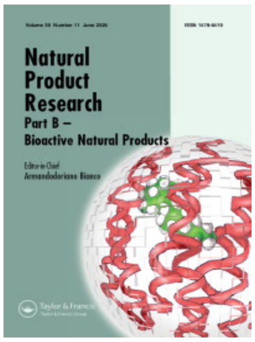

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UPLC-QTOF-MS/mS profiling and antioxidant activity of Ngoc Linh ginseng (*panax vietnamensis*) hairy root extract from zebrafish: insights into Keap1/Nrf2-dependent mechanisms

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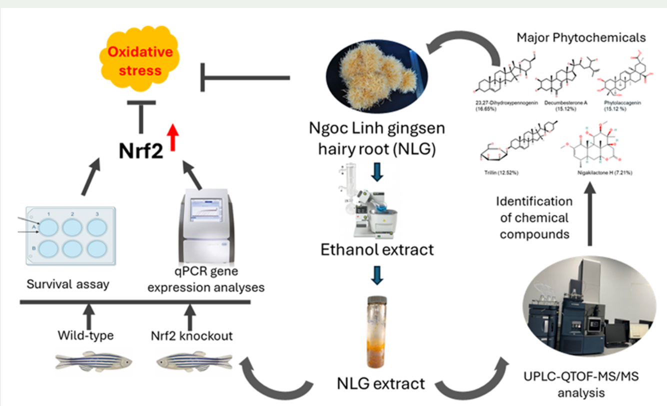
ABSTRACT

Panax vietnamensis (Ngoc Linh ginseng) hairy root extract (PV-HRE) was comprehensively analysed *via* UPLC–QTOF–MS/MS. The analysis revealed 41 phytochemical constituents, predominantly saponins, with the major bioactive compounds identified as 23,27-dihydroxypennogenin, decumbesterone A, phytolaccagenin, trillin, and nigakilactone H. By employing a zebrafish model under hydrogen peroxide-induced oxidative stress, we demonstrated that PV-HRE pre-treatment significantly enhanced larval survival. Molecular investigations revealed Keap1/Nrf2 pathway-dependent mechanisms characterised by substantial upregulation of the antioxidant genes glutathione S-transferase pi 1 (*gstp1*) and peroxiredoxin 1 (*prdx1*). Comparative experiments with Nrf2-deficient zebrafish (*nfe2l2a*^{dl703}) confirmed the critical role of the pathway in mediating protective cellular responses. These findings substantiate the therapeutic potential of *Panax vietnamensis* hairy root extract as a sophisticated natural intervention for oxidative stress-related pathological conditions.



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1. Introduction

Panax vietnamensis Ha et Grushv., an endemic medicinal herb indigenous to Vietnam, represents a pivotal source of bioactive phytochemicals with substantial pharmacological significance. Panax vietnamensis hairy roots (line 5) were cultivated at the Biotechnology Centre of Ho Chi Minh City following established protocols (Ha Thi Loan et al. 2019). The parent plant material was collected from Kon Tum province, Vietnam, and authenticated by Dr. Ha Thi Loan at the Biotechnology Centre of Ho Chi Minh City, Vietnam. A voucher specimen (PV-2023-05) has been deposited in the herbarium of the Biotechnology Centre of Ho Chi Minh City. Due to increasing anthropogenic pressures and natural population depletion, advanced biotechnological interventions have emerged as strategic approaches for sustainable metabolite production. Specifically, hairy root cultures established through *Agrobacterium rhizogenes*-mediated transformation offer an efficient alternative for producing valuable compounds.

Oxidative stress, characterised by persistent reactive oxygen species (ROS) accumulation and compromised cellular antioxidant mechanisms, represents a fundamental pathophysiological phenomenon implicated in the progression of multiple diseases (Deshmukh et al. 2017). The Keap1/Nrf2 signalling cascade constitutes a sophisticated cellular defense mechanism in which Nrf2 transcription factor dynamics are regulated through complex protein interactions. Under normal cellular conditions, Nrf2 remains cytosolic and is sequestered by Keap1. During oxidative challenge, conformational modifications facilitate Nrf2 nuclear translocation, enabling the transcriptional activation of antioxidant response elements (AREs) and the subsequent upregulation of critical cytoprotective genes, including *gstp1* and *prdx1* (Nakajima et al. 2011; Nguyen et al. 2016).

