

***Ganoderma Neo-Japonicum Imazeki* in Bu Gia Map National Park: Studies on Nutritional Compositions, Active Biological Compounds, and Antioxidant Properties**

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ABSTRACT

Ganoderma neo-japonicum Imazeki, 1939 is a special mushroom species growing on tropical bamboo bushes and has been used in folk medicine to prevent and treat many diseases. Our investigation provided valuable scientific evidence about nutritional composition, active biological compounds, and antioxidant properties, helping to evaluate this mushroom's medicinal value properly and contributing to the conservation and propagation of precious mushroom species in Bu Gia Map National Park. *G. neo-japonicum* contains a high total polysaccharide of 72.4 %, carbohydrate of 74 g/100 g, and high mineral contents like Na, Ca, and Mg. Additionally, the ethanol extract of *G. neo-japonicum* exhibits a substantial total polyphenol content, quantified at 284.91 mg GAE/mg DW, along with a total flavonoid content of 73.12 mg CAE/g DW and a total saponin content of 230.23 mg OAE/g DW. Consequently, this extract demonstrates potent antioxidant properties, evidenced by an IC₅₀ value of 11.70 µg/mL in the DPPH assay and an EC₅₀ value of 124.68 µg/mL in the FRAP assay. Interestingly, the presence of adenosine with a concentration of 104 mg/kg plays a significant role in using this mushroom in food and medicine because this is a bioactive compound found in Cordyceps mushrooms and has a variety of bioactivity such as possible cardioprotective and therapeutic agents for chronic heart failure.

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1. Introduction

Ganoderma neo-japonicum Imazeki, 1939 (*G. neo-japonicum*) belongs to the phyla Basidiomycete, order Agaricales, family Ganoder-mataceae, and genus *Ganoderma* [1] (Fig.1). *G. neo-japonicum* (other name is *Ganoderma bambusicola*), also known as Lingzhi lò ô, Lingzhi nứa. The mushroom is distributed mainly in Asian countries and has been used in folk medicine to prevent and treat many different diseases [2]. *G. neo-japonicum* is a special mushroom species, growing on tropical bamboo bushes, growing and developing in the rainy season (July to September) in Bu Gia Map National Park, Binh Phuoc. Local people exploit mushrooms illegally and sell them on the market at very high prices to use it to support liver disease and cancer. The situation of exploitation and use of *G. neo-japonicum* mushroom is unplanned; control is still difficult; this will lead to the risk of affecting the natural resources of the national park in general and the Gene source of this mushroom species in particular. Besides, there has yet to be any scientific publication on this species' composition, activity, and medicinal potential in Vietnam. In about 31 years (1991 to 2021), there have only been 36 scientific publications about this species in the world; this number is too modest for *Ganoderma lucidum* (Lingzhi mushroom) [3]. Currently, this species is underutilized when compared to *G. lucidum*; therefore, it is not common in Vietnam; major reasons are that it is rare, expensive, and very difficult and time-consuming to cultivate its fruiting bodies. However, in recent decades (2008 to present), this mushroom has attracted significant attention for its ethnic medicinal uses, especially in Southeast Asian regions

such as Malaysia, Korea, Taiwan [2], [3]. Many scientific publications have shown that *G. neo-japonicum* exhibits many valuable medical properties such as anti-oxidation, anti-inflammatory, anti-cancer, anti-hyperglycemia, hand-leg-mouth disease, liver protection, neuropathy, diabetes [3], [4]. Polysaccharides (α/β -D-glucans), triterpenoids and sterols/ergosterols are the three main groups of compounds in this category, which have been extensively researched and published. Although few studies have been conducted on the polyphenol, flavonoid, and saponin groups found in mushrooms, these bioactive compounds mainly contribute to their antioxidant properties [3], [4]. The antioxidant capacities are usually related to low-molecular-weight compounds, particularly the phenolic fractions [5]. Therefore, we focus on research on the chemical composition, qualitative and quantitative, of these groups of compounds, thereby studying the antioxidant activity of *G. neo-japonicum*. Addition, this study aims to determine some nutritional and mineral components and some biocompounds in mushrooms, therefore evaluating and comparing *G. neo-japonicum* to other *Ganoderma* species. The research results provide scientific evidence, helping to evaluate this mushroom's medicinal value properly and contributing to the conservation and propagation of precious mushroom species in Bu Gia Map National Park.



