization of enzymatic hydrolysis conditions

3.3.1. Enzyme/epimedium flavonoids ratio

The effect of the enzyme/Epimedium flavonoids ratio on hydrolysis was investigated, and the result was shown in Fig. 4A. The results showed that when the ratio of enzyme/Epimedium flavonoids increased from 0.2:1 to 1:1, the conversion rate of Epimedin C gradually increased to 100 %. When the ratio of enzyme to Epimedium flavonoids was higher than 1:1, the conversion rate continued to be 100 %, and the yield of Rhamnosyl Icariside II was not significantly improved. Considering the conversion rate and cost factors, the ratio of enzyme/Epimedium flavonoids at 1:1 was finally selected as the best conversion condition.

3.3.2. Buffer

As shown in Fig. 4B, the hydrolysis efficiencies of Epimedium flavonoids in the three buffers, HOAc-NaOAc, CPBS, and PBS, were compared. Epimedium flavonoids were completely hydrolyzed in the three buffers at the same pH, but the yield of Rhamnosyl Icariside II was the highest in HOAc-NaOAc; therefore, it was selected as the most effective enzymatic hydrolysis buffer.

3.3.3. pH of HOAc-NaOAc buffer

Fig. 4C shows the effect of the pH of the HOAc-NaOAc buffer on hydrolysis after 8 h. The experimental results show that the Epimedin C in the reaction system is completely hydrolyzed when pH $\geq$ 5. The yield of Rhamnosyl Icariside II reached maximum at pH 5.5; therefore, this was chosen as the optimal hydrolysis pH.

3.3.4. Reaction temperature

In a previous experiment, we found that an excessively long reaction time led to the additional hydrolysis of Rhamnosyl Icariside II

