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ENHANCED ANTIOXIDANT METABOLITES AND ENZYME ACTIVITIES IN VIETNAMESE NGOC LINH GINSENG (*Panax vietnamensis* Ha et Grushv) INDUCED BY YEAST EXTRACT

Van Phuong Nguyen[1], Ngan Anh Pham[1], Xuan Tu Dinh[2], Thi Phuong Linh Huynh[3] and Thi Phuong Nga Mai[1,✉]

[1]*University of Science and Technology of Hanoi, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nghia Do, Hanoi, Vietnam*

[2]*Technology Incubation Center, No 115 Tran Duy Hung, Yen Hoa Ward, Hanoi, Vietnam*

[3] *University Montpellier, Campus Triolet, Pl. Eugène Bataillon, 34090 Montpellier, France*

✉To whom correspondence should be addressed. Email: mai-thi-phuong.nga@usth.edu.vn

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ABSTRACT

Ngoc Linh ginseng (*Panax vietnamensis* Ha et Grushv) is a valuable medicinal plant known for its richness in ginsenoside saponins and exceptional pharmacological properties. However, excessive harvesting in the wild has led to serious depletion of natural populations and raised significant conservation concerns. As a result, alternative strategies are needed to ensure its sustainable use and preservation. *In vitro* cultivation has emerged as a sustainable approach to enhance bioactive compound production. This study investigated the effects of yeast extract (YE) elicitation at concentrations of 0.1 g/L (YE1) and 0.2 g/L (YE2) on antioxidant enzyme activity and secondary metabolite accumulation in *P. vietnamensis* to improve cultivation efficiency and phytochemical yield. Results revealed that, regarding antioxidant enzyme activities, YE1 significantly boosted catalase (CAT) activity, while YE2 increased peroxidase (POD) activity, suggesting distinct oxidative stress responses of these two enzymes towards yeast extract elicitation. In terms of chemical compound production, gallic acid, aescin, and quercetin were used as standard compounds to build the standard curve in phytochemical measurement. The results showed that YE1 increased total phenolic content (25 mg gallic acid-equivalent/g extract) and total saponin content (>600 mg aescin-equivalent/g extract), while both YE1 and YE2 significantly increased total flavonoid content (56–57 mg quercetin-equivalent/g extract) compared to the control. A strong positive correlation between the total saponin, total flavonoid, and total phenolic content was obtained, with correlation coefficients of r = 0.96, r = 0.63, and r = 0.83, respectively. Therefore, yeast extract is considered a promising biological elicitor to enhance the phytochemical potential and the sustainable cultivation of Vietnamese Ngoc Linh ginseng.

Keywords: antioxidant enzyme, catalase, peroxidase, *Panax vietnamensis* Ha et Grushv, secondary metabolites, yeast extract.

INTRODUCTION

Ginseng, from the *Panax* genus within the Araliaceae family, is considered a globally valued medicinal plant due to its bioactive ginsenosides, which have exhibited anti-inflammatory, antioxidant, and adaptogenic properties (Nguyen and Tran, 2019). While

Panax ginseng (Asian ginseng) and *Panax quinquefolius* (American ginseng) are more popular and currently dominate the international market for pharmaceuticals, cosmetics, and nutraceuticals, *P. vietnamensis* Ha et Grushv., or Ngoc Linh ginseng, stands out for its chemical profile, particularly its high content of ocotillol-type saponins like majonoside-R2, absent in other Panax species (Ung *et al*., 2018). It was discovered in 1973 in Vietnam's Ngoc Linh Mountain, named after its natural habitat. Since then, this species has gained attention for its pharmacological and biotechnological potential, making it a critical resource for natural product research. Its ability to produce high levels of bioactive compounds under specific conditions makes it a promising candidate for sustainable cultivation and industrial applications, addressing global demand for high-quality medicinal plants (Nguyen *et al*., 2019).

