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Original Article

Effects of thermal processing on the bioactivity of *Panax vietnamensis* extract from Son La, Vietnam

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Abstract

Thermal processing is widely applied to improve the functional properties of ginseng through structural transformation of ginsenosides. This study evaluated the effects of controlled thermal treatment (75 °C in 70% ethanol) on the phytochemical composition and bioactivity of *Panax vietnamensis* Ha et Grushv. cultivated in Son La, Vietnam. The major saponins investigated in this study included majonoside R2 (ocotillol-type); the protopanaxatriol (PPT)-type ginsenosides Rg1, Re, and Rh1, along with notoginsenoside R1; and the protopanaxadiol (PPD)-type ginsenosides Rb1 and Rd. High-performance liquid chromatography analysis revealed that thermal treatment resulted in a significant reduction in key polar ginsenosides, specifically a 56.6% decrease in notoginsenoside R1 (from 0.99% to 0.43%) and a 35.9% decrease in ginsenoside Rg1 (from 3.15% to 2.02%), accompanied by the relative enrichment of less-polar derivatives formed through deglycosylation. Rd acted as an intermediate in the conversion pathway, while the characteristic ocotillol-type saponin Majonoside R2 showed notable thermal stability with partial structural modification. These compositional changes were strongly linked to enhanced antioxidant activity, demonstrated by reduced DPPH IC_{50} values after processing. The results demonstrate that integrated hydrothermal extraction, which replicates the traditional practices currently used by ginseng farmers, effectively modulates the polarity balance of the saponin matrix and enhances the biofunctional quality of *P. vietnamensis*. This approach provides a practical, scientifically validated framework for developing value-added ginseng products through a thermal process that significantly improves antioxidant potential.

Keywords: Antioxidant, Bio-activity, Ginsenoside transformation, Ocotillo-type saponins, *Panax vietnamensis,* Thermal processing

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Introduction

Panax vietnamensis Ha and Grushv., (Vietnamese ginseng), is one of the most important medicinal plants in East Asian traditional medicine, widely valued for its adaptogenic, immunomodulatory, and anti-ageing properties (Attele et al., 1999), which is a rare and endemic species recognized for its unique saponin profile, particularly its high content of ocotillol-type saponins such as majonoside R2 (Nguyen et al., 1993; Shin et al., 2000). The pharmacological effects of ginseng are primarily attributed to ginsenosides, a diverse group of triterpenoid saponins whose composition and abundance vary according to species, age, cultivation conditions, and post-harvest processing (Christensen, 2009; Qi et al., 2011).

Thermal processing has long been employed to enhance the medicinal value of ginseng, as exemplified by the transformation of fresh ginseng into red or black ginseng through repeated steaming and drying cycles (Radad et al., 2006; Huang et al., 2023). Heat treatment induces structural modifications of ginsenosides, including deglycosylation, dehydration, and isomerization, leading to the formation of less-polar compounds which are associated with enhanced antioxidant, anti-inflammatory, anticancer, and neuroprotective effects (Hwang et al., 2014; Liu et al., 2020). While extensive studies have investigated the effects of thermal processing on *P. ginseng* and *P. notoginseng* (Wang et al., 2012; Qui et al., 2018; Ye et al., 2023), systematic research on *P. vietnamensis* remains limited. To date, investigations into this species have primarily focused on the dynamic changes of saponins during steaming processes, where it has been observed that polar ginsenosides (such as Rb1, Rc, Rd, Re...) rapidly decrease while less-polar "rare" ginsenosides (such as Rg3, Rg1 and Rh) increase. While systematic research on *Panax* species has documented the structural changes induced by dry steaming and drying cycles, studies on *P. vietnamensis* remain limited and primarily focused on these traditional steaming methods (Hwang et al., 2014). A significant research gap exists regarding the chemical transformations occurring during solvent-based thermal processing. This gap is particularly notable given that hydrothermal processing in solvents is the predominant practice employed by ginseng farmers for the practical recovery of concentrates and the production of medicinal beverages (Minh-Tu, 2025). Surveys of traditional cultivation in regions like Sam Ta, Son La, reveal that these non-mechanized methods are central to local value-added production. By evaluating ethanol-based thermal treatments that mimic these real-world farming practices, this study provides the first scientific evidence on how such conditions rebalance the unique ocotillol and dammarane saponin profiles to optimize the therapeutic potential of Vietnamese ginseng. Given its distinct chemical composition, particularly the presence of majonoside R2 and related ocotillol-type saponins, the response of *P. vietnamensis* to thermal treatment may differ significantly from that of other *Panax* species (Le et al., 2014; Minh-Tu, 2025). Remarkable results from studies on steamed Vietnamese ginseng reveal that while PPD and PPT-type saponins undergo significant degradation, their characteristic ocotillol-type saponins, specifically Majonoside R2 (MR2), which constitutes over 5% of the plant's dry weight, are relatively stable during steaming. Furthermore, steamed *P. vietnamensis* has demonstrated significantly enhanced antiproliferative activity against A549 lung cancer cells and a continuous increase in free radical scavenging activity, which is closely associated with the accumulation of transformed ginsenosides (Le et al., 2014).

