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Original Article

Bioreactor scale-up and bioactivity evaluation of *Psammosilene tunicoides* hairy roots

Rui Jin[1†], Chang An[2†], Bingrui Wang[3†], Changbin Liu[1], Xiaopeng Fan[4], Zongshen Zhang[1*]
[1] Laboratory of Pharmaceutical Plant Cell Culture Research, School of Biological Engineering, Dalian Polytechnic University, Dalian 116034, China
[2] Fujian Provincial Key Laboratory of Haixia Applied Plant Systems Biology, Center for Genomics, College of Life Science, Fujian Agriculture and Forestry University, Fuzhou 350002, China
[3] College of Plant Science & Technology, Huazhong Agricultural University, Wuhan 430070, China
[4] Hangzhou Institute of Advanced Technology, Hangzhou 310018, China

[†] *These authors contributed equally to this work*
**Corresponding author's email:* zhangzs@dlpu.edu.cn
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Abstract

Psammosilene tunicoides is a well-known representative of "Miao medicine" in China, has high market demand and application potential. However, wild resources are scarce and increasingly endangered due to overharvesting. To address this issue, this study investigates the potential of bioreactor-cultivated hairy roots (HRs) of *P. tunicoides* as a sustainable alternative to wild medicinal resources. We first examined the bioreactor cultivation of *P. tunicoides* HRs, with emphasis on their growth, bioactive compound accumulation, and biological activities. HRs were cultured in 10 L bioreactors for up to 45 days. At 30 days of culture, the results showed that the biomass and growth rate of HRs reached 138.19±2.77 g and 0.233±0.06 g/d, respectively, with a saponin content of 11.41±0.02 %, which exceeded the levels observed in five-year-old wild. *P. tunicoides*. In addition, the antioxidant and antibacterial activities of the HRs were evaluated. Before deglycosylation, the DPPH radical scavenging rate was 65%, increasing to 83% after deglycosylation. Similarly, hydroxyl radical scavenging rates were 65% and 80%, and superoxide anion scavenging rates were 48.2% and 81.6% before and after deglycosylation, respectively. The deglycosylated saponins exhibited scavenging effects comparable to vitamin C, likely due to the removal of impurities by AB-8 macroporous adsorption resin. Antibacterial tests showed that deglycosylated saponins had a higher inhibitory effect on gram-positive bacteria (*S. aureus* > *M. luteus* > *E. coli* > *P. aeruginosa*) than on gram-negative bacteria. Taken together, the consistent enhancement in saponin levels, antioxidant capacity, and antibacterial performance underscores the pharmacological robustness of bioreactor-cultivated HRs. These findings indicate that bioreactor-cultivated *P. tunicoides* HRs possess similar biological activities to wild *P. tunicoides*, supporting the feasibility of using cell engineering methods to sustainably replace rare and endangered wild medicinal resources.

Keywords: *Psammosilene tunicoides*, Hairy roots, Bioreactor cultivation, Antioxidant activity, Antibacterial activity

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Introduction

Hairy roots (HRs) are adventitious roots induced by *Agrobacterium rhizogenes* infection of plant explants. They possess several advantages, including rapid growth, hormone autotrophy, and genetic stability (Gantait and Mukherjee, 2021). Large-scale cultivation of medicinal plant HRs via plant cell engineering provides an important raw material source for natural compound production and significantly contributes to the protection and sustainable utilization of wild resources (Gutierrez-Valdes et al., 2020). HR culture technology not only enhances biomass production but also promotes the accumulation of secondary metabolites, making it a promising platform for natural product biosynthesis (Yu et al., 2023). Compared with cell and tissue cultures, in vitro root organ cultures offer multiple advantages, including stronger organ-specific accumulation of medicinal compounds, higher metabolic stability, no requirement for illumination (thus reducing energy consumption), and simpler, more stable production processes (Alcalde et al., 2022; Babich et al., 2020). This approach, known as "root organ engineering", has become a crucial strategy for the sustainable exploitation of rare and endangered medicinal plant resources.

Bioreactors are closed, sterile systems equipped with nutrient media and inflow/outflow modules that allow precise control of culture conditions, including pH, aeration, dissolved oxygen, and temperature (Stiles and Liu, 2013). Compared with conventional shake-flask cultures, they ensure homogeneity, enable real-time monitoring, and support large-scale, stable biomass and metabolite production (Paek et al., 2005). In plant biotechnology, they have been successfully applied to scale up the production of HRs, suspension cells, and other organ cultures, providing a reliable platform for biomass accumulation and secondary metabolite biosynthesis (Srivastava and Srivastava, 2007). Thus, the use of bioreactors represents a crucial technological step toward the industrialization and sustainable exploitation of rare medicinal plants.