Before the discovery by scientists, Ngoc Linh ginseng was used by ethnic minorities in Central Vietnam, especially the Xe Dang ethnic group, as a wild root, which they called "small wormwood root" or "medicinal plant", to treat many diseases according to traditional medicine. During the war, Ngoc Linh ginseng was used to treat wounds, malaria, colds, etc. Nowadays, economically, it supports many rural livelihoods through the cultivation and trade of medicinal plants, contributing to a herbal medicine market worth hundreds of millions of dollars annually (Le *et al.,* 2018). Its roots and rhizomes contain over 52 dammarane-type triterpene saponins, including 26 unique vina-ginsenosides (R1–R25) and ocotillol-type saponins like majonoside R2, which constitute over 50% of its saponin content (Nhung et al., 2024). Overexploitation has rendered wild populations endangered, and traditional cultivation is slow, requiring at least four years for commercial viability. This scarcity has driven research to enhance biomass and ginsenoside production, supporting conservation and meeting rising demand (Titova *et al*., 2024).

Research indicates that ginseng plants often produce higher levels of bioactive compounds, including phenolics, flavonoids, and saponins, under stressful environments like drought or temperature fluctuations (Xu *et al*., 2023). These compounds, along with antioxidant enzymes like catalase (CAT) and peroxidase (POD), serve as defense mechanisms, protecting the plant against oxidative stress and environmental challenges. *In vitro*, stress responses can be induced using elicitors to enhance phytochemical and enzyme activity. Among these, yeast extract has shown significant promise for stimulating the production of total phenolic content (TPC), total flavonoid content (TFC), and total saponin content (TSC) in many plants (Lescano *et al*., 2025). Studies have demonstrated that yeast extract (YE), with its complex composition of amino acids and peptides, triggers stress-like responses, upregulating biosynthetic pathways without compromising plant viability (Nguyen and Tran *et al.,* 2019). For instance, 150 mg/L YE in culture medium increased phytochemical content in adventitious root cultures, highlighting its potential for scalable production (Nguyen *et al.,* 2024). However, the optimal concentration of YE for maximizing TPC, TFC, TSC, CAT, and POD activity remains under investigation, necessitating further research to standardize its application.

While various elicitors and conditions have been explored, this study focuses specifically on YE in Schenk and

Hildebrandt (SH) medium (Schenk and Hildebrandt, 1972) to enhance TPC, TFC, TSC, and antioxidative enzyme activities in *Panax vietnamensis*. Some studies have tested other environmental factors like LED lighting to stimulate phytochemical production, while others have optimized the SH medium with growth regulators and additives to support biomass growth (Nguyen *et al*., 2024). Yeast extract, however, was chosen due to its cost-effectiveness and efficacy. Trong *et al*. (2017) reported that YE at 150 mg/L, combined with casein hydrolysate, increased biomass by 1.8–2.6 times in cell suspension cultures, with elevated TPC and TFC levels.

This study aimed to evaluate the effectiveness of yeast extract in enhancing phytochemical accumulation and anti-oxidant enzyme activities (CAT and POD) in *P. vietnamensis in vitro* cultures and to identify its optimal concentration in SH medium. The effects of yeast extract on plant morphology, phytochemical content, and antioxidant responses were assessed to support the development of efficient and sustainable *in vitro* cultivation protocols.

MATERIALS AND METHODS

Materials

Plant material

In vitro 1-month-old *P. vietnamensis* Ha et Grushv, planted in SH medium (Duchefa, Haarlem, Netherlands) supplemented with hormones NAA 0.1 mg/L and BAP 1 mg/L (Sigma-Aldrich, USA), was used as material. All plants were grown at 24 ± 2°C throughout the experiment and exposed to a 16h/8h light/dark photoperiod in the culture room.

Methods

In vitro culture and elicitor treatment of P. vietnamensis Ha et Grushv

From the initial seedling flasks, the plants were subcultured in SH medium supplemented with hormones consistent with the original environment. After an additional one month of stabilization, the plants were grown in medium supplemented with 0.1 g/L YE (YE1) and 0.2 g/L YE (YE2) (Sigma-Aldrich, USA) and continued to culture for another one month. After one month, the plants were harvested for phytochemical and antioxidant enzyme activity analysis.

Antioxidant enzyme activities

Crude protein/enzyme extraction was performed according to the protocol of Chen and Zhang (2016). The fresh sample was extracted in Phosphate-Buffered Saline (PBS) buffer (pH 7.8) (Thermo Fisher Scientific, USA) and stored at 4°C to maintain enzyme activity.