Currently in Vietnam, processed ginseng products are diverse and abundant, including dried sliced ginseng, ginseng concentrates, ginseng beverages, and ginseng powders. In addition, *P. vietnamensis* has been successfully transplanted to Son La province, yet there are no studies reporting products harvested from this region. Therefore, this study aims to evaluate the effects of ethanol-based thermal processing mimic to real condition of ginsengfarmers on the ginsenoside composition of *P. vietnamensis* collected from Sam Ta, Son La province, with particular emphasis on changes in major saponins (R1, Rg1, Re, MR2, Rb1, Rd and Rh1) (Minh-Tu, 2025). The results provide insights into the relationship between processing conditions and phytochemical changes, with implications for bioactivity and the scientific basis for applying processing technologies in health-supporting foods.

Material and Methods

Plant material

Rhizomes and leaves of *Panax vietnamensis* Ha and Grushv. aged 6 years of the mature growtth stage were collected from Sam Ta village, a highland area of Son La province, Vietnam (altitude ~ 1,700 m) in May

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2025, coinciding with the active vegetative and flowering phase of the plant's seasonal cycle. The cultivar has been officially certified by the Ministry of Agriculture and Rural Development of Vietnam, with a plant variety protection period of 20 years from 2022. Voucher specimens (KTTP_SAM 052025) were deposited at Hanoi University of Science and Technology, Vietnam. Samples were dried using a freeze dryer, then ground using a hammer mill.

Prior research has demonstrated that thermal processing alters ginsenoside profiles, transforming major polar ginsenosides into less-polar forms with enhanced biological activities (Le et al., 2014; Hwang et al., 2014). Solvent and temperature combinations, including heat with ethanol, have been reported to influence the type and yield of transformed ginsenosides (Jang et al., 2017). Thermal processing by ethanol extraction at various temperatures for durations of 6, 8, 10, and 12 hours was conducted to investigate the transformation of major ginsenosides and enhancement of bioactivity. To evaluate the simultaneous recovery and structural conversion of saponins, thermal processing and extraction were integrated into a single hydrothermal step, mimicking the traditional value-added production methods used by ginseng farmers. Approximately 20 g of dried ginseng rhizomes or leaves' powders were subjected to 70 ^{o}C/boiling with 1000 mL of ethanol in a 2L flask for extraction, the extracts were recovered by vacuum evaporator under 40 ^{o}C (Jang et al., 2017; Minh-Tu, 2025). While this integrated design provides a holistic representation of the chemical identity found in traditionally processed ginseng concentrates, it carries the methodological limitation of preventing the isolated measurement of extraction rates versus thermal effect.

Total saponin determination

The total saponin content of the extracts was determined using the vanillin-sulfuric acid colorimetric method. Briefly, an aliquot of the extract was mixed with a 5% (w/v) vanillin-ethanol solution, followed by the addition of concentrated sulfuric acid. The mixture was heated to facilitate a chemical reaction that induces the formation of a characteristic purple-red complex in the strong acid medium. After cooling to room temperature, the absorbance of the solution was measured at a wavelength of 544 nm using a spectrophotometer. The total saponin concentration was calculated based on a standard calibration curve (using ginsenoside Re) (Le et al., 2014).

Total polyphenol content determination

The total polyphenol content (TPC) of the *Panax* extracts was determined using the Folin-Ciocalteu colorimetric method as described by Hwang et al. (2014). Briefly, a 0.1 mL aliquot of the appropriately diluted extract was mixed with 2 mL of 2% Na_2CO_3 and 0.1 mL of 50% Folin-Ciocalteu phenol reagent in a test tube. After the mixture was allowed to react for exactly 30 minutes, the absorbance was measured at 750 nm using a spectrophotometer. The concentration was calculated based on a calibration curve prepared using gallic acid as a standard at concentrations ranging from 20 to 200 µg/mL. Results were expressed as milligrams of gallic acid equivalents per gram (mg GAE/g) of the sample. (Hwang et al., 2014).