Psammosilene tunicoides, a medicinal plant endemic to southwestern China, belongs to the family Caryophyllaceae and genus *Psammosilene* (Zhang et al., 2011). Its rhizomes are rich in triterpenoids, triterpenoid saponins, and volatile oils, and exhibit anti-inflammatory, analgesic, hemostatic, and anti-rheumatic activities (Li et al., 2023; Wen et al., 2020). With the growth of the wellness industry and the rising "returning to nature" trend, market demand for *P. tunicoides* has increased annually. However, its long natural growth period (5-7 years), overharvesting, and habitat destruction have led to severe depletion of wild resources. As a result, it has been listed as a rare and endangered species in the latest Key Protected Wild Plant List of China and classified as a national second-level protected plant species. Despite the increasing demand, studies on establishing stable HRs culture systems of *P. tunicoides* remain limited, and systematic attempts to scale up HRs production in bioreactors are still lacking. In contrast, species such as *Panax ginseng* and *Bupleurum falcatum* have already achieved industrial production through bioreactor scale-up and made significant progress in the sustainable exploitation of rare and endangered plants (Kusakari et al., 2012; Paek et al., 2009).

Here, we present the first successful establishment of a bioreactor-based scale-up system for *P. tunicoides* HRs, representing a significant advancement toward their commercial application. By systematically evaluating growth performance, major bioactive compound content, and antioxidant and antibacterial activities, this study not only fills a critical knowledge gap but also provides a solid foundation for future industrial-scale production and the sustainable conservation of wild resources.

Material and Methods

Material

P. tunicoides HRs were prepared and preserved by the Laboratory of Cell Engineering for Rare and Endangered Medicinal Plants at Dalian Polytechnic University. The suspension culture was carried out according to previously reported methods (Li et al., 2022).

Scaling-up cultivation of HRs

Exponential phase HRs of *P. tunicoides* from 250 mL suspension cultures were inoculated into a 10 L bioreactor containing 7.0 L of liquid media at an inoculation amount of 100 g fresh weight. The cultures were maintained in complete darkness at 25±1 ℃ with a controlled agitation speed of 80 rpm, an aeration rate of 100 mL/min, and dissolved oxygen (DO) maintained above 40% air saturation

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by adjusting airflow and agitation. The pH was automatically regulated at 7.0±0.1. To prevent excessive foaming, 0.01% (v/v) polypropylene glycol was added as an antifoam agent. Cultures were harvested at 15, 30, and 45 days. The biomass and specific growth rate were calculated as follows:

Biomass (g, DW) = Harvest weight (g) - Inoculum weight (g)

Growth rate (g/d) = Biomass (g) / (Inoculum weight (g) × Culture time (d))

Extraction, qualitative and quantitative detection of total saponin

The extraction of total saponins was performed following established procedures described in earlier studies (Shehzad et al., 2011), with minor modifications. Powdered HRs (sieved through a 60-mesh screen, 1 g dry weight) were soaked in 50 mL of 75% ethanol for 60 min. This step was repeated three times using reflux condensation at 80 ℃ in a water bath for 2 hours each time. All filtrates were collected, combined, and evaporated to dryness under reduced pressure. The residue was then dispersed in 25 mL of water and successively extracted with petroleum ether (25 mL) and n-butanol (25 mL), each repeated three times. The n-butanol phase was collected and subjected to rotary evaporation to obtain the total saponin extract. The extract was dissolved in methanol and transferred to a 10 mL volumetric flask to prepare the test solution (Mwangi et al., 2024).

Spectrophotometric determination

The total saponin content was determined using the vanillin-perchloric acid colorimetric method (Su et al., 2021). A standard solution of oleanolic acid (AR) was prepared at 1 mg/mL in methanol. The absorbance values of different concentrations of oleanolic acid were measured at 540 nm, yielding a calibration curve with the equation $y = 0.0865x + 0.015$ ($R^2 = 0.999$). The total saponin content was calculated as follows:

Content of total saponins (mg/g) = (C × V × N) /M

C*:* Concentration of total saponins (mg/mL)
V: Volume of the sample (mL)
N: Dilution factor
M: Mass of the sample (g).

Thin-layer chromatographic (TLC) analysis

A 20 × 5 cm silica gel plate was activated in an oven at 100 ℃ for 10 min for use in TLC analysis. The freshly prepared eluent was poured into the chromatographic chamber and allowed to saturate for 30 min. The silica gel plate was then placed in the chamber at an appropriate angle, ensuring the liquid level was below the spotting position. When the eluent had almost reached the top of the plate, the plate was removed and dried with a hairdryer. The plate was subsequently immersed in the coloring agent for a short time and then dried again with a hairdryer. The eluent consisted of a mixture of chloroform-methanol-water (7:3:0.5, v/v/v), and the coloring agent was 10% concentrated sulfuric acid.

High performance liquid chromatographic (HPLC) analysis

HPLC analysis was performed according to previously reported methods (Shehzad et al., 2011), with minor modifications. The analysis was conducted using a Zhonghuida C18 column (φ 250 mm × 4.6 mm, 5 μm). The mobile phase consisted of acetonitrile (A) and water (B), with gradient elution performed as specified in Table 1. The column temperature was maintained at 35°C, and detection was carried out at a wavelength of 203 nm. The injection volume was 10 μL, with a flow rate of 1.0 mL/min.