The POD and CAT activity assays were conducted following Chen and Zhang's protocol (2016). A reaction buffer contained 0.2% guaiacol (Acros Organics, USA), 100 mM PBS (pH 7.0), and 30% H_2O_2 (Merck, Germany) (for POD), or without 0.2% guaiacol (for CAT). For the reaction solution, 50 µL of crude enzyme solution was combined with 1 mL of the reaction buffer. Absorbance of POD and CAT was measured every 15 seconds for 1 minute at 470 nm and 240 nm, respectively, using a SpectraMax® iD5 UV-Vis spectrophotometer (Molecular Devices, USA).

The enzyme activities were calculated as follows:

$$\text{CAT activity} = \Delta A240 * \frac{V}{Vt * 0.1 * t * Cp}$$

$$\text{POD activity} = \Delta A470 * \frac{V}{Vt * 0.01 * t * Cp}$$

Where:

ΔA470 and ΔA240 are the absorbance alterations measured at 470 nm and 240 nm for every 15 seconds over 1 minute;

V: crude enzyme solution total volume;

Vt: the amount of crude enzyme used in the testing tube;

t: reaction time;

Cp: the concentration of crude protein (mg/mL);

0.01 and 0.1: One unit of POD or CAT is defined as the amount of enzyme that increases 0.01 or 0.1, respectively, of absorbance at 470 nm or 240 nm per minute.

Phytochemical tests

Sample extraction

To prepare samples for phytochemical testing, plant materials were extracted using methanol, following the protocol of Le *et al.* (2024). The plant extracts were evaporated at 70°C for several days to eliminate all methanol. The obtained dry extract was used for phytochemical analysis.

Total phenolic content

TPC was determined following the method described by Pérez *et al.* (2023), with minor modifications. TPC was quantified by reaction with the Folin-Ciocalteu reagent (Sigma-Aldrich, Germany), resulting in the formation of a blue complex stabilized by sodium carbonate (Na_2CO_3; Xilong, China). A standard calibration curve was established using gallic acid (Merck, Germany) as the reference phenolic compound. For sample analysis, the extracted sample was mixed with 10% (v/v) Folin–Ciocalteu reagent (light-protected) and 6% (w/v) Na_2CO_3, and the mixture was incubated at 40 °C for 15 minutes. Absorbance was recorded at 765 nm against a reagent blank. The TPC was calculated using the gallic acid standard curve and expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract).

Total flavonoid content

The TFC was determined using the colorimetric method described by Bhaigyabati *et al*. (2014). This technique involves the formation of a colored complex with sodium nitrite ($NaNO_2$) (Xilong, China) and aluminum chloride ($AlCl_3$) (Xilong, China), stabilized by sodium hydroxide (NaOH) (Xilong, China) and ethanol (Xilong, China), with absorbance measured against a standard curve. Quercetin was used as the reference flavonoid compound. For analysis, the sample was mixed with 10% $NaNO_2$ and incubated at room temperature for 6 minutes.

Subsequently, 10% $AlCl_3$ was added, followed by another 6-minute incubation at room temperature. Then, 1M NaOH and 30% ethanol were added, and the mixture was incubated for 30 minutes at room temperature. Absorbance was measured at 510 nm against the blank. The TFC was quantified by comparing the absorbance of the sample to the quercetin standard curve. The results were calculated using the standard curve equation and expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g extract).

Total saponin content

TSC was determined following the method described by Khoang *et al.* (2022), with minor modifications. The technique involves a colorimetric assay using a reagent mixture of acetic acid and sulfuric acid (H_2SO_4) (Xilong, China) to form a colored complex. Aescin (Merck, Germany) was used as the reference saponin compound to establish a standard calibration curve. A reagent solution was prepared by mixing acetic acid and H_2SO_4 in a 1:1 ratio. For analysis, the sample was combined with the reagent mixture and incubated at 60°C for 30 minutes. Absorbance was measured at 527 nm. The TSC was quantified by comparing the absorbance of the sample to the aescin standard curve. The results were calculated using the standard curve equation and expressed as milligrams of aescin equivalent per gram of extract (mg AE/g extract).

Correlation

The linear relationship between parameters was analyzed using Microsoft Excel to show the Pearson correlation coefficient. The resulting correlation coefficients ranged from −1 to +1, indicating negative, no, or positive linear correlations, respectively.