HPLC analysis of ginsenosides

Ginsenosides determination was analyzed at the National Institute of Medicinal Plant. High-performance liquid chromatography with a PDA detector at 196 nm was performed on a C18 column (150 × 4.6 mm, 3 µm). The mobile phase ran at 1.0 mL/min with a 10 µL injection. Standard ginsenosides (R1, Rg1, Re, MR2, Rb1, Rd and Rh1) were dissolved in methanol. Samples were extracted in n-butanol and methanol, filtering through a 0.22 µm filter before injection. All analytical procedures were conducted in accordance with established pharmacopoeia protocols to ensure the accuracy of the representative data with the linearity range of R^2>0.999; LOD of 0.1- 0.5 µg/mL; LOQ of 0.3 -1.5 µg/mL and recovery of 95-102%. (Jang et al., 2017; Minh-Tu, 2025).

DPPH radical scavenging assay

The antioxidant activity of the samples was determined by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay following the method described by Hwang et al. (2014) with slight modification. The radical scavenging activity was calculated as (1-B/A) × 100, where A represents the absorbance of the control (DPPH solution without the extract) and B represents the absorbance of the test sample (DPPH solution containing the extract). IC_{50} (inhibitory concentration, 50 %) values were also calculated.

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Griess assay – Nitric oxide (NO) production

Nitric oxide (NO) production was assessed in LPS-stimulated RAW 264.7 macrophages using the Griess reaction. Cells were seeded in 96-well plates, allowed to adhere overnight, and then treated with LPS together with samples for 24 hours. After incubation, culture supernatants were collected and reacted sequentially with sulfanilamide and N-(1-naphthyl) ethylenediamine solutions. Absorbance was recorded at 540 nm, and nitrite levels were quantified using a sodium nitrite standard curve. Inhibition of NO production was expressed as a percentage decrease relative to LPS control, following the method described by Dat et al. (2019).

Evaluation of ginsenoside transformation

To evaluate the efficiency of thermal processing, induced ginsenoside transformation, transformation yields were calculated based on changes in the concentrations of precursor (polar) and product (less-polar) ginsenosides before and after treatment. Transformation was evaluated using diagnostic ratios given in Table 1.

Table-1. Ratios of ginsenosides for processing efficiency.

Ratio Type	Biomarkers involved	Significance
Onset of thermal degradation (Le et al. (2014))	R1/(Rg1+Re)	Indicates the transformation of protopanaxatriol (PPT)-type saponins.
Intermediate processing (Yao et al. (2021))	Rd/Rb1	Indicates the start of PPD-type deglycosylation.
Advanced processing (Yao et al. (2021))	Rg3/Rd	A high ratio indicates the accumulation of potent "rare" saponins.
PPT-type efficiency (Le et al. (2014))	Rh1/(Rg1+Re)	Measures the transformation of the major PPT components.
Vietnamensis specific (Le et al. (2014))	MR2/R1	Monitors the transformation of the unique ocotillol-type core.

Statistical analysis

Experiments of chemical profile and bioassay were conducted in triplicate, and results are expressed as mean ± standard deviation. Statistical differences among treatments were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's test. Differences were considered significant at $p < 0.05$.

Results and Discussion

Screening on saponin and polyphenol content of *P. vietnamensis's* extract

The dynamic changes in total saponin content and polyphenol content under varying extraction and thermal conditions were investigated with ethanol concentrations of 70%, 80%, and 96%, which are the most effective solvents for balancing the extraction of polar ginsenoside precursors and the less-polar derivatives generated during processing (Table 2). The temperatures of 70°C and boiling were selected to investigate the threshold for thermal transformation. While moderate heat facilitates extraction, boiling provides the activation energy necessary to catalyze the deglycosylation and dehydration reactions that convert common ginsenosides into more bioactive, less-polar "rare" forms.

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Table-2. Effect of thermal-solvent conditions on the phytochemical profiles of *P. vietnamensis* rhizome extract.

