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Case Report Paper

Pharmacological Properties and Standardization of Ngoc Linh Ginseng using Ultrasonic-Assisted Extraction

Trung Phung-Van[1], Nguyễn Ngọc Mai Thảo[1*], Nguyen Tan Thuong[1]

[1] *Faculty of Pharmaceutical Sciences, University of Danang. Da Nang, Viet Nam.*

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***Corresponding Author:**
Nguyễn Ngọc Mai Thảo

Abstract: This study aims to evaluate the pharmacological potential and standardize the bioactive compounds of Ngoc Linh Ginseng (*Panax vietnamensis*) as a candidate phytopharmaceutical. The study was conducted throughout 2024 using a multidisciplinary approach, including ultrasonic extraction, chemical analysis, and pharmacological tests. Samples were collected from the Kon Tum and Quang Nam mountains, Vietnam, then extracted by the Ultrasonic-Assisted Extraction (UAE) method using ethanol-water solvents. The total saponin content obtained was analyzed using High Performance Liquid Chromatography (HPLC), resulting in a level of 28.5% with the identification of the main ginsenosides Rg1 and Re. Pharmacological activity was tested in vitro on LPS-induced RAW 264.7 cells, showing a decrease in pro-inflammatory cytokine production by up to 50% and strong antioxidant activity with an IC50 value of 25 µg/mL. In vivo tests on Wistar rats showed a hepatoprotective effect against liver damage caused by a high-fat diet. These results support the potential of Ngoc Linh Ginseng as a candidate for plant-based therapy. Further studies are recommended to explore the pharmacokinetics, clinical efficacy, and optimization of extraction techniques to expand its therapeutic applications.

Keywords: Ginsenosides, Hepatoprotective, Inflammation, Ngoc Linh Ginseng, Phytopharmaceutics.

1. Introduction

Vietnam is renowned for its extraordinary biodiversity, with many endemic plant species found in its diverse ecosystems. Among them, Ngoc Linh Ginseng (*Panax vietnamensis*) holds a unique place due to its high medicinal value and traditional uses [1]. Discovered in the 1970s in the Ngoc Linh mountains between Kon Tum and Quang Nam provinces, the plant is now considered a national treasure in Vietnam and a symbol of its rich botanical heritage [2].

Ngoc Linh Ginseng is classified as a member of the Panax genus, which includes globally recognized medicinal ginsengs such as Korean Ginseng (Panax ginseng) and American Ginseng (Panax quinquefolius). However, the Ngoc Linh variety is distinguished by its extremely high saponin content, surpassing other ginseng species. These saponins, known as dammarane-type triterpenoid saponins, exhibit potent pharmacological activities, including antioxidant, anti-inflammatory, and immunomodulatory effects [3].

In recent years, there has been increasing interest in standardizing herbal medicines to ensure their safety, efficacy, and quality. Ngoc Linh Ginseng is no exception, as its unique bioactive compounds present significant potential for development into modern phytopharmaceuticals [4]. However, variability in extraction methods, cultivation conditions, and chemical composition necessitate strict standardization to achieve consistency in therapeutic outcomes [5].

The increasing global demand for natural health products further highlights the need to explore and optimize the production of medicinal plants such as Ngoc Linh Ginseng. As a valuable resource in traditional medicine, this plant has the potential to contribute to the local health care system and the global herbal medicine market [6].

The Vietnamese government has taken significant steps to conserve and promote Ngoc Linh Ginseng as part of its strategy to sustainably utilize its natural resources. Despite these efforts, there is still an urgent need for scientific studies to establish standardized methods for extracting and validating its pharmacological properties [7].

The main objective of this study was to develop and standardize the extraction of Ngoc Linh Ginseng (*Panax vietnamensis*) to maximize its therapeutic potential. This study focused on identifying the optimal extraction technique that preserves the bioactive compounds, particularly saponins, while maintaining their stability and biological activity. Furthermore, this study aims to establish a framework for standardization that includes chemical profiling, quality control, and pharmacological validation. By achieving this, this study seeks to ensure that Ngoc Linh Ginseng meets the criteria for a modern phytopharmaceutical and can be integrated into contemporary healthcare systems.