Statistical analysis

Data were presented as mean ± standard deviation. All the experiments were biological triply replicated. The statistical analysis was performed using one-way ANOVA and the Tukey post-hoc test, with $p < 0.05$ considered a significant difference. The data was visualized using GraphPad Prism software.

RESULTS

Enzyme activities

The results of POD and CAT activities achieved via spectrophotometry are shown in Figure 1. YE treatment enhanced the POD and CAT activities compared to the control. POD activity remained relatively constant between control and YE1, but significantly increased approximately two times in samples treated with YE2 ($p < 0.001$), indicating an upregulation in response to the YE2 treatment. The POD reached approximately 3100 U/mg protein in the sample treated with 0.2 g/L YE. Conversely, CAT activity was highest in YE1, suggesting a strong induction of CAT in this condition, while control and YE2 showed much lower CAT levels. The highest CAT activity was reached at approximately 1000 U/mg protein in the sample treated with 0.1 mg/L YE. These contrasting enzyme activity profiles suggest a differential oxidative stress response induced by YE treatments, where YE2 prominently enhances POD-mediated defense mechanisms, while YE1 favors CAT activity, which may indicate that YE1 and YE2 trigger different signaling cascades or induce reactive oxygen species (ROS) at different intensities or cellular compart-ments, leading to enzyme-specific responses. While YE1 appears to promote a rapid CAT

response, potentially mitigating high bursts of H_2O_2, YE2 favors POD activation, which may contribute not only to ROS scavenging but also to secondary defense processes such as lignification. Together, these findings highlight a differential oxidative stress response to YE treatments, implying that plants fine-tune their antioxidant enzyme activities depending on the nature and strength of the elicitor.

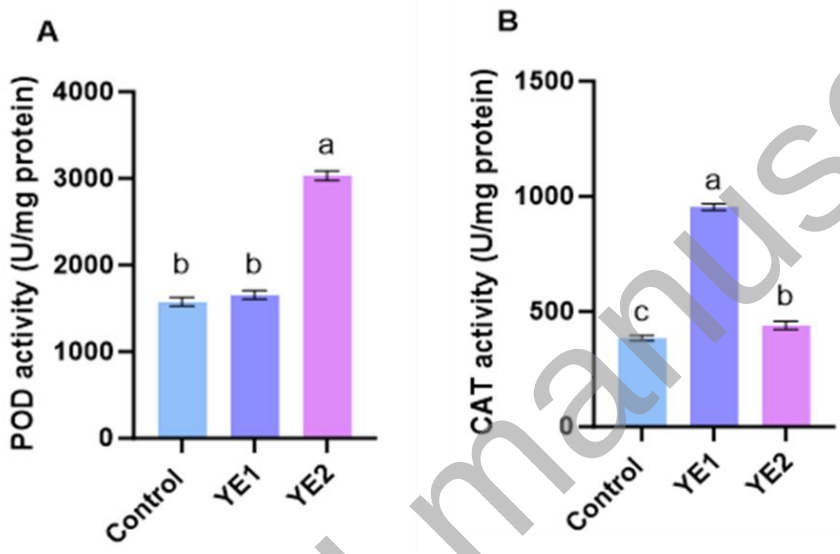

Figure 1. The effects of different yeast extract concentrations on the antioxidant enzyme activities of *P. vietnamensis* (A) Peroxidase activity, (B) Catalase activity. YE1: samples treated with 0.1 g/L yeast extract; YE2: samples treated with 0.2 g/L yeast extract. Different letters indicate significant differences with $p < 0.05$.

Phytochemical production

Total phenolic content

The TPC was presented in Figure 2. The TPC analysis revealed a specific dose-dependent elicitation. The control sample registered a TPC of around 15 mg ± 0.16 GAE/g extract. The YE1 samples showed a dramatic increase, reaching approximately 25 ± 0.29 mg GAE/g extract, representing the highest TPC among all samples. Conversely, the TPC in the YE2 samples (0.2 g/L YE) decreased to about 16 mg ± 0.31 GAE/g extract. The statistical differences confirm that 0.1 g/L YE statistically enhanced phenolic synthesis, while a higher concentration (0.2 g/L) did not confer the same benefit, but still produced higher TPC than the control, indicating a precise concentration window for this specific elicitation.