No.	Ethanol concentration (%)	Temperature (°C)	Time (h)	Total saponins (mg/g) (UV-Vis)	Total phenolic content (mg GAE/g)
1	70	70	6	102.20^{a}±3.93	3.65^{a}±0.30
2			8	105.45^{b}±4.34	2.85^{b}±0.25
3			10	107.60^{c}±4.55	1.95^{c}±0.15
4			12	106.68^{c}±4.10	1.15^{d}±0.10
5	80	70	6	108.50^{a}±3.15	3.25^{a}±0.30
6			8	111.20^{b}±4.55	2.95^{b}±0.22
7			10	113.40^{c}±4.80	2.45^{c}±0.20
8			12	112.85^{c}±4.25	2.15^{d}±0.25
9		boiling	6	104.30^{a}±3.77	3.05^{a}±0.20
10			8	110.15^{b}±3.29	2.15^{b}±0.18
11			10	115.80^{c}±3.17	1.65^{c}±0.12
12			12	113.22^{d}±2.91	1.25^{d}±0.15
13	96	70	6	105.80^{a}±3.17	3.05^{a}±0.20
14			8	108.45^{b}±4.61	2.15^{b}±0.15
15			10	110.10^{c}±4.10	1.45^{c}±0.12
16			12	108.70^{d}±4.40	0.85^{d}±0.09
17		boiling	6	98.70^{a}±3.71	2.05^{a}±0.18
18			8	102.40^{b}±4.12	1.35^{b}±0.15
19			10	105.30^{c}±4.60	0.95^{c}±0.10
20			12	103.20^{d}±5.20	0.55^{d}±0.08

Different letters within the same independent variable in the same condition of extraction not limited to duration, indicate statistical differences at a 95% confidence.

The results presented in the table demonstrate that thermal processing and solvent concentration significantly influence the phytochemical profile of the *P. vietnamensis* extract. The total saponin content follows a distinct upward trend as temperature and duration increase. Reaching a peak of 115.80±3.17 mg/g at 10 hours under boiling conditions with 80% ethanol. The crude extraction yield, representing the total mass of the recovered extract relative to the starting dry material at the defined processing condition (80% ethanol boiling for 10 hours) was 28,75 % (w/w).

Saponin dynamics during thermal treatment

To investigate the dynamic of saponin, the thermal treatment focused on the 8-to-10-hour thermal window using 80% ethanol to capture the peak accumulation of less polar ginsenoside while ensuring the stability of Majonoside R2 (MR2). Leaves are included because, in practical and traditional applications, leaves are frequently processed together with rhizomes (Lee et al., 2021).

The results in Table 3 demonstrate that in rhizomes, the total saponin content gradually increased during thermal processing, rising from 12.04% to 12.56% after 10 hours of boiling. This trend is largely attributed to the remarkable stability of Majonoside R2 (MR2), the characteristic ocotillol-type saponin of *P. vietnamensis*, which remained consistently high at approximately 7.3% (Figure 1). The stability of MR2 can be explained by the absence of a heat-labile glycosidic linkage at the C-20 position, making it less susceptible to thermal hydrolysis compared with other ginsenosides (Le et al., 2014; Nguyen et al., 2024).

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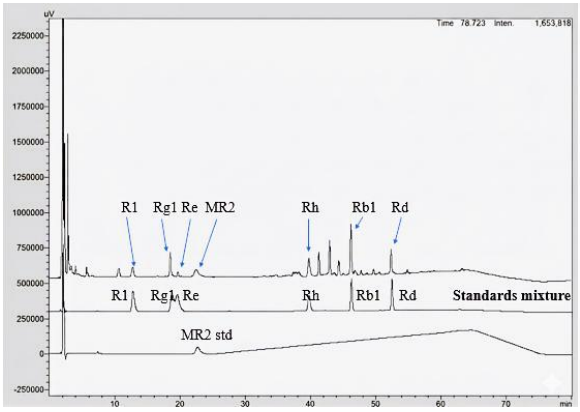
Table-3. Dynamic changes in individual ginsenosides of *Panax vietnamensis* rhizomes and leaves.