Table-1. Gradient elution program of the mobile phases.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0–20	20	80
20–31	20→32	80→68
31–40	32→43	68→57
40–70	43→100	57→0

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Determination of antioxidant capacity

The total saponin extract was dissolved in an appropriate amount of deionized water and then absorbed onto the AB-8 macroporous adsorption resin multiple times. Deglycosylation was performed with 8 times the column volume of deionized water, followed by elution with 6 volumes of 70% ethanol. The eluate was collected, concentrated, and dried. The DPPH free radical scavenging ability was determined according to previously reported methods (Pavithra and Vadivukkarasi, 2015). The hydroxyl radical scavenging ability was assessed using the Fenton method (Basit et al., 2023). The superoxide anion free radical scavenging ability was evaluated using the pyrogallol autoxidation method (Khadivi-Derakhshan et al., 2024).

Bacteriostatic test

Escherichia coli, *Staphylococcus aureus*, *Xanthomonas maltophilia*, and *Pseudomonas aeruginosa* were used as test strains. The filter paper disc diffusion method was employed. Filter paper discs (6 mm in diameter) were immersed in the saponin solutions for 24 h prior to use. Each Petri dish contained: (i) a blank control disc (sterile deionized water), (ii) discs soaked in saponin solutions at different concentrations (10, 20, and 30 mg/mL) before deglycosylation, and (iii) discs soaked in saponin solutions at the same concentrations (10, 20, and 30 mg/mL) after deglycosylation. Plates were incubated at 37 ± 1 °C for 24 h, and inhibition zone diameters were measured in millimeters.

Statistical analysis

Data were recorded using Excel 2010. All experiments were performed in triplicate, and results are expressed as mean ± standard deviation (SD). Statistical analyses were conducted using one-way ANOVA followed by Tukey's post-hoc test in SPSS 26.0, with differences considered significant at $p < 0.05$. Graphs were plotted using Origin 2021.

Results

Biomass, growth rate, and total saponin content of HRs cultured in 10 L bioreactors

Figure 1 presents the biomass (DW), growth rate, and total saponin content of *P. tunicoides* HRs cultured in a 10 L bioreactor. After 15, 30, and 45 days of culture, the biomass reached 35.67 ± 1.52 g, 138.19 ± 2.77 g, and 204.49 ± 3.51 g, with corresponding growth rates of 0.115 ± 0.04 g/d, 0.233 ± 0.06 g/d, and 0.223 ± 0.07 g/d, respectively. Table 2 shows that HRs cultured for 30-45 days contained higher total saponin levels than wild medicinal materials, following the order: 45 days > 30 days > 5-year-old wild *P. tunicoides* > 15 days. These findings indicate that large-scale bioreactor culture not only enables substantial biomass production but also promotes the accumulation of total saponins. Notably, the lowest saponin content was observed at 15 days, suggesting a strong correlation between saponin accumulation and culture duration. In the early growth stage, primary metabolism predominates and suppresses secondary metabolism, including saponin biosynthesis. As growth slows and cells begin to senesce, secondary metabolism becomes more active, leading to enhanced saponin production.