Total flavonoid content

TFC was presented in Figure 3. The TFC increased significantly with YE, with control plants at about 28 ± 1.247 mg QE/g extract. YE1 (0.1 g/L) and YE2 (0.2 g/L) reached approximately 57 ± 1.3 mg QE/g and 56 ± 0.39 mg QE/g, respectively, both significantly higher than the control ($p < 0.05$), but statistically similar to each other. This suggests both concentrations effectively boost flavonoid accumulation in *P. vietnamensis*.

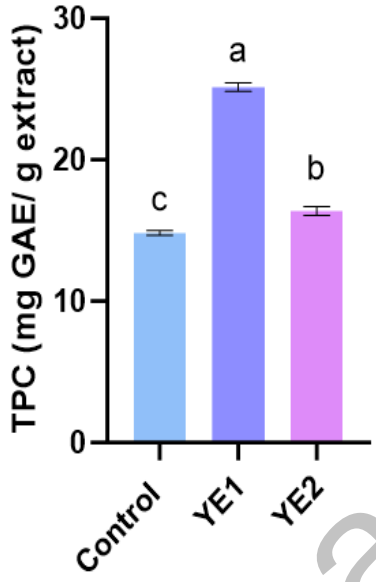

Figure 2. The effects of different yeast extract concentrations on the total phenolic content (TPC) of *P. vietnamensis*, measured as mg gallic acid equivalent (GAE) per g extract. YE1: samples treated with 0.1 g/L yeast extract; YE2: samples treated with 0.2 g/L yeast extract. Different letters indicate significant differences with $p < 0.05$.

Total saponin content

The study investigated the impact of YE elicitation on the TSC in *P. vietnamensis*. Figure 4 showed that YE addition significantly influenced total saponin content (TSC). The control samples exhibited a baseline TSC of approximately 350 ± 52 mg AE/g extract. Both YE treatments led to a notable increase in saponin accumulation. Specifically, the YE1 samples demonstrated the highest TSC, exceeding 617 ± 95.7 mg AE/g extract. The YE2 samples also showed an elevated TSC, around 534 ± 23.82 mg AE/g extract, which was significantly higher than the control but slightly lower than the YE1 samples. The presence of statistically significant differences ($p < 0.05$) between the samples confirms that both YE concentrations significantly enhanced saponin production compared to the control, with YE1 generally showing a superior or comparable effect to YE2. This indicates that YE at these concentrations acts as an effective elicitor for the biosynthesis of saponins, the bioactive compounds in *P. vietnamensis*.

Correlations

The correlation matrix revealed significant relationships among the biochemical parameters in *P. vietnamensis*, as described in Figure 5, offering insights into co-regulation. There were very strong positive correlations among the major secondary metabolites: TSC with TFC ($r = 0.96$) and TSC with TPC ($r = 0.83$). TPC and TFC also showed a strong positive correlation ($r = 0.63$). These strong positive associations suggest that elicitation strategies effectively promote the simultaneous accumulation of these diverse medicinal compounds, likely through interconnected biosynthetic pathways or shared physiological responses.

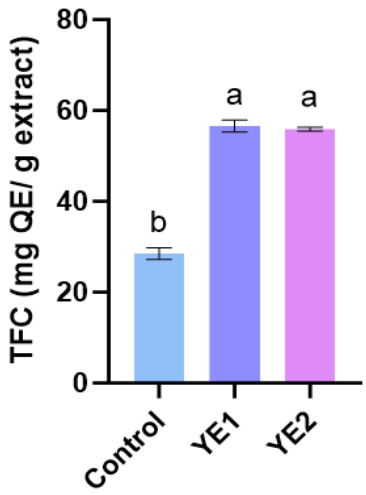

Figure 3. The effects of different yeast extract concentrations on the total flavonoid content (TFC) of *P. vietnamensis*, measured as mg quercetin equivalent (QE) per g extract, YE1: samples treated with 0.1 g/L yeast extract; YE2: samples treated with 0.2 g/L yeast extract. Different letters indicate significant differences with $p < 0.05$.