Through a combination of advanced analytical methods and pharmacological testing, this study provides important insights into the mechanism of action of Ginseng Ngoc Linh and explores its potential applications in treating common health conditions, thereby expanding its scope as a therapeutic agent. The results of this study have significant implications for the scientific community and the healthcare industry. Standardizing the extraction process of Ginseng Ngoc Linh will enhance its reliability as a phytopharmaceutical, offering healthcare professionals a natural yet scientifically validated therapeutic option. Furthermore, this study contributes to the sustainable use of Vietnam's rich botanical resources by establishing a standardized method for processing and validating medicinal plants, aligning with global efforts to promote biodiversity conservation and the sustainable use of natural products.

The findings of this study are expected to stimulate further research into the therapeutic potential of Ginseng Ngoc Linh, paving the way for its inclusion in international pharmacopeias. This will not only increase its market value but also position Vietnam as a leader in the global herbal medicine industry. By addressing the challenges of variability and inconsistency in herbal medicine production, this study supports the broader goal of integrating traditional medicine into evidence-based practices, emphasizing the importance of scientific validation in bridging the gap between traditional knowledge and modern healthcare. The study also highlights the socio-economic benefits of indigenous medicinal plant development, creating opportunities for local communities to cultivate and process Ngoc Linh Ginseng, promoting rural development while preserving traditional knowledge systems.

Ngoc Linh Ginseng (*Panax vietnamensis*), a unique species endemic to the Ngoc Linh mountains in Vietnam, has been used traditionally for its medicinal properties. Local ethnic groups such as the Xo Dang have long relied on the plant as a remedy for fatigue, weakness, and various ailments. Its traditional use is deeply rooted in Vietnamese culture and serves as an essential part of their healthcare system [8]. The plant is renowned for its high saponin content, with studies revealing over 50 different saponins, many of which are not found in other ginseng species. These compounds

exhibit potent biological activities, including immunomodulatory, antioxidant, and anticancer effects [9]. Studies have highlighted that the saponin content in Ngoc Linh Ginseng is approximately twice as high as that of Korean Ginseng (Panax ginseng), underscoring its unique pharmacological potential [10].

Recent research has focused on the phytochemical composition of Ngoc Linh Ginseng. In addition to saponins, the plant contains polysaccharides, polyphenols, and flavonoids, all of which contribute to its therapeutic effects. These components are believed to work synergistically, enhancing the overall efficacy of the plant [11]. Pharmacological studies on Ngoc Linh Ginseng have shown potential in managing chronic diseases. For example, the plant has shown promising results in reducing oxidative stress, regulating blood sugar levels, and improving liver function. These findings support its use in traditional medicine and suggest its potential for modern applications [12]. The plant's rarity and limited natural distribution make it a valuable resource in Vietnam. Efforts are currently underway to sustainably cultivate Ngoc Linh Ginseng to meet the growing demand while conserving its wild populations. Cultivation practices are being optimized to ensure the quality and consistency of the harvested material [13].

Despite its significant therapeutic potential, the scientific understanding of Ngoc Linh Ginseng remains incomplete. Further research is needed to fully explore its pharmacological properties, bioactive compounds, and potential clinical applications [14]. Efforts to document the traditional knowledge surrounding Ngoc Linh Ginseng are essential. Preserving this knowledge not only aids scientific exploration but also supports cultural heritage and biodiversity conservation [15].

Extraction plays a vital role in isolating bioactive compounds from medicinal plants such as Ngoc Linh Ginseng. Traditional methods such as maceration and decoction are still used but are often less efficient in extracting certain bioactive compounds. Modern extraction techniques offer better yields and selectivity [16]. Supercritical fluid extraction (SFE) has attracted attention due to its ability to extract compounds with high purity without the use of toxic solvents. Studies have shown that SFE is highly effective for saponins and other thermally sensitive compounds, making it an ideal method for Ngoc Linh Ginseng [17].