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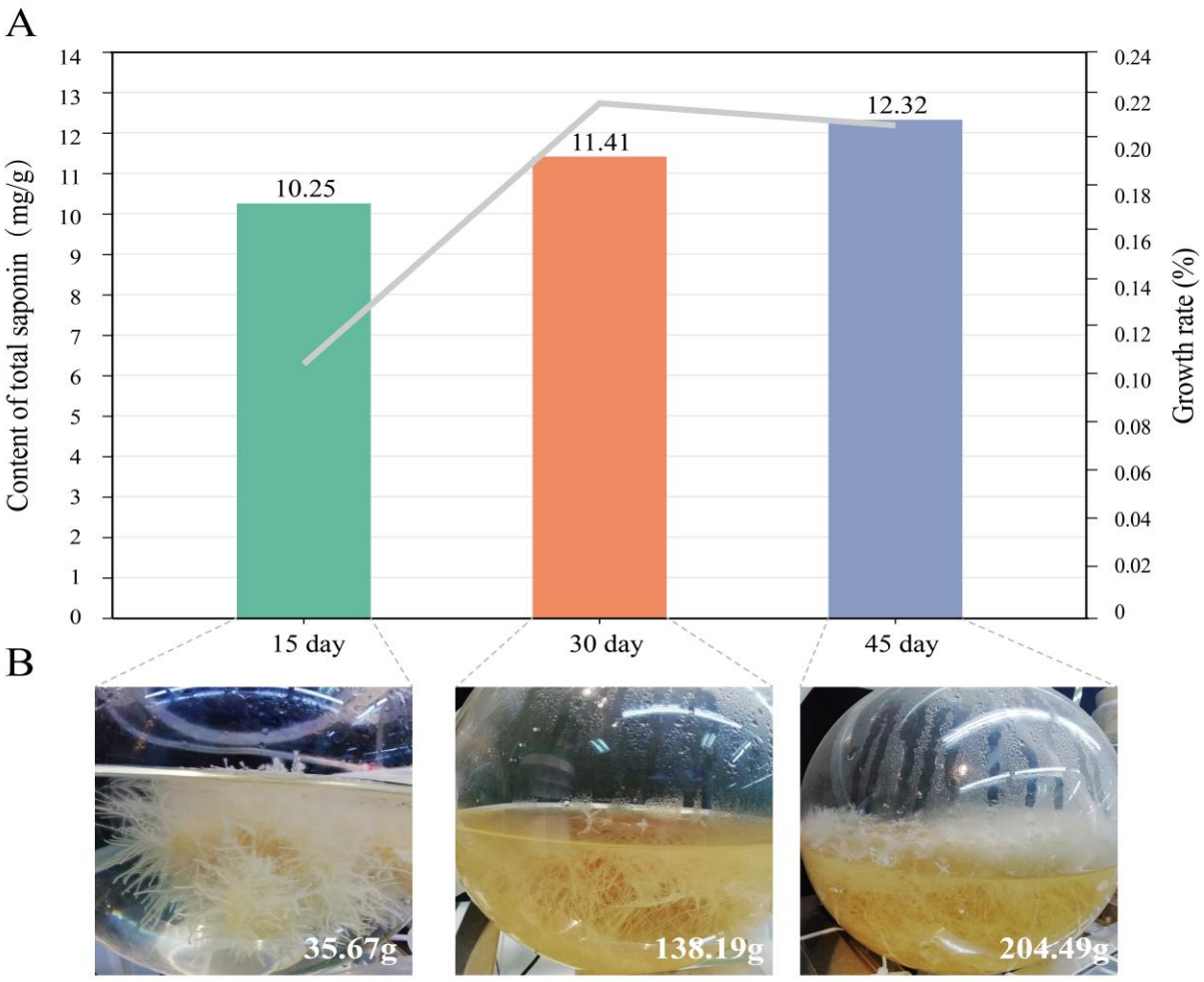

Figure-1. Biomass, growth rate (%), and total saponin content (mg/g) of 10 L bioreactor-cultured HRs. (A) Content of total saponin (mg/g) and growth rate (%) represented by bar and line charts; (B) HRs cultured in a 10 L bioreactor at different time points.

Table-2. Comparison of total saponin content between bioreactor-cultured HRs and wild *P. tunicoides.*

Metrics	**Bioreactor-cultured HRs**			**Wild *P. tunicoides***
Culture time	15 d	30 d	45 d	5 y
Biomass (g, DW)	35.67±1.52	138.19±2.77	204.49±3.51	-
Growth rate (%)	0.115±0.04	0.233±0.06	0.223±0.07	-
Content of total saponin (mg/g)	10.25±0.13 b	11.41±0.18 a	12.32±0.13 c	10.86±0.17 b

Note: Different lowercase letters indicate significant differences between treatments ($P < 0.05$), as below.

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TLC and HPLC analyses of oleanolic acid and total saponins from wild and HRs

As shown in Figure 2A, the oleanolic acid standard and all four tested samples displayed good separation with clear bands on TLC. Comparison with the standard revealed that each sample contained oleanolic acid, along with other saponins and unidentified components. Owing to quantitative spotting, the relative saponin content could be preliminarily estimated based on the size and intensity of the TLC spots. The results indicated that HRs cultured in a bioreactor for 45 days exhibited the highest saponin content, whereas wild *P. tunicoides* showed the lowest. Moreover, HRs contained markedly higher levels of low-polarity saponins compared with 5-year-old wild materials. Since low-polarity saponins are more likely to disrupt cell membrane integrity, this suggests stronger pharmacological activity in HRs than in wild roots.

To further validate these findings, Figure 2B presents the HPLC chromatograms. The oleanolic acid standard exhibited a peak at a retention time of 60.9 min, and similar peaks were detected in all four samples, confirming the presence of oleanolic acid. Based on peak intensity and area, the oleanolic acid content was highest in HRs cultured for 45 days, followed by those cultured for 30 days, then the 5-year-old wild *P. tunicoides* root, and finally the 15-day cultured HRs.