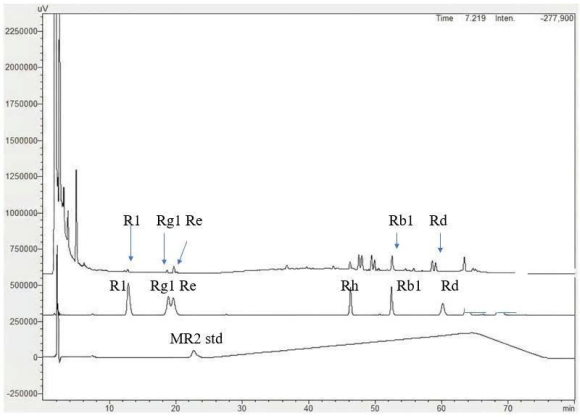
Saponin ginsenoside (%w/dw)	**Polarity**	***P. vietnamensis's* rhizomes**			***P. vietnamensis's* leaves**		
		Fresh**	80% EtOH- boiling		Fresh	80% EtOH- boiling	
			8 h	10 h		8 h	10 h
Protopanaxatriol (PPT. $C_{47}H_{80}O_{18}$)							
Notoginsenoside R1	↓ decrease	0.99	0.62	0.43	0.05	0.03	0.02
Ginsenoside Rg1		3.15	2.45	2.02	0.08	0.04	0.02
Ginsenoside Re		0.01	0.65	1.29	0.12	0.19	0.26
Protopanaxadiol (PPD)							
Ginsenoside **Rb1**	↓ decrease	0.30	0.58	0.86	0.21	0.31	0.42
Ginsenoside **Rh1**		0.1	0.06	0.03	0.04	0.02	Nd*
Ginsenoside **Rd**		0.85	0.71	0.59	0.92	0.88	0.84
Ocotillol							
Majonoside R2		7.27	7.31	7.34	Nd	Nd	Nd
Total		**12.04**	**12.38**	**12.56**	**1.42**	**1.47**	**0.72**

*nd: not detected.

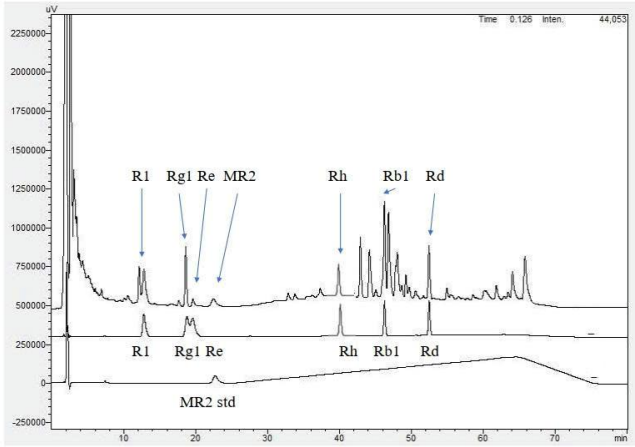
**ultrasonic extraction in a water-shake bath at a frequency of about 40 Hz in MeOH for approximately 2 hours, 50 °C.



Fresh rhizome



Fresh leaf



Rhizome extract at 10 hours

Figure-1. HPLC chromatograms of rhizome and leaf samples.

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In contrast, distinct transformation patterns were observed among the dammarane-type saponins. The protopanaxatriol (PPT) group, represented by Notoginsenoside R1 and Ginsenoside Rg1, showed a gradual decline during heating, consistent with previous reports indicating that PPT-type ginsenosides are relatively less stable under thermal conditions. Conversely, Ginsenoside Rb1 and Ginsenoside Re exhibited an increase in concentration in rhizomes during the early stages of processing.

In contrast to rhizomes, the leaves showed a substantially lower initial concentration of total saponins (1.42%) and lacked the presence of MR2, highlighting a clear phytochemical distinction between aerial and underground parts of the plant. A slight increase in total saponin content was observed after 8 hours of processing (1.47%), suggesting that moderate heating may initially facilitate the release or conversion of bound saponins. However, prolonged heating resulted in a sharp decline in total saponins, reaching 0.72% after 10 hours. This reduction indicates that leaf-derived saponins may be more susceptible to thermal degradation, possibly due to the cleavage of glycosidic bonds and dehydration reactions occurring at the C-3 and C-20 positions under extended boiling conditions (Hwang et al., 2014; Yao et al., 2021). Nevertheless, the increase in Ginsenoside Rb1 in leaves (from 0.21% to 0.42%) reflects a transformation pattern as that observed in rhizomes, suggesting that thermal processing can promote the conversion of precursor saponins into more stable forms even in leaf extracts. Overall, these findings confirm that rhizomes remain the primary source of stable and abundant saponins, particularly MR2, while leaves may still represent a supplementary source of selected ginsenosides if processed under optimized conditions.