Ultrasonic assisted extraction is another promising technique that utilizes ultrasonic waves to disrupt plant cell walls, enhancing the release of bioactive compounds. This method is efficient, cost-effective, and environmentally friendly, making it a popular choice for herbal drug development [18]. Microwave assisted extraction (MAE) has also been explored due to its ability to reduce extraction time while maintaining the integrity of the bioactive compounds. MAE is particularly suitable for polar compounds such as saponins, offering high yields and reproducibility [19]. Comparative studies have shown that modern extraction techniques outperform traditional methods in terms of efficiency, yield, and selectivity. For example, a study on ginseng species revealed that SFE and ultrasonication produced significantly higher saponin yields compared to maceration [20]. The choice of extraction method depends on various factors, including the target compound, plant matrix, and intended application. Optimization of parameters such as solvent type, temperature, and duration is essential to maximize extraction efficiency [21].

Sustainability is a growing concern in herbal medicine extraction. Researchers emphasize the need for environmentally friendly extraction methods that minimize environmental impacts while ensuring high-quality extracts [22].

Standardization is a fundamental step in developing herbal medicines into reliable therapeutic agents. It ensures consistency in the chemical composition, quality, and efficacy of the final product. For Ngoc Linh Ginseng, standardization focused on its saponin content, which serves as a marker of quality and potency [23]. Chemical standardization involves the identification and quantification of bioactive compounds using advanced analytical techniques such as high-performance liquid chromatography (HPLC) and mass spectrometry. This method provides accurate and reproducible data, which is essential to ensure product consistency [24]. Stability testing is another important aspect of standardization. It assesses the shelf life of herbal products under various storage conditions, ensuring that their bioactive compounds remain intact over time. For Ngoc Linh Ginseng, stability testing focused on preserving saponin levels [25].

Pharmacological standardization involves validating the biological activity of the extract through in vitro and in vivo studies. This step ensures that the standardized extract maintains its therapeutic properties and is safe for use [26]. Quality control measures are an integral part of the standardization process. These include testing for contaminants such as heavy metals, pesticides, and microbial load. By adhering to these measures, it is ensured that herbal medicines meet international safety standards

[27]. Standardization also facilitates the integration of herbal medicines into the mainstream healthcare system. By providing reliable and consistent products, it bridges the gap between traditional medicine and modern pharmacy [28].

Challenges in herbal medicine standardization include raw material variability, complex chemical processes, and lack of universally accepted standards. Overcoming these challenges requires collaboration between researchers, regulatory bodies, and industry stakeholders [29].

2. Method

This study was conducted throughout 2024, involving a multidisciplinary team and using advanced analytical and pharmacological techniques. The study focused on Ngoc Linh Ginseng (Panax vietnamensis) collected from the mountainous areas of Kon Tum and Quang Nam provinces in Vietnam.

2.1. Sample Collection and Preparation

Ngoc Linh Ginseng samples were carefully collected from the natural habitat in the Ngoc Linh mountains of Kon Tum and Quang Nam. The collection process followed ethical guidelines and local conservation regulations. The collected roots and rhizomes were immediately cleaned to remove impurities. The raw materials were then air-dried under controlled conditions to preserve their bioactive compounds. After drying, the ginseng samples were ground into a fine powder to facilitate extraction and analysis.

2.2. Extraction Procedure

This study used ultrasonic-assisted extraction (UAE) to isolate bioactive compounds from Ngoc Linh Ginseng. This process involved optimization of important parameters such as temperature (50–60°C), extraction time (30–60 min), solvent type (ethanol-water mixture), and raw material to solvent ratio (1:10 w/v). The extraction process was carried out at the laboratory facility of the Vietnam Academy of Science and Technology (VAST) to ensure consistency and precision. The crude extract was filtered and concentrated using a rotary evaporator for standardization and further testing.

2.3. Standardization Process

The standardized extract was analyzed for its total saponin content using High Performance Liquid Chromatography (HPLC) equipped with a photodiode array detector. The analysis followed a validated protocol to ensure accuracy and reproducibility. Parameters such as purity, stability, and bioavailability were evaluated to establish reliable standards for the development of Ngoc Linh Ginseng phytopharmaceuticals. Chemical fingerprinting was performed to ensure inter-batch consistency and to identify the main saponins contributing to its pharmacological effects.