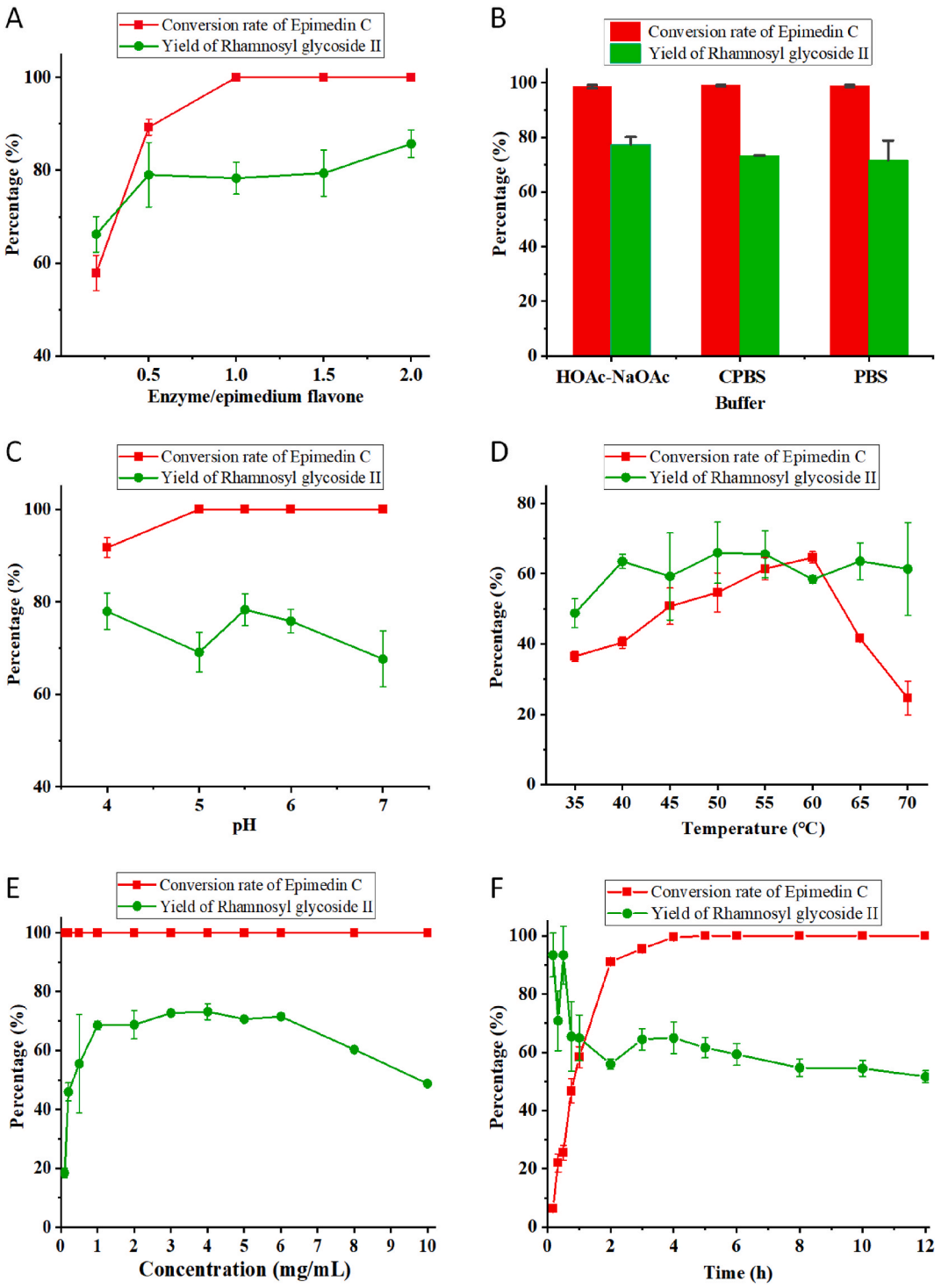

Fig. 4. Optimization of enzymatic hydrolysis conditions. (A) Enzyme/Epimedium flavonoids ratio, (B) buffer, (C) pH of the buffer, (D) reaction temperature, (E) concentration of Epimedium flavonoids, (F) reaction time.

in the system, and pointed out that the content of Rhamnosyl Icariside II in the reaction system was the highest when Epimedine C was completely consumed. Therefore, it was feasible to determine the optimal hydrolysis temperature based on the conversion rate of Epimedin C at a hydrolysis time of 1 h, with the results being shown in Fig. 4D. The conversion rate of Epimedin C increased with the increase in reaction temperature, reaching a maximum at 60 °C, while the conversion rate of Rhamnosyl Icariside II reached a

maximum at 50 °C and then began to decline. Considering this, 55 °C was selected as the optimal temperature for enzymatic hydrolysis.

3.3.5. Epimedin C concentration

The effects of different concentrations of the Epimedium extracts on the conversion efficiency of the reaction system were compared. The results showed that the yield of Rhamnosyl Icariside II was the highest when the concentration of Epimedium flavonoids was 4 mg/mL (Fig. 4E).

3.3.6. Reaction time

According to our previous research, Rhamnosyl Icariside II gradually degrades with increasing reaction time; therefore, a low reaction time is vital for an improved yield. The experimental results showed that the hydrolysis of Epimedin C was complete and the yield of Rhamnosyl Icariside II reached its maximum at 4 h; therefore, it was chosen as the best reaction time (Fig. 4F).

3.4. Production of Rhamnosyl Icariside II

As shown in Fig. S1, the primary flavonoid glycosides in the *E. wushanense* extracts were completely converted into secondary flavonoid glycosides. The reaction solution was boiled to inactivate the enzyme and extracted with n-butanol. The n-butanol was removed by vacuum concentration, and then the crude product was obtained as a yellow solid (13.1 g, yield 84.1 %, purity 61.3 %; Fig. S2). The crude product was purified by a silica gel column eluted by ethyl acetate: methanol: water (9.26 g, yield 67.4 %, purity 74.5 %; Fig. S3) and further dynamic axial compression column chromatography purification (Fig. S4). Finally, high purity Rhamnosyl Icariside II was obtained by removing the solvent under reduced pressure as a yellow solid (5.86 g, yield 82.5 %, purity 99.1 %; Fig. S5). The HRMS, ^{1}H NMR and ^{13}C NMR spectra of the product were shown Figs. S6–8. The related characterization data are as follows: yellow solid, mp 182–183 °C, molecular formula $C_{33}H_{40}O_{14}$, HRMS *m/z*: 661.24677 $[M + H]^+$. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.58 (s, 1H, 5-OH), 10.87 (s, 1H, 7-OH), 7.87 (d, J = 8.7 Hz, 2H, 2′, 6′-H), 7.13 (d, J = 8.7 Hz, 2H, 3′, 5′-H), 6.34 (s, 1H, 6-H), 5.38 (s, 1H, rha-1′-H), 5.16 (s, 1H, 2″-H), 4.96 (d, J = 4.6 Hz, 1H, rha-1-H), 4.90–4.84 (m, 2H), 4.70–4.61 (m, 2H), 4.50 (d, J = 5.0 Hz, 1H), 4.12 (s, 1H), 3.86 (s, 3H, 4′-OCH_3), 3.68 (s, 1H), 3.64–3.53 (m, 1H), 3.21–3.03 (m, 4H, 1″-CH_2), 1.68 (s, 3H, 5″-CH_3), 1.63 (s, 3H, 4″-CH_3), 1.11 (d, J = 5.9 Hz, 3H, rha-6′-H), 0.82 (d, J = 4.8 Hz, 3H, rha-6-H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 178.39 (C4), 162.17 (C7), 161.80 (C5), 159.35 (C4′), 157.18 (C2), 154.25 (C9), 134.86 (C3), 131.51 (C3″), 130.89 (C2′, 6′), 122.77(C1′), 122.73 (C2″), 114.55 (C3′, 5′), 106.45 (C8), 104.61 (C10), 102.08 (rha C1′), 101.18 (rha C1), 98.83 (C6), 75.99 (rha C2), 72.40 (rha C4′), 71.83 (rha C4), 71.12 (rha C3′), 70.95 (rha C2′), 70.72 (rha C3), 70.59 (rha C5′), 69.28 (rha C5), 55.96 (OCH_3), 25.88 (C5″), 21.64 (C1″), 18.25 (C4″), 18.08 (rha C6′), 17.96 (rha C6). These data are consistent with that of a previous report [18,26], which proved that the compound was Rhamnosyl Icariside II, with a purity of 99.1 %.