This investigation employs a comprehensive, multidisciplinary approach to elucidate the antioxidant properties of Ngoc Linh ginseng hairy root extract. Using advanced analytical methodologies, including UPLC–QTOF–MS/MS for precise phytochemical characterisation, we implemented a zebrafish model to systematically evaluate the molecular mechanisms underlying oxidative stress mitigation. Our research paradigm encompasses hydrogen peroxide-induced oxidative stress assessment, gene expression profiling of Nrf2 target genes, and comparative survival analyses in wild-type and Nrf2-deficient zebrafish models, thereby providing nuanced insights into the therapeutic potential of PV-HRE as a sustainable source of natural antioxidant interventions.

2. Results and discussion

2.1. Yield extraction and phytochemical profiling

The extraction process of PV-HRE was performed *via* ultrasonic-assisted extraction with 70% ethanol as the solvent. This method yielded 22.53 g of extract from 50 g of dried biomass, resulting in a recovery efficiency of 45.06% (Table S1). The high extraction yield (45.06%) demonstrated the efficiency of the ultrasonic-assisted extraction method in isolating bioactive compounds from hairy root biomass. This approach is suitable for obtaining substantial quantities of secondary metabolites for further analysis (Faraz et al. 2020; Nguyen and Phuong 2021).

Comprehensive UPLC–QTOF–MS/MS analysis identified a total of 41 distinct compounds within the PV-HRE. These compounds are distributed across 14 chemical classes, including saponins, terpenoids, steroids, flavonoids, and alkaloids, which reflects the extensive phytochemical diversity of the extract (Table S2; Figure 1A). Among the identified compounds, several have been previously reported to possess antioxidant properties. For example, saponins such as ginsenoside Rf and trillin have been shown to exhibit antioxidant effects in various models (Shi et al. 2019; Ratan et al. 2021). Additionally, flavonoids and terpenoids identified in the extract may also contribute to its overall antioxidant capacity. However, further studies are needed to isolate and evaluate the antioxidant activity of individual compounds to determine their specific contributions.

Among these classes, saponins were the most abundant class, contributing significantly to the bioactivity of the extract. Based on peak area ratios, the five most abundant compounds were 23,27-dihydroxypennogenin (16.65%), decumbesterone A (15.12%), phytolaccagenin (15.12%), trillin (12.52%) and nigakilactone H (7.21%) (Figure 1B). Together, these compounds accounted for approximately 66.62% of the total peak area, suggesting their potential contribution to the extract's antioxidant activity. Although we estimated compound abundance using peak area ratios, future studies should employ internal or external standards to precisely quantify these bioactive compounds and determine their exact concentrations and purities.

Other significant compounds included ginsenoside Rf (RT: 9.62 min, m/z: 801.5012) and eclalbasaponin IX (RT: 26.02 min, m/z: 703.406), which are known for their pharmacological properties (Murthy et al., 2017). The chromatographic and mass spectrometric characteristics of phytolaccagenin (RT: 11.80 min, m/z: 533.3473) and nigakilactone H (RT: 11.66 min, m/z: 425.2147) were determined, providing valuable information for further identification and isolation of these compounds with known therapeutic properties (Zhou et al. 2017). Collectively, these compounds represent diverse phytochemical classes with documented biological activities, including antioxidant, anti-inflammatory, and neuroprotective properties.

The extraction efficiency and chemical diversity observed in this study have significant implications. The high extraction yield (45.06%) and rich bioactive compound content demonstrates the practical feasibility of scaling PV-HRE production for pharmaceutical and nutraceutical applications, potentially reducing production costs while maintaining therapeutic efficacy (Dar et al. 2023). The diverse chemical classes identified, particularly the dominant saponins, highlight the potential of the extract as a source of therapeutic agents that target oxidative stress and inflammation. This aligns with previous studies on the bioactive properties of similar compounds, further supporting the relevance of PV-HRE in addressing health-related challenges (Le et al. 2018).