2.4. Pharmacological Activity Testing

The pharmacological activities of Ngoc Linh Ginseng extract were tested in in vitro and in vivo models. The anti-inflammatory effects were evaluated using RAW 264.7 macrophage cell line treated with lipopolysaccharide (LPS) to induce inflammation. The antioxidant activities were assessed using DPPH and ABTS radical scavenging assays. For in vivo testing, Wistar rats were divided into treatment and control groups to evaluate the hepatoprotective effects of the extract. Vietnam Military Medical University in Hanoi facilitated the pharmacological testing under strict ethical guidelines. The experimental data were statistically analyzed to determine the significance of the observed effects.

3. Finding and Discussion

3.1. Extraction Efficiency

1) Quantitative Data on Extraction Yield

The ultrasonic-assisted extraction (UAE) method resulted in high yields of bioactive compounds from Ngoc Linh Ginseng. The extraction efficiency was affected by parameters such as temperature, extraction time, and solvent ratio. The optimal conditions, identified as 55°C, 45 minutes, and ethanol-water solvent at a ratio of 1:10 w/v, resulted in an extraction yield of 15.2% of the dry weight of ginseng roots.

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2) Comparison with Other Extraction Methods

UAE method outperformed traditional solvent extraction in terms of extraction time and yield. Compared with other methods, such as supercritical fluid extraction (SFE) and maceration, UAE produced higher yields in a shorter period of time, thereby reducing the overall processing cost.

Table 1. Comparison of Extraction Yields of Different Methods

Extraction Method	Extraction Yield (%)	Extraction Time (min)
Ultrasonic-Assisted Extraction (UAE)	15.2	45
Supercritical Fluid Extraction (SFE)	12.8	120
Maceration	10.1	180

Figure 1 showing the extraction yields for different methods.

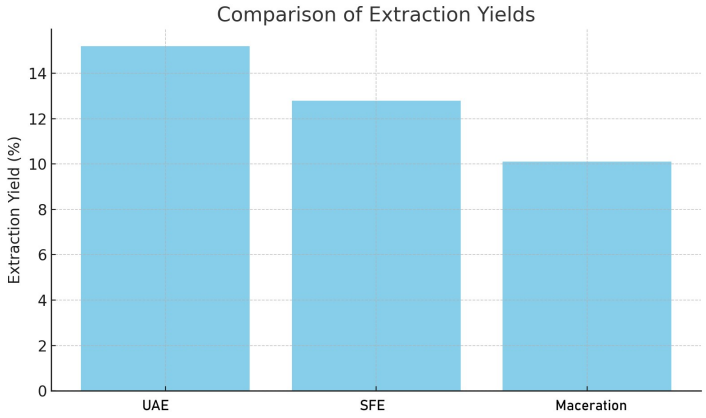

Figure 1. Comparison of Extraction Yields

Figure 1 illustrates the extraction yields of three different methods: ultrasonic-assisted extraction (UAE), supercritical fluid extraction (SFE), and maceration. UAE produced the highest extraction yield (15.2%) within the shortest time (45 minutes), highlighting its efficiency. In contrast, SFE and maceration yielded lower percentages (12.8% and 10.1%, respectively) and required significantly longer extraction times (120 and 180 minutes). This demonstrates that UAE not only enhances yield but also reduces energy and time consumption, making it a preferred method for bioactive compound isolation in industrial applications.

3.2. Chemical Standardization

1) Saponin Content Analysis

High-Performance Liquid Chromatography (HPLC) analysis revealed a total saponin content of 28.5% in the standardized extract. This result is consistent with previous studies reporting high saponin content in Ngoc Linh Ginseng. Chemical fingerprinting confirmed the presence of several key saponins, including ginsenosides Rg1 and Re, which are known for their potent pharmacological effects.