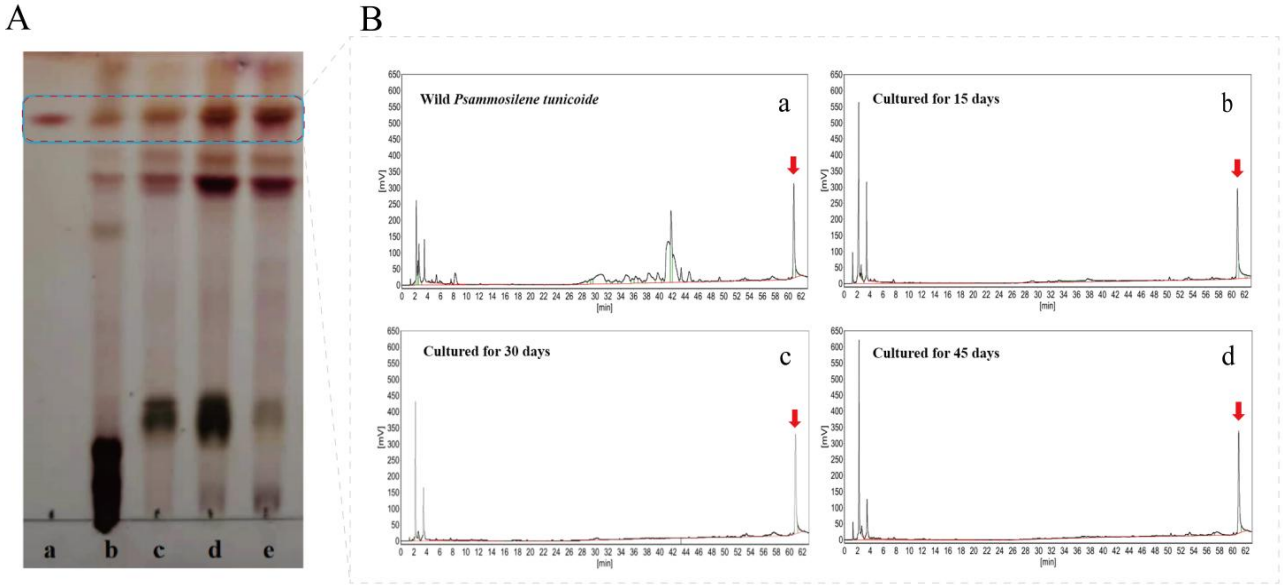

Figure-2. TLC and HPLC analysis of crude total saponins from wild plants and hairy roots. (A) TLC profiles of total saponins (a: Marker, oleanolic acid; b: wild material; c: 15 d; d: 30 d; e: 45 d); (B) HPLC chromatograms of total saponins from wild plants and hairy roots.

Effect of deglycosylation treatment on purity of the total saponin from *P. tunicoides* HRs

Based on the total saponin content results from 10 L bioreactor cultured for different periods, crude saponins from HRs cultured in 10 L bioreactor for 45 days were selected for deglycosylation. The crude saponins appeared caramel-like after extraction and rotary evaporation (Figure 3A). Following deglycosylation and drying, they became powdery, increasing the purity of the saponins and facilitating subsequent pharmacological studies.

The total saponins extracted from HRs were treated with macroporous adsorption resin for deglycosylation. The purity of the saponins increased from 40.60±0.28% to 84.05±0.74% after treatment, approximately doubling the original purity. This demonstrates that the AB-8 resin is effective for deglycosylation.

The antioxidant activity of total saponins from HRs of *P. tunicoides*

Figure 3B shows the DPPH radical scavenging ability of total saponins from HRs before and after deglycosylation. The results indicate that within the concentration range of 0.1 to 0.8 mg/mL, the

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scavenging ability increases rapidly with increasing concentration. Between 0.8 to 1.6 mg/mL, the scavenging rate of total saponins, both before and after deglycosylation, increases more slowly. At 1.6 mg/mL, the scavenging rates are 65.29±1.96% (before deglycosylation) and 83.27±2.50% (after deglycosylation). This shows that total saponins from HRs can scavenge DPPH radicals both before and after deglycosylation. At the same concentration, deglycosylated saponins have a higher scavenging ability than the non-deglycosylated saponins but lower than that of ascorbic acid (Vitamin C, Vc), used as a positive control.

Figure 3C presents the hydroxyl radical scavenging ability of HRs-derived total saponins before and after deglycosylation. The scavenging ability is dose-dependent but shows different trends. Before deglycosylation, the scavenging rate remains between 40% and 50% when the saponin concentration is 0.8–1.6 mg/mL. After deglycosylation, the scavenging rate increases steadily from 60.70±1.82% to 79.19±2.38% at the same concentration. The scavenging ability ranks as Vc > after deglycosylation > before deglycosylation, indicating enhanced efficacy post-deglycosylation.

Figure 3D shows the superoxide anion radical scavenging capacity of total saponins from HRs before and after deglycosylation. Within the concentration range of 0.1 to 0.8 mg/mL, the scavenging capacity is positively correlated with concentration. Before deglycosylation, the scavenging rate increased from 10.68±0.32% to 48.18±1.45%. After deglycosylation, it increased from 11.41±0.34% to 81.56±2.44%. At 0.6 mg/mL, the scavenging capacity of deglycosylated saponins exceeded that of Vc, indicating enhanced efficacy post-deglycosylation.