Ginsenosides ratio and antioxidant and inflammatory activities relation

Table 4 presents a comparative analysis of the ginsenosides' ratio and bioactivity profiles in *P. vietnamensis* rhizomes and leaves subjected to 80% ethanol boiling for 8 and 10 hours. By tracking specific indicators, such as the thermal stability of the species-specific marker Majonoside R2 and the deglycosylation of polar precursors (Rd/Rb1), the data illustrates how thermal processing redistributes the chemical identity of both plant parts to enhance antioxidant and anti-inflammatory potencies. Furthermore, the inclusion of leaves highlights their value, which exhibits significant chemical sensitivity and therapeutic potential when processed alongside traditional rhizomes.

Table-4. Ginsenoside profile variation in rhizomes and leaves of *P. vietnamensis* under thermal processing.

Ratio type/Bioactivity	***P. vietnamensis's* rhizomes**			***P. vietnamensis's* leaves**		
	Fresh	80% EtOH boiling		Fresh	80% EtOH boiling	
		8 h	10 h		8 h	10 h
Onset of thermal degradation: R1/(Rg1+Re)	0.11	0.20	0.13	0.25	0.13	0.07
Intermediate thermal processing**:** Rd/Rb1	2.83	1.22	0.69	4.38	2.84	2.00
PPT-type efficiency transformation of the major PPT components: Rh1/(Rg1+Re)	0.03	0.02	0.01	0.20	0.09	Nd*
PPD vs. PPT precursor balance: Rb1/Rg1	0.10	0.24	0.43	2.63	7.75	21.00
Vietnamensis specific transformation of the unique ocotillol-type core: MR2/R1	20.19	11.79	17.07	Nd*	Nd*	Nd*
(R1, Rb1, Rd)	1.51	1.91	1.88	1.18	1.22	1.28
(Re, Rg1, Rh1)	3.26	3.16	3.34	0.24	0.25	0.28

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(R1, Rb1, Rd, Re, Rg1, Rh1, MR2)	12.04	12.38	12.56	1.42	1.47	0.72
Antioxidant DPPH IC50, μg/ml	1.12 ± 0.12	0.78± 0.09	0.61 ± 0.07	1.85 ± 0.18	1.45 ± 0.11	1.10 ± 0.10
NO production's inhibition, (Griess IC50, μg/ml)	58.34 ± 2.15	45.06± 1.13	36.80 ± 1.02	62.50 ± 3.40	56.20 ± 2.25	50.10 ± 1.80

In rhizomes, PPD/PPT precursor balance increased progressively from 0.10 (fresh) to 0.43 (10 hours with heat treatment), reflecting a relative enrichment of PPD-type saponins compared with highly polar PPT-type components. Simultaneously, the Rd/Rb1 ratio decreased (2.83 to 0.69), indicating dynamic transformation within the PPD pathway and a shift toward less polar derivatives. This compositional rearrangement corresponded with a significant enhancement in antioxidant capacity: DPPH IC_{50} decreased from 1.12 ± 0.12 μg/mL (fresh) to 0.61 ± 0.07 μg/mL (10 hours with heat treatment). A similar trend was observed for NO inhibition, where IC_{50} declined from 58.34 ± 2.15 to 36.80 ± 1.02 μg/mL. The inverse relationship between the Rb1/Rg1 ratio and IC_{50} values suggests that increasing the relative abundance of less polar PPD-type saponins contributes positively to bioactivity.

The R1/(Rg1+Re) ratio fluctuated but generally indicated partial degradation and rearrangement of primary PPT-type saponins during heating. Moreover, the Rh1/(Rg1+Re) ratio declined in rhizomes (0.03 to 0.01), suggesting that prolonged heating does not simply accumulate intermediate PPT derivatives but promotes further structural conversion.

The MR2/R1 ratio remained high in rhizomes (20.19 to 17.07), indicating preservation of the ocotillol-type core during processing. The stability of Majonoside R2 may play a synergistic role in maintaining strong antioxidant capacity, as the rhizome consistently exhibited superior bioactivity compared to leaves. In contrast, MR2 was not detected in leaves after processing, and although leaf extracts showed moderate improvement in DPPH and NO inhibition, their IC_{50} values remained higher than those of rhizomes. This difference reinforces the importance of the ocotillol-type component in the overall pharmacological potential of *P. vietnamensis*.