4. Conclusions

In this study, a method for obtaining high purity Rhamnosyl Icariside II by efficiently catalyzing the total flavonoids of *E. wushanense* was developed and the conditions were optimized. Specifically, six commercial enzymes were screened, and it was concluded that the snailase had the best catalysis performance effect. The catalytic conditions for snailase activity were optimized. Finally, it was found that the yield of Rhamnosyl Icariside II was the highest, when the concentration of Epimedium flavonoids was 4 mg/mL, the ratio of snailase/Epimedium flavonoids was 1:1 (*w:w*) and the reaction time was 4 h at 55 °C in sodium acetate buffer with pH = 5.5. Based on the above conditions, the substrate concentration was scaled up for industrial production. The reaction raw material *E. wushanense* extracts containing 20 mg/mL Epimedin C was subjected to enzyme conversion. After simple purification processes, Rhamnosyl Icariside II monomer compound with a purity of 99.1 % was obtained with a total yield of 46.8 %.

We found that snailase can hydrolyze Epimedin C into Rhamnosyl Icariside II, which could be used to catalyze the hydrolysis of β-glucosidic bonds at C7 of other flavonoids. In this study, its catalytic efficiency and stability were more effective than that of the glucosidases reported in other papers [27,28]. *E. wushanense* extracts were obtained by the conventional extraction method, and the extracts could be used directly as a raw material without any purification, suggesting the feasibility and potential of high adaptability to substrates of enzymatic hydrolysis method. Finally, based on the optimum conditions for enzyme transformation, a scaled-up pilot of the enzymatic hydrolysis method was carried out based on industrialization cost and transformation efficiency. The yield and purity of the product show that this method can be used for the large-scale production of high-purity Rhamnosyl Icariside II.

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Wang Chen: Writing - review & editing, Conceptualization. **Gege Liu:** Writing - original draft, Methodology, Conceptualization. **Yue Zhang:** Software, Data curation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Wang Chen reports equipment, drugs, or supplies was provided by Shaanxi Jinhuifang Traditional Chinese Medicine Technology Company. Wang Chen has patent #CN202310471432. X pending to Shaanxi University of Technology. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23805.