The prominence of 23,27-dihydroxypennogenin, decumbesterone A, phytolaccagenin, trillin and nigakilactone H in the chemical profile reinforces their likely contribution to the extract's bioactivity. Previous studies have demonstrated that 23,27-dihydroxypennogenin, decumbesterone A, and phytolaccagenin activate specific antioxidative and anti-inflammatory pathways, suggesting that their high abundance in PV-HRE is a key driver of its therapeutic potential (Valdés-González et al. 2023). The presence of additional pharmacologically active constituents, such as ginsenoside Rf (known to modulate AMPK signalling) and eclalbasaponin IX (with documented

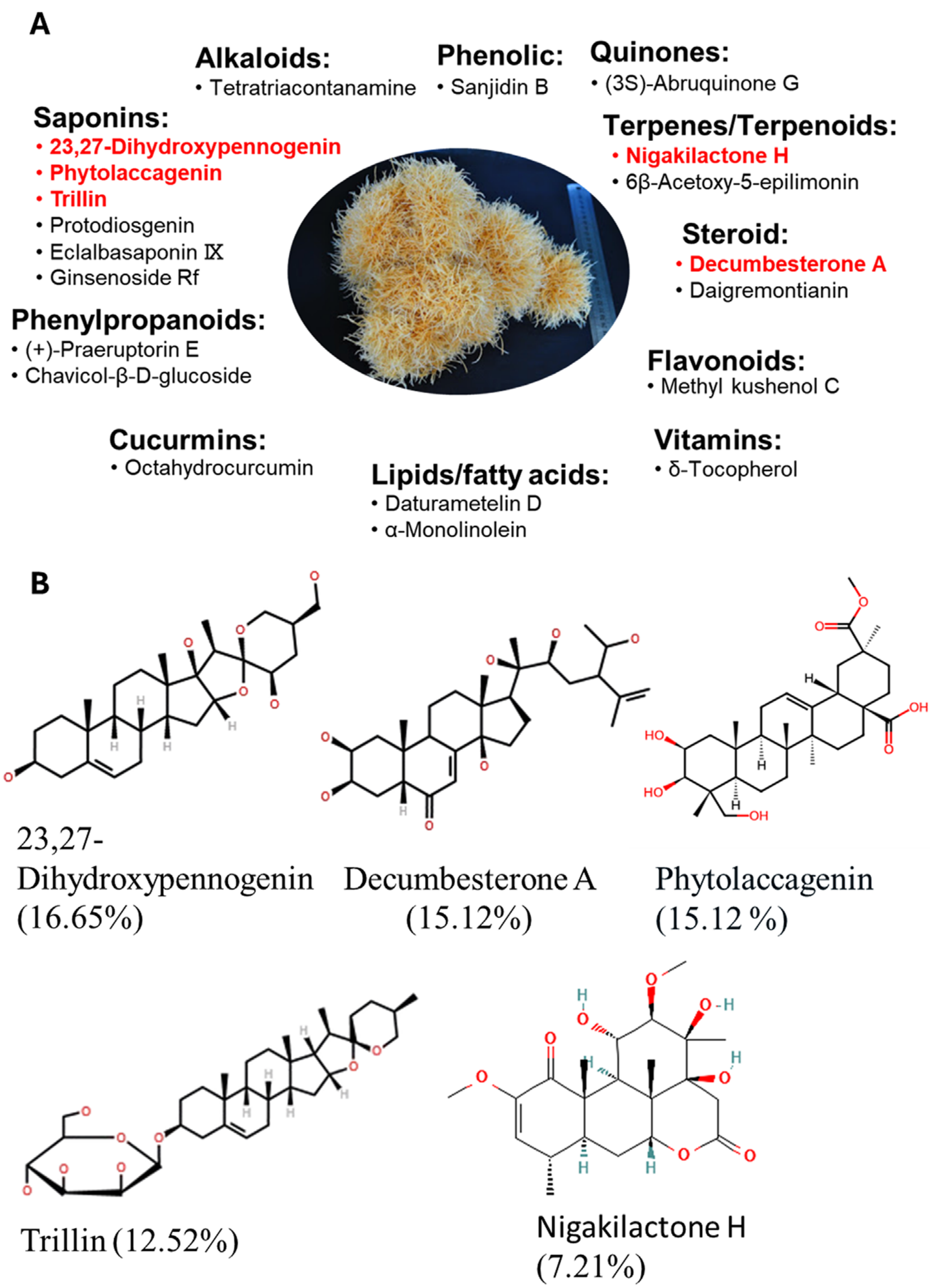

Figure 1. UPLC–QTOF–MS/MS Analysis of PV-HRE: (A) base peak intensity chromatogram identifying 41 compounds and (B) Five most abundant compounds: 23,27-dihydroxypennogenin, decumbesterone A, phytolaccagenin, trillin, and nigakilactone H.

anti-inflammatory properties), further adds to the extract's value as therapeutic agent capable of simultaneously affecting multiple cellular pathways (Shi et al. 2019).