Figure 1. *Ganoderma neo-japonicum* Imazeki, 1939 in Bu Gia Map National Park, Binh Phuoc, Vietnam.

2. Materials and Methods

2.1. Samples and Chemicals

Aluminum chloride (AlCl_3), Potassium ferricyanide ($\text{K}_3[\text{Fe}(\text{CN})_6]$) and trichloroacetic acid (TCA) were bought from Merk, Germany. 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, Vitamin C (ascorbic acid, AA) were collected from Sigma Chemical Co. (St. Louis, U.S.A.). Solvents and other chemicals were in analytical grades. *G. neo-japaponicum* was identified by MSc. Khuong Huu Thang who has been working in the Bu Gia Map National Park. *G. neo-japaponicum* (dried fruiting bodies) was collected on 03/03/2024 and deposited in an Organic lab at the Department of Chemical Technology, Faculty of Chemical and Food Technology, HCMC University of Technology and Education, Ho Chi Minh City, Vietnam.

2.2. Nutritional profile

This study was conducted at the Eurofins Sac Ky Hai Dang companies in May 2024. The nutritional profile of *G. neo-japonicum* was determined, including protein, moisture, lipid, carbohydrate, polysaccharide, unsaturated fatty acid, and ash. Besides, minerals like Ca, Mg, K, Zn, Cu, and Se were quantitated in dried mushrooms.

2.3. Preparation of samples

100 g of *G. neo-japonicum* powder was percolated by soaking with ethanol 70 % solvent with raw material: solvent ratio of 1:10 (w/v) at room temperature (35 ± 5 °C) for 24 hours and changing the solvent, repeated three times. Liquid extracts were collected and evaporated (110 rpm, 50 °C, 50 - 60 minutes) to get the crude extract and dried at a temperature of 45 – 50 °C. The extracts were stored in a dark glass jar in the refrigerator at a temperature of 4 °C before using further studies. The extraction yield of the extracts was estimated using the expression $H(\%) = \frac{m}{m'} \times 100$. In which H, m, and m' were extraction yield (%), weight of the dried extract (g), and weight of dried powder (g)

2.4. Phytochemical analysis

2.4.1. Qualitative phytochemical analysis

The extracts were preliminary investigated functional groups of polyphenols, coumarins, flavonoids, saponins, tannins, carotenoids, and organic acids based on the characteristic reactions of functional groups with reagents [6]. Ganoderic acid A, a typical triterpenoid in mushrooms, was analyzed at the Eurofins Sac Ky Hai Dang companies.

2.4.2. Determination of total polyphenol (TPC), flavonoid (TFC), and saponin (TSC) contents

The total compound contents were quantified using UV-Vis analysis by measuring the absorption of colored complexes at their maximum wavelength. The data were then calculated using the calibration curve of the standard substances [7], [8]. To brief, 0.2 g of the extract was mixed with 9.80 mL of methanol: water extract (70 °, 7:3 v/v), incubated at 70 °C for 10 minutes and subsequently vortexed for 5 min. The mixture was centrifuged at 3500 rpm for 10 min to collect the supernatant. Mixing 0.2 mL of the supernatant with 9.80 mL of distilled water, 1300 µL of the extract, 1 mL of Folin–Ciocalteu reagent (10 %), and incubation for 5 min, adding 700 µL of Na₂CO₃ (10 % (w/v)). The mixture was stirred and incubated in the dark for 30 min at room temperature (35 ± 5 °C), and the absorbance was measured at 760 nm using a UV spectrometer (UH5300, Hitachi, Japan). The TPC is calculated by calculating the equivalent of the gallic acid ($y = 0.096x + 0.0365$, $R^2 = 0.998$), represented by mg GAE/g of dry weight (mg GAE /g DW), calculated as the below equation.

$$TPC = \frac{C \cdot V_m}{m \cdot (100 - w_m) \times 10}$$

In which V_m , m, and w_m are the volume of extracted solution sample (mL), mass of sample (g), and sampling moisture (%), respectively.