References

[1] H.-P. Ma, X.-R. He, Y. Yang, M.-X. Li, D.-J. Hao, Z.-P. Jia, The genus Epimedium: an ethnopharmacological and phytochemical review, J. Ethnopharmacol. 134 (3) (2011) 519–541, https://doi.org/10.1016/j.jep.2011.01.001.
[2] J. Jiang, B.-J. Zhao, J. Song, X.-B. Jia, Pharmacology and clinical application of plants in epimedium L, Chinese Herbal Medicines 8 (1) (2016) 12–23, https://doi.org/10.1016/S1674-6384(16)60003-5.
[3] X.-J. Chen, Z.-H. Tang, X.-W. Li, C.-X. Xie, J.-J. Lu, Y.-T. Wang, Chemical constituents, quality Control, and bioactivity of epimedii folium (yinyanghuo), Am. J. Chin. Med. 43 (5) (2015) 783–834, https://doi.org/10.1142/s0192415x15500494.
[4] J. Jiang, J. Song, X.-B. Jia, Phytochemistry and ethnopharmacology of epimedium L. Species, Chinese Herbal Medicines 7 (3) (2015) 204–222, https://doi.org/10.1016/S1674-6384(15)60043-0.
[5] L.-L. Wang, Y. Li, Y.-B. Guo, R.-F. Ma, M. Fu, J.-Z. Niu, S.-H. Gao, D.-W. Zhang, Herba epimedii: an ancient Chinese herbal medicine in the prevention and treatment of osteoporosis, Curr. Pharmaceut. Des. 22 (3) (2016) 328–349, https://doi.org/10.2174/1381612822666151112145907.
[6] S. Huang, N. Meng, B.-Q. Chang, X.-H. Quan, R.-Y. Yuan, B. Li, Anti-inflammatory activity of Epimedium brevicornu Maxim ethanol extract, J. Med. Food 21 (7) (2018) 726–733, https://doi.org/10.1089/jmf.2017.4088.
[7] J. Ding, Y.-H. Tang, Z.-Y. Tang, X.-B. Zu, L. Qi, X.-B. Zhang, G. Wang, Icariin improves the sexual function of male mice through the PI 3K/AKT/eNOS/NO signalling pathway, Andrologia 50 (1) (2018), e12802, https://doi.org/10.1111/and.12802.
[8] S.-K. Yan, B. Wu, Z.-Y. Lin, H. Jin, J.-H. Huang, Y. Yang, X.-M. Zhang, Z.-Y. Shen, W.-D. Zhang, Metabonomic characterization of aging and investigation on the anti-aging effects of total flavones of Epimedium, Mol. Biosyst. 5 (10) (2009) 1204–1213, https://doi.org/10.1039/B816407J.
[9] Y.-F. Liu, H. Yang, J. Xiong, J.-J. Zhao, M.-M. Guo, J. Chen, X. Zhao, C. Chen, Z.-X. He, Y. Zhou, L. Xu, Icariin as an emerging candidate drug for anticancer treatment: current status and perspective, Biomed. Pharmacother. 157 (2023), 113991, https://doi.org/10.1016/j.biopha.2022.113991.
[10] Y. Chen, J.-Y. Wang, X.-B. Jia, X.-B. Tan, M. Hu, Role of intestinal hydrolase in the absorption of prenylated flavonoids present in Yinyanghuo, Molecules 16 (2) (2011) 1336–1348, https://doi.org/10.3390/molecules16021336.
[11] J. Zhou, Y.-H. Ma, Z. Zhou, Y. Chen, Y. Wang, X. Gao, Intestinal absorption and metabolism of epimedium flavonoids in osteoporosis rats, Drug Metabol. Dispos.: the biological fate of chemicals 43 (10) (2015) 1590–1600, https://doi.org/10.1124/dmd.115.064386.
[12] Y. Lu, Q. Luo, X. Jia, J.-P. Tam, H. Yang, Y. Shen, X. Li, Multidisciplinary strategies to enhance therapeutic effects of flavonoids from Epimedii Folium: integration of herbal medicine, enzyme engineering, and nanotechnology, Journal of Pharmaceutical Analysis 13 (3) (2023) 239–254, https://doi.org/10.1016/j.jpha.2022.12.001.
[13] L. Zhang, T. Wang, B.-S. Zhao, J.-X. Zhang, S. Yang, C.-L. Fan, P. Li, Effect of 2 ″-O-rhamnosyl icariside II, baohuoside I and baohuoside II in herba epimedii on cytotoxicity indices in HL-7702 and HepG2 cells, Molecules 24 (7) (2019) 1263, https://doi.org/10.3390/molecules24071263.
[14] E.S. Kim, M.K. Kim, H.K. Kang, Y.I. Park, M.S. Dong, D.H. Kim, H.S. Chung, Flavonol glycosides with antioxidant activity from the aerial parts of Epimedium koreanum Nakai, Nat. Prod. Sci. 14 (4) (2008) 233–238.
[15] X.-J. Ping, S.-W. Ji, Separation and simultaneous quantification of seven prenylflavones from organs of different epimedii using gradient reverse-phase HPLC, Natural Product Research and Development 22 (5) (2010) 820–825, https://doi.org/10.16333/j.1001-6880.2010.05.028.
[16] H.-F. Zhang, L.-H. Yan, Q.-W. Zhang, Z.-M. Wang, Flavonoids from leaves of Epimedium pubescens, China J. Chin. Mater. Med. 38 (12) (2013) 1942–1946, https://doi.org/10.4268/cjcmm20131220.
[17] X.-L. Xin, G.-J. Fan, Z. Sun, N. Zhang, Y. Li, R. Lan, L. Chen, P.-P. Dong, Biotransformation of major flavonoid glycosides in herb epimedii by the fungus Cunninghamella blakesleana, J. Mol. Catal. B Enzym. 122 (2015) 141–146, https://doi.org/10.1016/j.molcatb.2015.05.021.
[18] C.-L. Feng, Y. Lu, Y.-Y. Zhou, H. Pang, Y.-P. Shen, H. Yang, Convenient preparation of 2 ″-O-rhamnosyl icariside II, a rare secondary flavonol glycoside, by recyclable and integrated biphase enzymatic hydrolysis, Phcog. Mag. 15 (60) (2019) 147–155, https://doi.org/10.4103/pm.pm_398_18.
[19] M. Dell'Agli, G.V. Galli, E. Dal Cero, F. Belluti, R. Matera, E. Zironi, G. Pagliuca, E. Bosisio, Potent inhibition of human phosphodiesterase-5 by icariin derivatives, J. Nat. Prod. 71 (9) (2008) 1513–1517, https://doi.org/10.1021/np800049y.
[20] Q. Xia, D.-J. Xu, Z.-G. Huang, J.-J. Liu, X.-Q. Wang, X. Wang, S.-Q. Liu, Preparation of icariside II from icariin by enzymatic hydrolysis method, Fitoterapia 81 (5) (2010) 437–442, https://doi.org/10.1016/j.fitote.2009.12.006.
[21] Y.-P. Shen, H.-Y. Wang, Y. Lu, L.-L. Xu, H.-W. Yin, J.-P. Tam, H. Yang, X.-B. Jia, Construction of a novel catalysis system for clean and efficient preparation of Baohuoside I from Icariin based on biphase enzymatic hydrolysis, J. Clean. Prod. 170 (2018) 727–734, https://doi.org/10.1016/j.jclepro.2017.09.192.
[22] C. Kornpointner, J. Scheibelreiter, H. Halbwirth, Snailase: a promising tool for the enzymatic hydrolysis of flavonoid glycosides from plant extracts, Front. Plant Sci. 13 (2022), https://doi.org/10.3389/fpls.2022.889184.