Thermal processing in 80% ethanol at boiling conditions markedly reshaped the saponin profile of *P. vietnamensis* rhizomes and leaves, promoting a transformation from more polar to relatively less polar constituents. Because ginsenoside polarity is largely determined by the number and position of sugar moieties attached to the dammarane skeleton. Heat-induced deglycosylation and structural rearrangement alter the balance between PPT-type (R1, Rg1, Re) and PPD-type (Rb1, Rd, Rh1) saponins. In rhizomes, the continuous increase in the Rb1/Rg1 ratio (0.10 to 0.43) and the decrease in Rd/Rb1 (2.83 to 0.69) indicate a redistribution within the PPD pathway and a relative enrichment of less polar components, while moderate changes in R1/(Rg1+Re) suggest concurrent transformation of PPT precursors. In leaves, the increase in Rb1/Rg1 was even more pronounced (2.63 to 21.00), confirming the higher thermal sensitivity of PPT-type saponins in this tissue. Notably, Majonoside R2 (MR2), the ocotillol-type saponin characteristic of *P. vietnamensis,* remained stable in rhizomes (MR2/R1: 20.19 to 17.07) but was not detected in leaves, highlighting both its tissue specificity and thermal resistance. These compositional shifts were accompanied by enhanced bioactivities, as reflected by reduced IC_{50} values for DPPH radical scavenging and NO inhibition in both rhizomes and leaves. The improvement in antioxidant and anti-inflammatory effects was particularly evident in rhizomes, suggesting a synergistic contribution of MR2 and the increased relative abundance of less polar PPD-type saponins. Overall, the data demonstrate that thermal processing enhances bioactivity primarily through polarity-driven rebalancing of the saponin matrix rather than simple concentration changes of individual compounds.

The thermal effect was observed from 70 ^{o}C to the boiling temperature of ethanol at durations of 6 to 12 hours which is sufficient to observe the transformation of ginsenoside (Jang et al., 2018; Yao et al., 2021). The increase of total saponin is driven by the extraction yield as the observation reported by Le et al. (2014) and Yao et al. (2021). However, the slight decline in saponin content observed after 12 hours (113.22±2.91 mg/g) indicates that prolonged exposure to heat eventually leads to thermal decomposition of the saponin structure (Hwang et al., 2014). Regarding the solvent, 80% ethanol proved more effective than 96% ethanol for maximizing total yield, as the presence of some water is often necessary to facilitate the

breakdown of the cell matrix and the diffusion of saponins (Hwang et al., 2014; Yao et al., 2021). Conversely, the total polyphenol shows a continuous decline as time and temperature increase (dropping from 3.65 to 1.25 mg GAE/g in the 80% ethanol) which suggests that while heat initially liberates bound phenolics extended boiling likely induces thermal oxidation or causes these compounds to be consumed in the advanced stages of the Maillard reaction alongside free amino acids which were determined in Son La's ginseng (Liu et al., 2020; Minh-Tu, 2025).

Importantly, these observations were obtained from *P. vietnamensis* cultivated in Son La, a highland region in northwestern Vietnam where this species has been successfully introduced and acclimatized outside its native habitat in the Ngoc Linh Mountain. Previous phytochemical studies have primarily focused on populations naturally growing in the Ngoc Linh area; therefore, the present results provide additional evidence that the transplanted population from Son La retains key chemical characteristics of the species, particularly the presence and thermal stability of the unique ocotillol-type saponin Majonoside R2 (Duc et al., 1994; Konoshima et al., 1998; Nguyen and Phuong, 2019).

The dynamic changes in ginsenoside ratios as seen in Table 4 further indicate that thermal processing promotes partial deglycosylation and structural conversion of dammarane-type saponins. The decrease in the PPT-related ratio R1/ (Rg1 + Re) together with the increase in PPD-related indicators such as the Rb1/Rd balance suggests that heat facilitates the conversion of polar ginsenosides into less-polar derivatives. Similar transformation pathways, including demalonylation and stepwise sugar cleavage at the C-20 or C-3 positions, have been widely reported during thermal processing of ginseng materials (Hwang et al., 2014; Yao et al., 2021; Zheng et al., 2017). These structural modifications are known to alter the biological properties of ginsenosides, as less-polar derivatives and certain PPD-type compounds often exhibit enhanced pharmacological activities compared with their precursor molecules (Tong et al., 2022). The bioactivity assays in this study also reflected these compositional modifications, as seen in Figure 2. Extracts subjected to controlled thermal treatment exhibited improved antioxidant activity, as indicated by the reduction in DPPH IC_{50} values, as well as enhanced nitric oxide inhibitory effects in comparison with fresh samples. Such improvements may result from both the enrichment of specific PPD-type ginsenosides and the formation of thermally generated metabolites with stronger biological potency, a phenomenon previously reported for heat-processed ginseng preparations (Kim et al., 2007; Hwang et al., 2014). Nevertheless, excessive heating may lead to degradation of heat-sensitive constituents, particularly phenolic compounds and certain PPT-type ginsenosides (Kim, 2022), emphasizing the importance of optimizing processing duration to balance transformation and preservation of active metabolites.