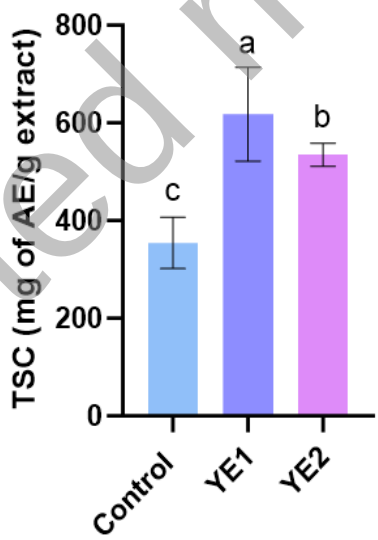

Figure 4. The effects of different yeast extract concentrations on the total saponin content (TSC) of *P. vietnamensis*, measured as mg aescin equivalent (AE) per g extract, YE1: samples treated with 0.1 g/L yeast extract; YE2: samples treated with 0.2 g/L yeast extract. Different letters indicate significant differences with $p < 0.05$.

Furthermore, CAT activity exhibits a remarkably strong positive correlation with TPC ($r = 0.99$) and a strong positive correlation with TSC ($r = 0.76$), indicating a close link between enhanced antioxidant defense and secondary metabolite production. Conversely, POD activity shows a moderate negative correlation with CAT ($r = -0.52$) and TPC ($r = -0.42$), but a moderate positive correlation with TFC ($r = 0.44$),

implying distinct or complementary roles for these antioxidant enzymes. Overall, the matrix underscores a synergistic upregulation of key bioactive compounds and a coordinated antioxidant response in *P. vietnamensis*, providing valuable information for optimizing its biotechnological cultivation.

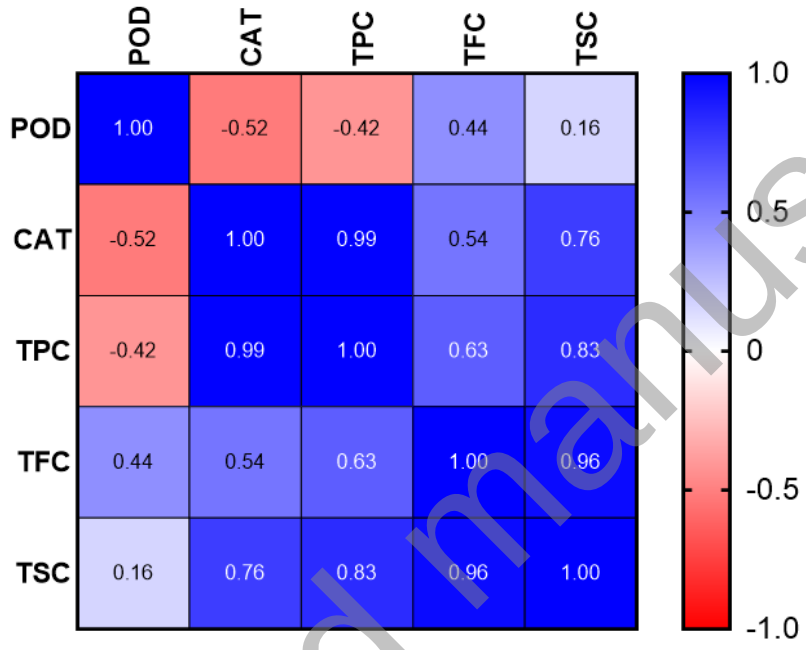

Figure 5. The correlation matrix displays the Pearson correlation coefficients (r-values) between five key biochemical parameters measured in *P. vietnamensis* plants: peroxidase (POD), catalase (CAT), total phenolic content (TPC), total flavonoid content (TFC), and total saponin content (TSC). The values range from -1 (strong negative correlation) to +1 (strong positive correlation), with 0 indicating no linear correlation.

DISCUSSION

The selection of the SH medium for *in vitro* culture

Another study shows that SH medium performs better than Murashige and Skoog (MS) medium in several key growth parameters. After 12 weeks of culture, plants grown on SH medium produced more leaves (3.73 vs. 2.93), more roots (2.73 vs. 2.47), and higher chlorophyll content (1.63 vs. 1.50 nmol/cm²). In addition, SH medium reduced undesirable callus formation (Pham *et al*., 2024). The medium's lower concentrations of ammonium (NH_4^+) and nitrate (NO_3^-) ions reduce pH fluctuations that can stress plantlets, unlike MS medium's higher nitrogen content, which may disrupt metabolic processes and damage sensitive cultures (Duong *et al*., 2013). When combined with YE (150 mg/L), SH medium enhanced phytochemical yields by mimicking stress-induced biosynthetic pathways, as evidenced by increased TPC and TFC in cell suspension cultures (Trong *et al*., 2017). These attributes make SH medium ideal for sustainable cultivation, ensuring high biomass, reliable phytochemical production, and effective

propagation of *P. vietnamensis* to meet medicinal demand while conserving this endangered species. Therefore, in our study, the SH medium was chosen for growing *P. vietnamensis*. During the subculture and growth time, the plants grew healthy, as the medium was proven to be suitable for this specific plant.