[23] C.-Y. Liu, R.-Y. Li, J. Peng, D. Qu, M.-M. Huang, Y. Chen, Enhanced hydrolysis and antitumor efficacy of Epimedium flavonoids mediated by immobilized snailase on silica, Process Biochem. 86 (2019) 80–88, https://doi.org/10.1016/j.procbio.2019.06.020.
[24] X. Jin, Z.-H. Zhang, E. Sun, X.-B. Jia, β-cyclodextrin assistant flavonoid glycosides enzymatic hydrolysis, Phcog. Mag. 9 (Suppl 1) (2013) S11–S18, https://doi.org/10.4103/0973-1296.117851.
[25] C. Liu, X. Gao, Y. Liu, M. Huang, D. Qu, Y. Chen, Icariin combined with snailase shows improved intestinal hydrolysis and absorption in osteoporosis rats, Biomed. Pharmacother. 94 (2017) 1048–1056, https://doi.org/10.1016/j.biopha.2017.07.163.
[26] S.S. Kang, Y.J. Kang, M.W. Lee, Flavonoids from epimedium koreanum, J. Nat. Prod. 54 (2) (1991) 542–546, https://doi.org/10.1021/np50074a029.
[27] Y.-P. Shen, M. Wang, J.-W. Zhou, Y.-F. Chen, L. Xu, M.-R. Wu, G.-H. Xia, J.-P. Tam, J.-N. Yu, X.-Y. Teng, H. Yang, X.B. Jia, Eco-efficient biphasic enzymatic hydrolysis for the green production of rare baohuoside I, Enzym. Microb. Technol. 131 (2019), 109431, https://doi.org/10.1016/j.enzmictec.2019.109431.
[28] Y.-B. Shen, M. Wang, Y.-F. Chen, L.-L. Xu, Y. Lu, Y.-Y. Zhou, J.P. Tam, F. Han, H. Yang, X.-B. Jia, Convenient preparation of sagittatoside B, a rare bioactive secondary flavonol glycoside, by recyclable and integrated biphase enzymatic hydrolysis, Enzym. Microb. Technol. 121 (2019) 51–58, https://doi.org/10.1016/j.enzmictec.2018.12.002.