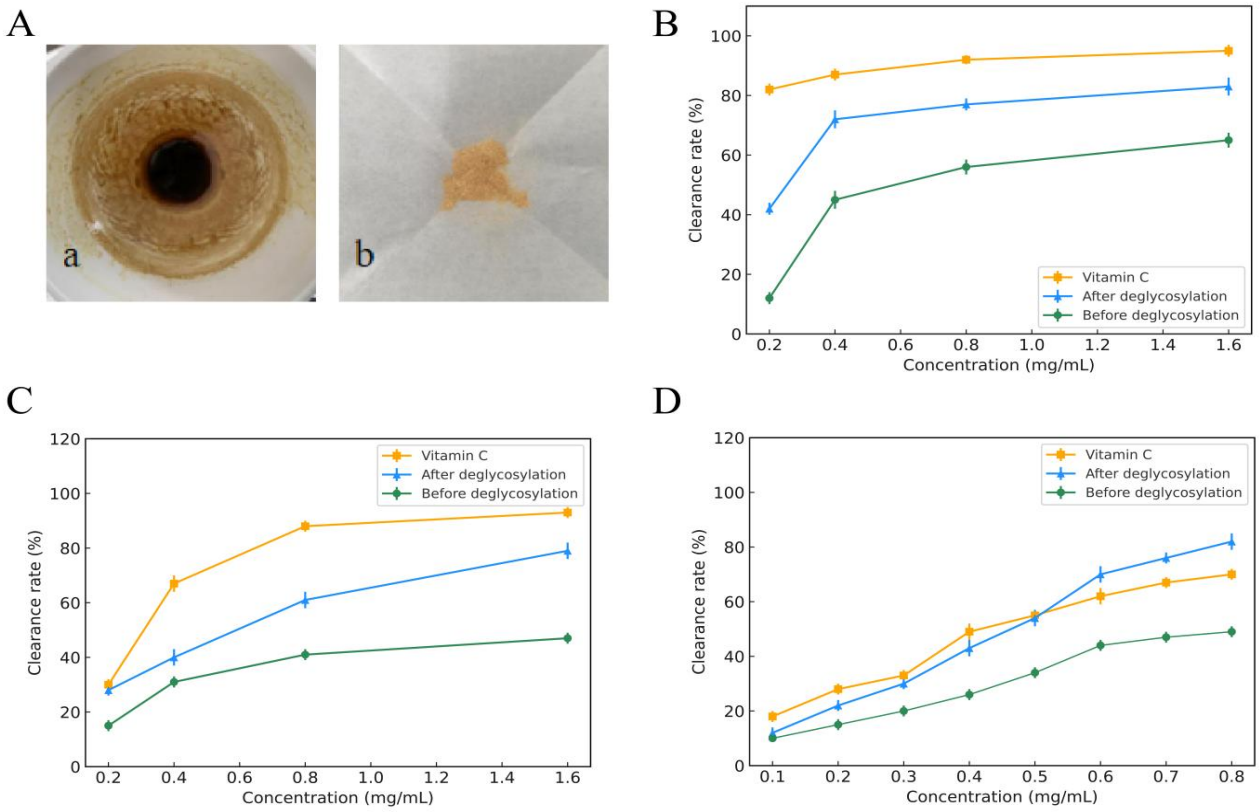

Figure-3. Effects of deglycosylation on crude saponins and their antioxidant activities. (A) Crude saponin powder (a: before desugarization; b: after desugarization); (B) DPPH radical scavenging activity of Vc and total saponins from HRs (before and after deglycosylation); (C) Hydroxyl radical scavenging activity of Vc and total saponins from HRs (before and after deglycosylation); (D) Superoxide anion radical scavenging activity of Vc and total saponins from HRs (before and after deglycosylation).

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Antibacterial effects of deglycosylated total saponins from HRs

Figures 4 and Table 3 show the antibacterial effects of glycosides from HRs against *E. coli*, *S. aureus*, *P. aeruginosa*, and *M. luteus*, both before and after deglycosylation. The results indicate that saponins from HRs exhibit antibacterial activity against these four bacteria, with the diameter of the inhibition zone positively correlated with the mass concentration. At a mass concentration of 30 mg/mL, the diameters of the inhibition zones before deglycosylation are 8.9±0.30 mm (*E. coli*), 11.4±0.40 mm (*S. aureus*), 7.8±0.15 mm (*P. aeruginosa*), and 10.2±0.25 mm (*M. luteus*). After deglycosylation, these diameters increase to 11.4±0.38 mm (*E. coli*), 17.1±0.36 mm (*S. aureus*), 8.9±0.66 mm (*P. aeruginosa*), and 13.7±0.64 mm (*S. luteus*). The antibacterial activity is consistently higher after deglycosylation, with the order of activity being: *S. aureus* > *M. luteus* > *E. coli* > *P. aeruginosa*. This suggests that saponins from HRs are more effective against gram-positive bacteria (*S. aureus* and *M. luteus*) than against gram-negative bacteria (*E. coli* and *P. aeruginosa*).