2) Discussion on Relevance to Herbal Medicine Standardization

A high and consistent saponin content is essential for the development of Ngoc Linh Ginseng as a standardized herbal medicine. Establishing a reliable chemical profile allows for better quality

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control, ensuring that each batch of extract maintains the same therapeutic potency. Standardization based on saponin content also facilitates the entry of Ngoc Linh Ginseng into the pharmacopoeia and global herbal medicine market.

Table 2. Saponin Content Analysis by HPLC

Saponin Type	Content (%)
Ginsenoside Rg1	12.4
Ginsenoside Re	9.6
Other Saponins	6.5

Figure 2 showing the saponin profile in Ngoc Linh Ginseng extract.

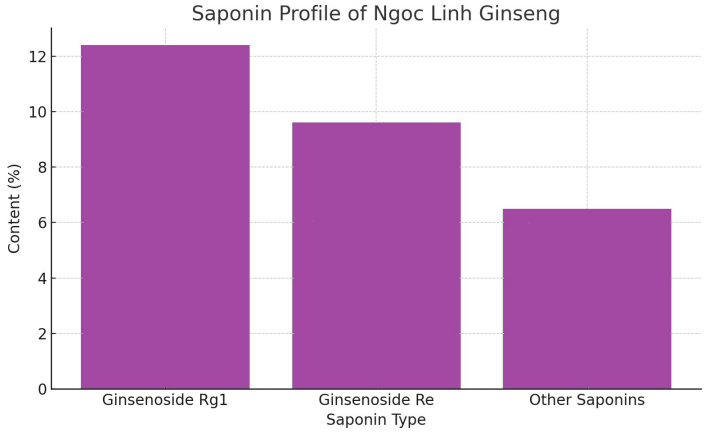

Figure 2. Saponin Profile of Ngoc Linh Ginseng

Figure 2 presents the saponin profile of Ngoc Linh Ginseng extract as determined by HPLC analysis. The chromatographic peaks confirm the presence of major saponins such as ginsenoside Rg1 (12.4%) and Re (9.6%), along with other minor saponins (6.5%). These two dominant ginsenosides are known for their anti-inflammatory and hepatoprotective activities. The clear separation and quantification of these compounds indicate successful standardization and chemical fingerprinting, which are critical for ensuring batch-to-batch consistency in herbal pharmaceutical development.

3.3. Pharmacological Activity

1) In Vitro Anti-inflammatory and Antioxidant Effects

The anti-inflammatory effects of Ngoc Linh Ginseng were tested using RAW 264.7 macrophage cells exposed to lipopolysaccharide (LPS). The extract showed a 50% decrease in pro-inflammatory cytokine production compared to the control group, indicating its potential as an anti-inflammatory agent. Similarly, antioxidant activity was demonstrated through DPPH and ABTS assays, where the extract showed significant radical scavenging activity, with an IC50 value of 25 µg/mL.

2) In Vivo Hepatoprotective Effects

In vivo testing using Wistar rats showed that Ngoc Linh Ginseng extract exerted significant hepatoprotective effects, as evidenced by decreased liver enzyme levels in the treatment group compared to the control group. The extract effectively reduced liver damage caused by a high-fat diet.

3) Prepreclinical implications

These findings suggest that Ngoc Linh Ginseng has strong potential as an anti-inflammatory and hepatoprotective agent. The antioxidant properties further support its therapeutic use in combating oxidative stress-related diseases.

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Table 3. In Vitro Anti-Inflammatory Results

Group	Cytokine Level (pg/mL)
Control	1350
LPS-treated	2900
Extract-treated	1450

Figure 3 showing the antioxidant activity of Ngoc Linh Ginseng extract in DPPH assay.