Similarly, flavonoid compounds react with AlCl₃ in an alkaline environment to form a yellow complex, TFC was measured with maximum absorbance at a wavelength of 550 nm. Saponin was oxidized by sulfuric acid with vanillin, producing a red-purple color, and the absorbance was measured at 572 nm. TFC and TSC were expressed as mg catechin equivalent/g dried weight of extract (mg CAE/g DW) with the equation catechin ($y = 0.0196x + 0.0056$, $R^2 = 0.9998$) and mg oleanolic equivalent/ g DW (mg OAE/g DW) with a standard equation of oleanolic acid ($y = 0.0169x - 0.0304$, $R^2 = 0.99987$).

2.5. Antioxidant assay

2.5.1. DPPH free radical scavenging assay

Two assays used for evaluating the antioxidant activity of *G. neo-japonicum* extract were the ferric reducing antioxidant power (FRAP) assay and the DPPH free radical scavenging assay [5], [7]. The extract (0.3 g) was mixed with 6.0 mL of ethanol: water extract (95°, v/v), shaken at room temperature. Afterwards, the extract was diluted to various concentrations (2.5, 5.0, 10, 25, 50 µg/mL). Mix 1.5 mL of the diluted extract sample with 1.5 mL of 0.15 mM DPPH (DPPH is diluted in 96° ethanol). Agitate

and incubate in the dark at room temperature (35 ± 5 °C) for 30 minutes. The absorbance was measured at 519 nm by Hitachi UH-530 spectrophotometer. Control samples at each concentration were prepared similarly but replaced the extract with 95% ethanol. The percentage of DPPH free radical inhibition (I %) was calculated as $(A_c - A_s/A_c) \times 100\%$, where A_c and A_s is the absorbance of the control sample and extract. The half maximal inhibitory concentration (IC_{50}) was determined using the mean data values from three determinations at 100, 50, 25, and 10 $\mu\text{g/mL}$ concentrations in 96 ° ethanol. Vitamin C was used as a positive control with IC_{50} of 10.27 $\mu\text{g/mL}$.

2.5.2. Ferric reducing/antioxidant power assay

The extract was diluted with distilled water to serial concentrations (25, 50, 100, 150, 200 $\mu\text{g/mL}$). Mix the diluted extracts with phosphate buffer $\text{KH}_2\text{SO}_4 - \text{K}_2\text{HSO}_4$ (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1 %). The mixture was incubated at 50 °C for 20 min. Then add 2.5 mL of TCA 10 % and centrifuge at 2000 rpm for 10 minutes. The supernatant 2.5 mL was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride 0.1 %. The absorbance was measured at 700 nm. The control samples at each concentration were made similarly, but the high-dilution solution was replaced with distilled water of the same volume. Vitamin C was a strong reducing control used in the FRAP assay and used as a positive control with EC_{50} of 13.15 $\mu\text{g/mL}$.

2.6. Quantitative bioactive compound analysis

Adenosine and cordycepin (3'-deoxyadenosine) are two bioactive compounds present in *Cordyceps* mushrooms. Adenosine exhibits several actions that support its potential use as a cardio protective and therapeutic agent for chronic heart failure. Similarly, cordycepin demonstrates a broad spectrum of biological activities, including neuroprotection, pulmonary and renal protection, antitumor, anticancer, and antileukemic properties. Additionally, cordycepin possesses high antioxidant activity, antibacterial and antifungal effects, anti-inflammatory properties, immunomodulatory effects, and prosexual activity [9]. Adenosine and codycepin were quantitated using HPLC (QTTT/kt3 153:2007) at the Eurofins Sac Ky Hai Dang companies.

2.7. Data analysis

All experiments were replicated thrice, enhancing the results expressed as mean \pm sample standard deviation. The data were analyzed using analysis of variance (ANOVA) with a significance level of $p < 0.05$, employing Excel 2010.