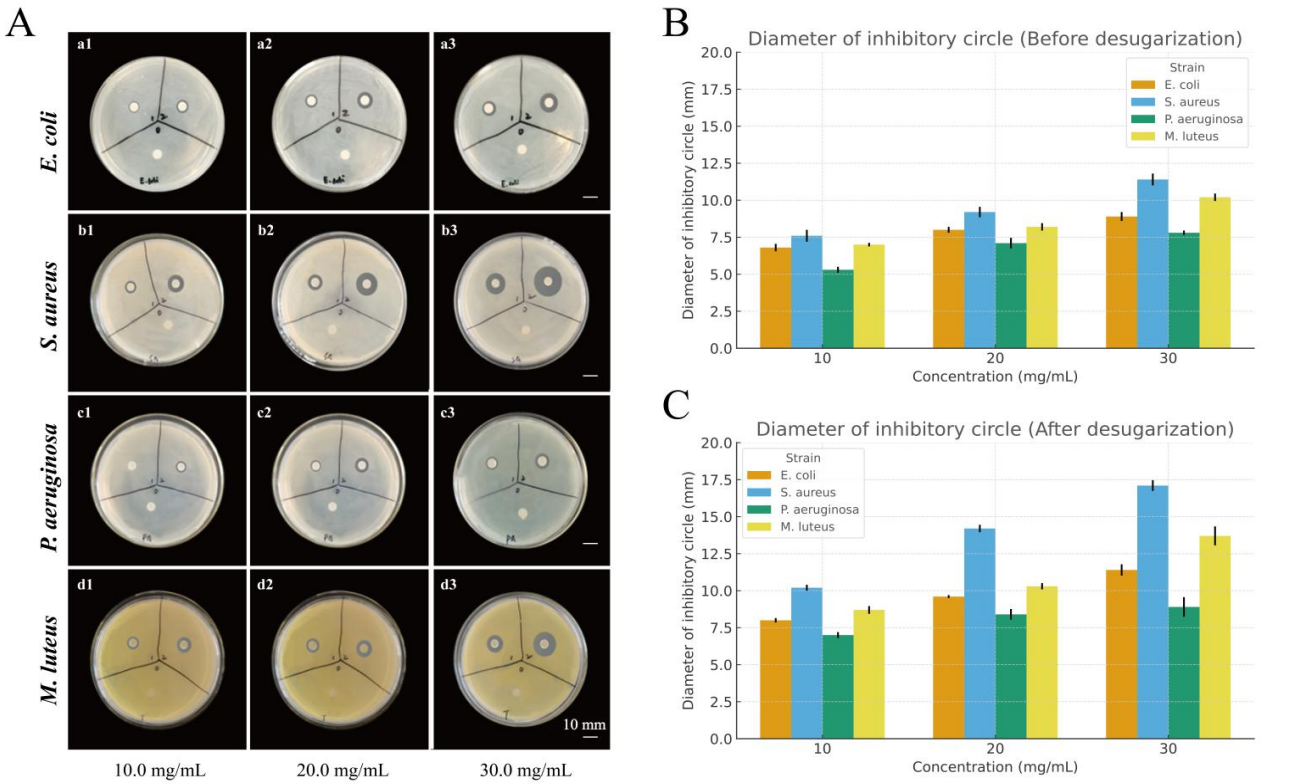

Figure-4. Antibacterial effects of total saponins from hairy roots before and after deglycosylation. (A) Inhibition of four bacterial strains (0: deionized water control; 1: before deglycosylation; 2: after deglycosylation; concentrations: 10.0, 20.0, and 30.0 mg/mL). Strains: A. E. coli; B. S. aureus; C. P. aeruginosa; D. M. luteus; (B) Statistical analysis of inhibition zone diameters before deglycosylation; (C) Statistical analysis of inhibition zone diameters after deglycosylation.

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Table-3. Inhibitory diameters before and after deglycosylation of total saponins of HRs.

Strain	Control	The diameter of the inhibition zone by concentration of total saponins (mm, mg/mL)					
		Total saponins before deglycosylation			Total saponins after deglycosylation		
		10	20	30	10	20	30
E. coli	6	6.8±0.25 b	8.0±0.20 b	8.9±0.30 c	8.0±0.15 c	9.6±0.10 c	11.4±0.38 c
S.aureus	6	7.6±0.40 a	9.2±0.35 a	11.4±0.40 a	10.2±0.20 a	14.2±0.25 a	17.1±0.36 a
P.aeruginosa	6	5.3±0.20 c	7.1±0.36 c	7.8±0.15 d	7.0±0.20 d	8.4±0.36 d	8.9±0.66 d
M.luteus	6	7.0±0.12 b	8.2±0.25 b	10.2±0.25 b	8.7±0.26 b	10.3±0.21 b	13.7±0.64 b

Discussion

P. tunicoides is a renowned representative of "Miao medicine" in China and a crucial raw material for many valuable Chinese patent medicines. It has a broad market demand and promising application prospects. Compared with cells or callus tissues, *P. tunicoides* HRs have advantages such as a short growth cycle, genetic stability, and robust secondary metabolism. The quality of *P. tunicoides* HRs is closely related to the content of active components, making the enhancement of these components a key issue. This study cultivated HRs using bioreactor amplification and demonstrated through rigorous quality evaluation and analysis that the cultured HRs can serve as a viable alternative to wild *P. tunicoides* for medicinal purposes.