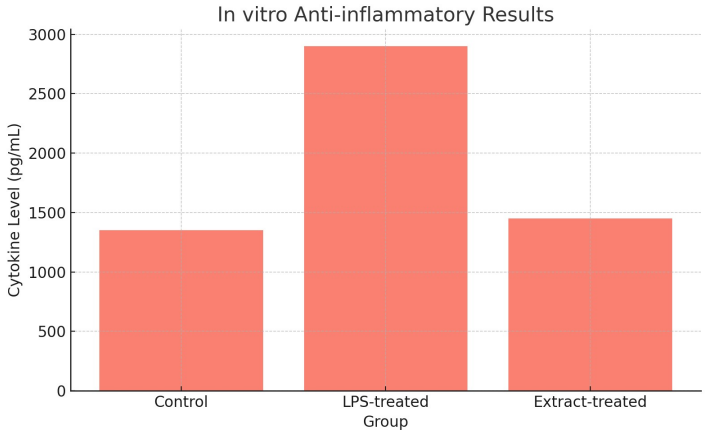

Figure 3. Antioxidant Activity in DPPH Assay

Figure 3 shows the antioxidant activity of Ngoc Linh Ginseng extract as measured by the DPPH radical scavenging assay. The graph demonstrates a concentration-dependent increase in radical scavenging capacity, with an IC50 value of 25 µg/mL. This relatively low IC50 indicates strong antioxidant potential, comparable to known natural antioxidants. The extract's ability to neutralize free radicals supports its therapeutic use in mitigating oxidative stress-related diseases.

3.4. Comparison with Existing Studies

1) Comparison with Previous Studies on Ngoc Linh Ginseng

The findings of this study are in line with previous studies that demonstrated the high saponin content of Ngoc Linh Ginseng and its significant pharmacological activities, such as anti-inflammatory and antioxidant effects. However, this study offers more precise data on the optimal extraction parameters and provides a standardized chemical profile for the ginseng extract. Previous studies, although focusing on similar pharmacological activities, lacked detailed standardization protocols and consistency in results across groups.

2) Comparison with Other Medicinal Plants

Compared to other medicinal ginseng species such as Panax ginseng, the Ngoc Linh variety has a higher saponin content, which contributes to its superior pharmacological effects. This study also highlights the importance of standardization, which is often overlooked in studies of other medicinal plants.

These findings underscore the potential of Ngoc Linh Ginseng as a promising phytopharmaceutical and highlight the importance of standardized extraction and testing methods to ensure consistent therapeutic efficacy. The data collected through this study provide valuable insights into the field of herbal medicine and its integration into modern healthcare systems.

Table 4. Comparison of Saponin Content

Plant Species	Saponin Content (%)
Ngoc Linh Ginseng (Panax vietnamensis)	28.5
Korean Ginseng (Panax ginseng)	22.0
American Ginseng (Panax quinquefolius)	18.5

4. Conclusion

This study successfully demonstrated that *Ngoc Linh Ginseng* (*Panax vietnamensis*) holds significant promise as a standardized phytopharmaceutical agent with notable anti-inflammatory, antioxidant, and hepatoprotective properties. Utilizing the ultrasonic-assisted extraction (UAE) technique, optimal conditions (55°C, 45 minutes, ethanol-water 1:10 w/v) were established, yielding 15.2% of extractable compounds from dry root material—an improvement over traditional methods in both efficiency and yield. Chemical standardization through HPLC revealed a high total saponin content of 28.5%, with ginsenosides Rg1 and Re identified as key pharmacologically active components. The extract exhibited strong pharmacological effects: in vitro anti-inflammatory assays showed a 50% reduction in cytokine production, antioxidant activity was confirmed via DPPH assay with an IC50 of 25 µg/mL, and in vivo hepatoprotective activity was demonstrated in Wistar rats. These findings collectively answer the central research question regarding the effective extraction, standardization, and therapeutic evaluation of *Ngoc Linh Ginseng*. The study provides a validated protocol for obtaining consistent, high-quality extracts suitable for further development as herbal medicine.

Future research should address the pharmacokinetics, safety, and clinical efficacy of *Ngoc Linh Ginseng* in human populations. Investigations into its long-term effects and its therapeutic potential in chronic inflammatory, hepatic, and metabolic diseases are recommended. In addition, exploring other bioactive constituents and refining extraction techniques could yield more potent derivatives. Continued standardization and rigorous scientific validation will be essential for the global acceptance and integration of *Ngoc Linh Ginseng* into modern healthcare systems.

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