The selection of the yeast extract elicitor and the concentrations

In our study, YE at the concentrations of 0.1 and 0.2 g/L was chosen to analyze the effects on the *P. vietnamensis* plant. According to Trong *et al.* (2017), YE supplementation in cell suspension cultures of *P. vietnamensis* increased biomass by 1.4 to 2.4 times, with the best results observed at 1.0 g/L, though lower concentrations, such as 0.1 and 0.2 g/L, also promoted substantial growth. This enhancement is attributed to YE acting as an elicitor, providing essential nutrients and organic compounds that stimulate cell division and metabolic activity. Furthermore, Nguyen *et al.* (2019) demonstrated that elicitation with YE at 150 mg/L in adventitious root cultures maximized root biomass and TSC, including key ginsenosides such as MR2, Rg1, and Rb1. The elicitor effect of YE likely triggers stress responses in plant cells, upregulating biosynthetic pathways for secondary metabolites, which are critical for the medicinal value of *P. vietnamensis*. These findings underscore the efficacy of low concentrations (0.1-0.2 g/L) of YE in optimizing bioactive compound production, making it a cost-effective and efficient strategy for large-scale *in vitro* cultivation of this valuable medicinal plant. Indeed, as consistently demonstrated, YE produces notable effects on the components being studied.

Yeast extract enhances antioxidant enzyme activities

According to the research results (Figure 1), the impact of YE on antioxidant activities is significant, consistent with other studies on YE in various plant species (Elshahawy *et al*., 2022). Enzyme activity analysis revealed distinct responses in POD and CAT activities. POD activity remained stable between the control and YE1 but significantly increased in YE2 ($p < 0.05$), indicating that the higher elicitor concentration (0.2 g/L) induces a stronger oxidative stress response, likely activating POD-mediated defense mechanisms such as ROS neutralization or cell wall lignification, as described in Vijayalakshmi & Shourie (2019). In contrast, CAT activity peaked in YE1, with much lower levels in the control and YE2, suggesting that 0.1 g/L tends to trigger CAT-mediated hydrogen peroxide (H_2O_2) detoxification. These contrasting profiles indicate differential stress responses, with YE1 favoring CAT-driven ROS scavenging and YE2 prioritizing POD-mediated defenses. This suggests that the two yeast extract concentrations activate distinct signaling pathways, with YE1 inducing a balanced stress response and YE2 triggering a more intense defense mechanism, possibly linked to secondary metabolite production or structural reinforcement.

Yeast extract enhances TPC, TFC, and TSC production

From Figures 2, 3, and 4, phytochemical analysis showed significant changes in secondary metabolite accumulation in the plant grown in medium supplemented with YE. TPC increased dramatically in YE1 to approximately 25 mg GAE/g extract, compared to 15 mg GAE/g in the control