In conclusion, the efficient recovery of bioactive compounds and the rich phytochemical diversity of PV-HRE lay a strong foundation for its further exploration. These findings emphasise the therapeutic potential of PV-HRE, particularly for oxidative stress-related conditions, and provide critical insights into its phytochemical makeup for future research and application (Nguyen and Phuong 2021).

2.2. Safety profile and antioxidant activity

The safety profile of the PV-HRE was evaluated in zebrafish larvae, with a dose-dependent assessment conducted at concentrations ranging from 200 to 1200 µg/mL. The results demonstrated that concentrations up to 600 µg/mL were well tolerated, with no significant adverse effects observed. However, exposure to relatively high concentrations (1000–1200 µg/mL) resulted in increased mortality rates (Figure S2), indicating potential toxicity at elevated doses. These findings highlight the importance of identifying an optimal concentration range for therapeutic applications.

The antioxidant activity of the PV-HRE was investigated under hydrogen peroxide-induced oxidative stress in zebrafish larvae. Pre-treatment with the extract at concentrations of 200 and 400 µg/mL significantly increased larval survival compared with that of the untreated group exposed to H_2O_2. Notably, the 400 µg/mL concentration had the greatest protective effect, with survival rates approaching those observed in the positive control group treated with sulforaphane (40 µM), a known Nrf2 activator (Figure S3A) (Shinkai et al. 2006). These results underscore the potent antioxidant properties of PV-HRE.

In Nrf2-deficient zebrafish (*nfe2l2a* knockout), the protective effects of PV-HRE and sulforaphane were absent, confirming that the antioxidant activity of the extract is mediated through the Nrf2 signalling pathway (Figure S3B). These findings provide mechanistic insights into the mode of action of the extract and reinforce its potential as a therapeutic agent for oxidative stress-related conditions.

The safety and antioxidant profiles of the PV-HRE extract are particularly relevant for its application in therapeutic contexts. The identification of a concentration threshold ensures the safe use of the extract, while the robust antioxidant activity supports its role in mitigating oxidative damage. These findings align with those of previous studies highlighting the critical role of Nrf2 in cellular defense mechanisms (Nguyen et al. 2020; Bian et al. 2023).

2.3. Mechanistic insights through Keap1/Nrf2 pathway activation

Molecular investigations revealed that the antioxidant effects of the PV-HRE extract were mediated *via* activation of the Keap1/Nrf2 signalling pathway. In wild-type zebrafish larvae, pre-treatment with the extract significantly upregulated the expression of key Nrf2 target genes, including *gstp1* and *prdx1* (Figure S4A and S4B). The gene expression levels observed following PV-HRE treatment were comparable to those induced by sulforaphane, further validating the role of the extract in activating this protective pathway.

In contrast, the expression levels of *gstp1* and *prdx1* remained unchanged in Nrf2-deficient zebrafish, providing direct evidence that the observed effects are Nrf2 dependent. These results highlight the critical role of the Keap1/Nrf2 axis in mediating the antioxidant activity of the PV-HRE extract. The ability of the extract to modulate this pathway suggests its potential utility in therapeutic interventions targeting oxidative stress and related pathologies (Alzain et al. 2023).

The mechanistic insights gained from this study emphasise the importance of the Keap1/Nrf2 pathway as a molecular target for PV-HRE. The upregulation of antioxidant genes provides a strong foundation for further exploration of the pharmacological potential of the extract. These findings not only reinforce the therapeutic importance of PV-HRE but also contribute to a broader understanding of its molecular mechanisms of action, paving the way for future applications in managing oxidative stress-related diseases.

3. Experimental

See Supplementary Material.

4. Conclusions

These findings establish the antioxidant efficacy of Ngoc Linh ginseng hairy root extract (PV-HRE) as a sustainable natural intervention for oxidative stress-related conditions. The unique phytochemical composition, including high-abundance saponins and other bioactive compounds, underscores its therapeutic potential. The Keap1/Nrf2 pathway-dependent mechanisms further position PV-HRE as a valuable candidate for managing oxidative stress and related disorders. This study not only highlights the potent antioxidant properties of PV-HRE but also highlights the utility of hairy root culture as a sustainable approach for producing high-value medicinal compounds, addressing conservation challenges associated with *Panax vietnamensis*. Future studies should focus on isolating and characterising the individual bioactive compounds in PV-HRE to elucidate their specific roles in antioxidant activity and their potential synergistic effects.

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Disclosure Statement

The authors declare that they have no competing interests.

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