Bioreactor cultivation of HRs offers high efficiency, low cost, and standardized production, and is not limited by seasons, climate, or geographical regions (Mirmazloum et al., 2024). A 10 L bioreactor was employed to cultivate Panax ginseng HRs, and optimization of medium composition significantly enhanced ginsenoside accumulation, with the maximum content reaching 9-12 mg/g DW, which was markedly higher than flask-cultured roots (Kochan et al., 2014). Similarly, licorice HRs cultured in a 10 L stirred-tank bioreactor achieved a 55-60-fold biomass increase in 60 days, with glycyrrhizic acid (GA) content reaching 0.1%, matching or exceeding flask-cultured roots (Zhang et al., 2009). These findings suggest that bioreactor-cultivated HRs have a competitive edge in biomass and effective component accumulation. In this study, 10 L bioreactors with a 45-day culture time produced the highest biomass and growth rate (138.19±2.77 g, 0.233±0.06 g/d) and saponin content (11.41±0.02%), surpassing five-year-old wild *P. tunicoides*. This demonstrates that bioreactor cultivation can produce high-quality *P. tunicoides* HRs, offering a viable alternative to wild resources.

In vitro experiments showed that the HRs of *P. tunicoides* exhibit antioxidant properties both before and after deglycosylation. At a concentration of 1.6 mg/L, the DPPH radical scavenging rate increased from 65% before deglycosylation to 83% after. Similarly, the hydroxyl radical scavenging rate rose from 65% to 80%, and the superoxide anion scavenging rate at 0.8 mg/L increased from 48.2% to 81.6% after deglycosylation. The deglycosylated saponins showed scavenging effects on DPPH radicals, hydroxyl radicals, and superoxide anions comparable to vitamin C. This enhancement in antioxidant activity may be due to the removal of impurities by AB-8 macroporous adsorption resin, as previously reported (Liu et al., 2021). Additionally, deglycosylated saponins exhibited stronger antibacterial activity against gram-positive bacteria than against gram-negative bacteria, with the overall order of activity being: *S. aureus* > *M. luteus* > *E. coli* > *P. aeruginosa*, consistent with earlier findings (Xue et al., 2020). These results indicate that bioreactor-cultured *P. tunicoides* HRs possess similar biological activity to wild *P. tunicoides*, supporting the use of cell engineering to sustainably replace rare and endangered wild medicinal resources. Furthermore, these findings not only demonstrate the biological activity of bioreactor-cultivated HRs but also highlight their potential as a sustainable source of medicinal compounds. This provides practical value for both pharmacological research and the commercial development of standardized *P. tunicoides* products.

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However, certain limitations should be noted. Although the established 10 L bioreactor system achieved high biomass, growth rate, and saponin content, further refinement of operational conditions (such as dissolved oxygen control, aeration strategies, and nutrient feeding) may enhance both productivity and bioactive compound accumulation. Moreover, the present study focused on in vitro antioxidant and antibacterial assays; comprehensive in vivo pharmacological evaluations and stability tests will be essential to fully validate their therapeutic equivalence to wild resources. Addressing these aspects in future research will not only strengthen the reliability of the present findings but also accelerate the translation of bioreactor-based HRs technology into industrial-scale production and conservation programs.

Conclusions

This study demonstrates the successful scale-up cultivation of *P. tunicoides* HRs in a 10 L bioreactor, achieving significant biomass production (138.19±2.77g) and high saponin content (11.41±0.02%), surpassing that of wild *P. tunicoides* harvested after five years. The deglycosylated saponins exhibited enhanced antioxidant activity, with DPPH, hydroxyl, and superoxide anion radical scavenging rates reaching 83%, 80%, and 81.6%, respectively, comparable to Vc. Additionally, the deglycosylated saponins showed stronger antibacterial effects against gram-positive bacteria (*S. aureus* and *M. luteus*) than gram-negative strains (*E. coli* and *P. aeruginosa*). These findings highlight the potential of bioreactor-cultivated *P. tunicoides* HRs as a sustainable and efficient alternative to wild resources, addressing the challenges of overharvesting and habitat destruction. The study provides a foundation for the industrial application of HRs cultures in producing bioactive compounds, contributing to the conservation of rare medicinal plants while meeting market demands.

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Conflict of Interest: None.

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Contribution of Authors
Jin R: Conceptualized the study, conducted experiments, analyzed data, and prepared the original draft.
An C: Assisted in methodology design, data collection, and contributed to writing and editing.
Wang B: Participated in data analysis, validation, and manuscript revision.
Liu C: Assisted in investigation and experimental work.
Fan X: Performed statistical analysis and contributed to result interpretation.
Zhang Z: Supervised the study, contributed to conceptualization and validation, revised the manuscript, and secured funding.

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