3. Results and Discussion

3.1. Nutritional profile

Dried mushrooms have moisture of 12.2 %, suitable for long-term preservation and acceptable to consumers [10]. *G. neo-japaponicum* contains the total protein, lipid, carbohydrate, and ash content of 11.2, < 0.3 (trace), 74.0, and 2.3 g/100 g dried mushroom, respectively (Table 1).

Compared to *G. lucidum*, total carbohydrate (42.8 g/100 g) was lower but higher in total protein (23.6 g/100 g), fat (5.8 g/100 g), and ash (18.7 g/100 g) [11]. In addition, the results indicated the unsaturated fatty acid content was small, with 0.145 % and metabolizable energy content (fat, protein or carbohydrates can supply energy) of 343 kcal/100 g. This data was higher than Oyster mushrooms cultivated in Bangladesh with a metabolizable energy content of less than 270 kcal/100 g [12].

In particular, *G. neo-japaponicum* contains high total polysaccharides of 72.34 %. Polysaccharides are a main popular component in *Ganoderma* genus, not yet studied in *G. neo-japaponicum*. However, the total polysaccharide content of 72.4 % indicates that this mushroom is a significantly valuable resource in food and medicine (Table 1). The extensive research has revealed that many mushrooms contain different biologically active polysaccharides with immunomodulation, prebiotic, antioxidant, and antimicrobial properties [13]. From the structural points of view, the *Ganoderma* genus's polysaccharides mainly comprise β -glucans, heteropolysaccharides and glycoproteins [3]. Additionally, the dried mushrooms contain various minerals, including calcium (1790 mg/kg), magnesium (507 mg/kg), potassium (3210 mg/kg), zinc (21.7 mg/kg), copper (10.7 mg/kg), and selenium (0.05 mg/kg)

(Table 1). These findings indicate that dried *G. neo-japonicum* is particularly rich in potassium, calcium, and magnesium.

In general, the results indicate that *G. neo-japonicum* is characterized by high levels of polysaccharides and carbohydrates, as well as significant mineral content, particularly potassium, calcium, and magnesium. These attributes suggest its potential applications in both the food and pharmaceutical industries.

Table 1. Nutritional content and identified active biological compounds in *G. neo-japonicum*

No.	Content	Unit	Method	Results
1	Total Carbohydrate	g/ 100 g	FAO Food and Nutrition paper 77 Chapter 2/section 2.3:2003	74.0
2	Lipid	g/ 100 g	EVN-R-RD-2-TP-3498 (Ref. FAO Food 14/7-1986)	Trace (<0.3)
3	Protein	%	TCVN 10034:2013 (ISO 1871:2009)	11.2
4	Metabolizable energy content: Fat, protein or carbohydrates can supply energy	kcal/ 100 g	FAO 2003 77	343
5	Unsaturated fatty acid	%	EVN-R-RD-2-TP-5631 (Ref. ISO 12966-2:2017 & AOAC 996.06)	0.145
6	Moisture	%	EVN-R-RD-2-TP-3496 (Ref. FAO Food 14/7-1986)	12.2
7	Total ash	g/ 100 g	EVN-R-RD-2-TP-3497 (Ref. FAO Food 14/7-1986)	2.30
8	Quanlitative analysis (Ganoderic A acid)		Ref.J.Pharm Biomed. Anal.54 (2011),717-721	Positive
9	Canxi (Ca)	mg/ kg	EVN-R-RD-2-TP-3507 (Ref. AOAC 968.08 (2011))	1790
10	Magie (Mg)	mg/ kg	EVN-R-RD-2-TP-3507 (Ref. AOAC 968.08 (2011))	507
11	Kali (K)	mg/ kg	EVN-R-RD-2-TP-3500	3210
12	Zinc (Zn)	mg/ kg	EVN-R-RD-2-TP-5254 (Ref. AOAC 2015.01)	21.7
13	Copper (Cu)	mg/ kg	EVN-R-RD-2-TP-5254 (Ref. AOAC 2015.01)	10.7
14	Polysaccharides (Total)	%	HD.PP.19/TT.LH	72.34
15	Selen (Se)	mg/ kg	EVN-R-RD-2-TP-20044 (Ref AOAC 2015.01& AOAC 2015.06)	0.05
16	Adenosine	mg/ kg	QTTN/Kt3 153:2017	104
17	Cordycepin	mg/ kg	QTTN/Kt3 153:2017	No detection (LOD=10)