(Figure 2), indicating that 0.1 g/L is preferable for phenolic biosynthesis. This pattern is strongly supported by Petrova *et al.* (2025), who found that yeast extract at 100 mg/L (0.1 g/L) significantly promoted TPC in *Arnica montana* shoots. However, TPC in YE2 (16 mg GAE/g) was nearly similar to the control, suggesting that the higher concentration exceeds the proper elicitation threshold, possibly causing feedback inhibition or metabolic redirection. This result is also supported by Ali *et al.* (2018). TFC increased significantly in both YE1 (about 57 mg QE/g) and YE2 (about 56 mg QE/g) compared to the control (about 28 mg QE/g) (Figure 3), with no significant difference between the two treatments, indicating that flavonoid biosynthesis is less sensitive to elicitor concentration and reaches a saturation point at 0.1 g/L. In addition, it has been found that treatment with 100 mg/L YE increased TPC levels and consistently enhanced phenol and flavonoid accumulation in various plant cultures, including *Aster scaber, Ocimum Basilicum L., Salvia virgata, Thymus lotocephalus,* and *Knautia sarajevensis* (Petrova *et al*., 2025). TSC, the primary bioactive compound in *P. vietnamensis*, also increased significantly, with YE1 exceeding 600 mg AE/g extract and YE2 reaching approximately 530 mg AE/g, both higher than the control (approximately 350 mg AE/g) (Figure 4). YE1 showed a superior effect, suggesting that 0.1 g/L properly activates saponin biosynthetic pathways, while YE2 may divert resources to other stress responses. However, in Nguyen *et al.'s* (2019) research on different medium content and culture time, the concentration of 0.15 g/L YE was proven to be more efficient in promoting saponin amount. Therefore, further studies on more concentrations in the range of 0.1-0.2 g/L YE should be conducted.

Correlation between the parameters

Correlation analysis revealed in Figure 5 exhibits strong positive relationships among secondary metabolites, with TSC and TFC showing a near-perfect correlation (r = 0.96), suggesting that the biosynthetic pathways for TSC and TFC in *P. vietnamensis* are tightly co-regulated, likely through shared stress-responsive signaling mechanisms triggered by YE elicitation. This correlation (r = 0.96) indicates that conditions enhancing saponin production, such as the activation of the mevalonate or methylerythritol phosphate pathways, also strongly promote flavonoid biosynthesis via the phenylpropanoid pathway. This correlation is also observed in Vittaya *et al.*'s (2022) research, with the r value between TSC and TFC being pretty high. TSC and TPC correlated strongly (r = 0.83), as expected in many other studies (Amooaghaie and Rajaie, 2025; Sardar *et al*., 2023; Desta *et al*., 2023). These associations suggest that yeast extract activates interconnected biosynthetic pathways, such as the phenylpropanoid pathway for phenolics and flavonoids and the mevalonate pathway for saponins, likely via shared stress signaling mechanisms like jasmonic acid or ROS. CAT activity exhibited a remarkably strong correlation with TPC (r = 0.99) and a strong correlation with TSC (r = 0.76), indicating that CAT supports secondary metabolite production by maintaining redox homeostasis, particularly in YE1, where TPC and TSC peaked. Conversely, POD activity showed a moderate negative correlation with CAT (r = -0.52) and TPC (r = -0.42) but a positive correlation with TFC (r = 0.44), suggesting that POD plays a complementary role, possibly in flavonoid stabilization or cell wall reinforcement, especially in YE2,

where POD activity was highest. In many studies, it has been suggested that TSC, TPC, and TFC are positively correlated with antioxidant activity, although with other plants and different antioxidant compounds (Vittaya *et al*., 2022).

The relationship between antioxidant enzymes and bioactive compounds highlights their roles in managing oxidative stress during elicitation. CAT's strong correlation with TPC and TSC suggests it facilitates secondary metabolite biosynthesis by detoxifying H_2O_2, preventing oxidative damage to biosynthetic enzymes. This is evident in YE1, where high CAT activity aligns with peak TPC and TSC. POD's negative correlation with TPC and increase in YE2 suggest it may prioritize stress defense (e.g., lignification) over phenolic accumulation at higher elicitor concentrations, potentially explaining the reduced TPC in YE2. The synergistic upregulation of TSC, TFC, and TPC in YE1 indicates that 0.1 g/L is the ideal concentration for enhancing the medicinal quality of *P. vietnamensis*.

Overall, our study first reveals a more nuanced and possibly concentration-specific activation of antioxidant enzymes, hinting at complex elicitor-plant interactions. Second, we identify a clear threshold beyond which elicitation does not improve or even suppresses certain phytochemicals - an important insight for elicitor dose optimization. Lastly, this study provides a more integrative biochemical picture with correlation data, which is valuable for understanding metabolic network responses.

CONCLUSION

The study emphasized the importance of yeast extract in the stimulation of secondary metabolite production and antioxidant enzyme activities. It also provides evidence of the strong correlation between some of these parameters, which gives more insight into the growth and development of an endemic plant in Vietnam. The bioactivity tests could be performed to examine the efficiency of metabolites produced by the *in vitro* plants grown in media with elicitors.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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