These results as in Figure 2 highlight that thermal processing serves not only as an extraction step but also as a key factor driving phytochemical transformation in *P. vietnamensis* grown in Son La. The observed stability of MR2 alongside the adaptive changes in dammarane-type ginsenosides suggests that this transplanted population preserves the distinctive chemical identity of Vietnamese ginseng while offering new insights into how environmental adaptation and processing conditions jointly influence its bioactive profile.

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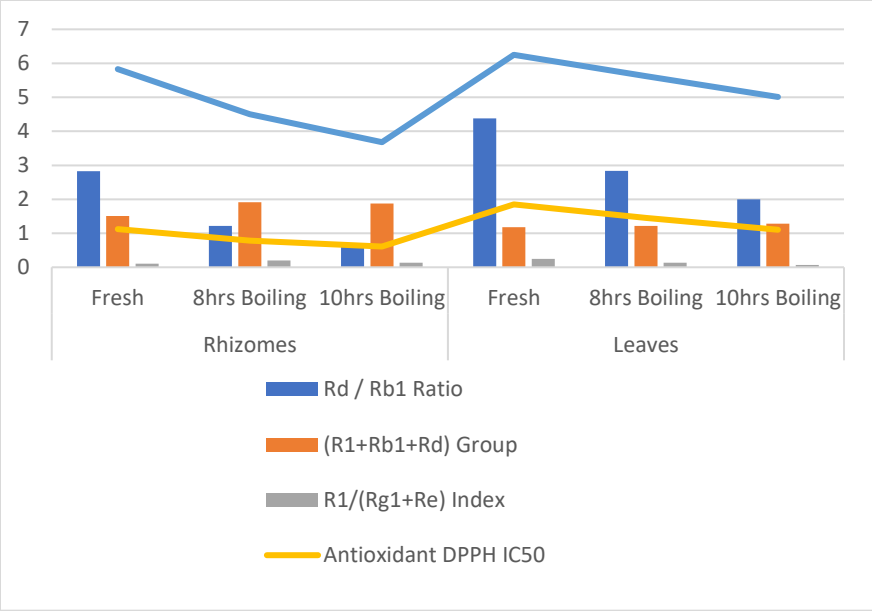

Figure-2. Phytochemical and bioactivities dynamic under thermal process.

Conclusion

This study demonstrates that thermal processing conditions markedly influence the phytochemical composition and bioactivity of extracts from *Panax vietnamensis*. Increasing extraction temperature and duration enhanced the release and transformation of saponins, with the highest yield obtained using 80% ethanol under boiling conditions for approximately 10 hours. In contrast, total polyphenol content declined progressively with increasing temperature and time, reflecting the heat sensitivity of this group. Detailed ginsenoside profiling further revealed distinct transformation patterns, where protopanaxatriol-type ginsenosides decreased while certain protopanaxadiol-type compounds increased during thermal processing, whereas the characteristic ocotillol-type saponin Majonoside R2 remained highly stable in rhizomes but was absent in leaves. These compositional changes were accompanied by shifts in diagnostic ginsenoside ratios and improvements in antioxidant and nitric oxide inhibitory activities, suggesting that controlled thermal treatment promotes the formation of more bioactive phytochemical profiles. Overall, the findings indicate that optimized hydrothermal extraction not only maximizes saponin recovery but also modulates the phytochemical balance and biological activity of Vietnamese ginseng.

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Contribution of Authors

Minh Tu NT: Designed experiments, collected data, wrote methodology, reviewed and edited the manuscript draft.

Le Hang HT: Collected, analysed and interpreted data and edited manuscript.

Ngoc Thu TT, Thao NT, Son VH & Huy NT: Supported in experiments and sample collection and edited the manuscript.

All authors read and approved the final draft of the manuscript.

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