3.2. Extraction extract

The moisture content of the dried mushroom and its ethanol extract were 12.21 % and 11.2 %, respectively. The extraction yield was 1.5 %, which is considered low. This suggests that the extraction

efficiency of low molecular weight compounds, such as polyphenols, from *G. neo-japonicum* was limited. During the extraction process, significant swelling of the mushrooms was observed, attributed to the high protein (11.2 %) and polysaccharide content (72.34 %). When compared to the extraction efficiency of Vietnamese *G. lucidum*, the efficiency for *G. neo-japonicum* was notably lower.

3.3. Phytochemical analysis

3.3.1. Qualitative phytochemical analysis

The preliminary qualitative functional group results show the presence of compound groups like polyphenols (++) , coumarins (++) , flavonoids (+) , saponins (+) , tannins (+) , carotenoids (-) , and organic acids (-) . Among them, polyphenols and coumarins had a moderately positive response; flavonoids, saponins, and tannins showed a trace amount; carotenoids and organic acids were absent in the extract. The phytochemical profile is quite similar to *G. lucidum* and *Ganoderma* genus in general, with the first interesting discovery of the presence of coumarins. Coumarins are derived from 1,2-benzopyrones and originate from the general phenylpropanoid pathway. Coumarins have been used as human therapeutics, symptomatic treatment of multiple sclerosis, photochemotherapy of T cell lymphoma, chemotherapy of multidrug-resistant tumors, organ transplants, and treatment of smokers for nicotine addiction [14]. Ganoderic acid A (+) , a typical triterpenoid in mushrooms, was detected in *G. neo-japonicum*. This compound has been found in *G. lucidum* and used as a folk remedy to prevent and treat various human diseases, including cancer and immunological disorders. It has the potential to enhance human vitality and promote longevity sustainably [15].

3.3.2. Quantitative bioactive compounds

The total polyphenol, flavonoid, and saponin content were 284.91 mg GAE/mg DW, 73.12 mg CAE/g DW, and 230.23 mg OAE/g DW, respectively. The TPC in Bu Gia Map National Park's ethanol extract is higher than in *G. neo-japaponicum* in China (101.01 mg GAE/g DW) [2]. Comparing these contents to *G. lucidum*, the TPC in *G. neo-japaponicum* (Bu Gia Map, Vietnam) was higher than *G. lucidum* ethanol extract (33.42–52.15 mg/g DW, in Vietnam), *G. lucidum* hot-water extract (63.51 mg/g DW, in Malaysia). In 2010, Park and Lee reported that *G. neo-japaponicum* possesses phenolic compounds like catechin, chlorogenic acid, gallic acid, p-coumaric acid, protocatechuic acid, quercetin, and vanillin [1].

On the other hand, the TFC and TSC have not been studied more in *G. neo-japaponicum* mushrooms; therefore, there was no publication about them. In our study, for the first time, the TPC and TSC of *G. neo-japaponicum* extract were noticed at 73.12 mg CAE/g DW and 230.23 mg OAE/g DW, respectively.

3.4. Antioxidant activity

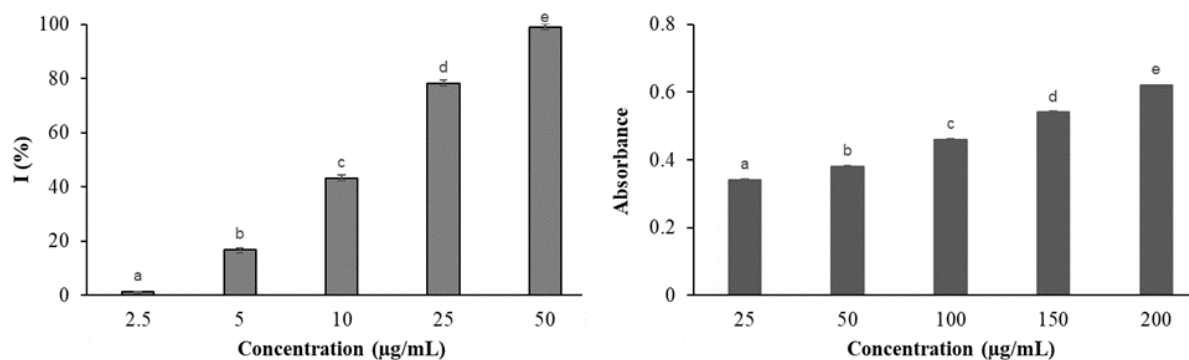


Figure 2. The results of DPPH assay (A) and FRAP assay (B) of *G. neo-japonicum* extracts. Means were presented as mean \pm standard deviation ($n = 3$) and were followed by different letters indicating significant differences at $p < 0.05$.

DPPH and FRAP assay are two common antioxidant methods, typical of two mechanisms: hydrogen atom transfer (HAT) and electron transfer (ET). The FRAP and DPPH assay results were shown in Fig. 2. For the DPPH assay, the percentage of DPPH free radical inhibition (I %) was from 1.22 % to 99.17 %, corresponding to the extract concentration of from 2.5 to 50 $\mu\text{g/mL}$ ($p < 0.05$) (Fig 2.A). The extract

showed potent antioxidants with IC₅₀ of 11.70 µg/mL. For the FRAP assay, the EC₅₀ was 124.69 µg/mL, and the absorbance at various concentration extracts had significant differences statistically (p<0.05) (Fig. 2B).

Interestingly, the antioxidant activities of *G. neo-japonicum* were more potent than those of *G. lucidum*. In the Dao et al study (2023), the IC₅₀ and EC₅₀ of five *G. lucidum* in Vietnam were around 41 to 82 µg/mL and 0.62 to 0.81 mg/mL [7]. This suggests that *G. neo-japonicum* possesses potent antioxidant properties and may serve as a therapeutic agent in preventing or decelerating aging and age-associated, oxidative stress-related degenerative diseases.

3.5. Quantitative adenosine and cordycepin in *G. neo-japonicum*

In Table 1, the content of adenosine was 104 mg/kg and there was no detection of cordycepin in *G. neo-japonicum*. In particular, cordycepin and adenosine are two widely known compounds in *Cordyceps* genus. They were considered to possess effective anti-cancer properties and other valuable bioactivities. The contents of cordycepin and adenosine in the fruiting bodies of *Cordyceps militaris* were 2.654 ± 0.02 mg/g and 2.45 ± 0.03 mg/g, respectively, while those in *Cordyceps sinensis* were 0.9801 ± 0.01 mg/g and 1.643 ± 0.03 mg/g, respectively [16]. Despite *G. neo-japonicum* having a lower adenosine content (104 mg/kg) compared to species within the *Cordyceps* genus, the findings indicate that the adenosine content remains significant, thereby enhancing the potential value of *G. neo-japonicum* in both food and medicinal applications.

4. Conclusions

Ganoderma neo-japonicum Imazeki, 1939 exhibits a high content of polysaccharides, total carbohydrates, and essential minerals such as sodium (Na), calcium (Ca), and magnesium (Mg). The ethanol extract of *G. neo-japonicum* is rich in bioactive compounds, including polyphenols, flavonoids, coumarins, and saponins, and shows a higher concentration of these compounds compared to *Ganoderma lucidum*. The extract demonstrates significant antioxidant activities, with an IC₅₀ value of 11.70 µg/mL in the DPPH assay and an EC₅₀ value of 124.68 µg/mL in the FRAP assay. These antioxidants are proposed to inhibit oxidative activities through two primary mechanisms: hydrogen atom transfer and electron transfer. Notably, the presence of adenosine at a concentration of 104 mg/kg underscores its potential in both culinary and medicinal applications. Consequently, it is imperative to enhance the understanding of the applicability of *G. neo-japonicum* in food and medicinal contexts, comparable to the well-known *G. lucidum* or *Cordyceps* mushrooms.

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Conflict of Interest

The authors declare no conflict of interest.

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