Ganoderma resinaceum as a Natural Source of Essential B Vitamins: HPLC Analysis and Nutritional Significance

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Abstract
Ganoderma resinaceum, a medicinal fungus with bioactive components present in it is not fully characterized for B-complex vitamins in the fruiting bodies. Identification and quantification of B-complex vitamins (thiamine [B[1]], riboflavin [B[2]], niacin [B[3]], and cobalamin [B1[2]]) were conducted using reverse-phase high performance liquid chromatography (RP-HPLC) with a C18 column and UV detection at 280 nm from dry extracts of the fruiting bodies. Acid and enzymatic hydrolysis were used to extract these vitamins according to each specific analyte and the antioxidant capacity of the extracts was assessed against the DPPH radical in the concentration range of 12.5-200 µg/mL, based on ascorbic acid as a reference standard. Four vitamins were detected by well-resolved chromatographic elution peaks at 3.804 (B1), 4.824 (B3), 11.932 (B2), and 15.076 min (B12). B1 had the highest concentration (30.6 mg/L), followed by B2 (26.9 mg/L), B3 (24.8 mg/L), and B12 (1.8 mg/L). B1, B2, and B3 had DPPH scavenging activity in a concentration-dependent manner which was similar to the DPPH scavenging activity of ascorbic acid at high concentrations, whereas B12 had low DPPH scavenging activity at all concentrations tested. Collectively, results from the current study support both the identification and quantification of the four B-complex vitamins, and their nutritional value as a natural food source of B-complex vitamins for use in the development of functional foods and nutraceuticals.

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Introduction

Recent attention has been directed towards medicinal mushrooms as significant functional foods and nutraceuticals because of their abundance of bioactive substances and their potential to promote the well-being of humans. Historically, these fungi have been utilized for centuries by various cultures for food and medicine; now there is scientific evidence from current research that supports the nutritional and therapeutic properties of medicinal mushrooms. Edible and medicinal mushrooms are considered "super foods," containing polysaccharides (especially β-glucans), terpenes, phenolic compounds, vitamins (such as B-complex vitamins and vitamin D2), trace minerals, and antioxidants that help produce immunomodulating, anti-inflammatory, antioxidant, and metabolic-regulating effects. Because of this, medicinal mushrooms are being increasingly used in functional food products that are designed to help prevent the onset of chronic diseases like heart disease, diabetes, and cancer [1, 2]. Medicinal mushrooms, including *Ganoderma lucidum*, Lentinula edodes, and Hericium erinaceus, have received significant research attention for their ability to enhance the immune system, modulate the microbiome of the gut, and promote the metabolic health of the body—primarily through their bioactive metabolites' synergies; therefore, whole mushroom extracts typically have greater efficacy than isolated bioactive metabolites. Additionally, advances in food biotechnology have created new mushroom-based powders, beverages, and enriched food products that provide numerous nutritional benefits while simultaneously providing preventive health benefits [3].

One of the more established groups of medicinal mushrooms in terms of research is Ganoderma due to the long history of use in Traditional Asian Medicine and the extensive body of pharmacological research that has been undertaken over the years. *Ganoderma lucidum* (Reishi or Lingzhi) is one of the most sought-after functional foods known for containing many different bioactive compounds, such as polysaccharides (particularly β-glucans), triterpenoids (specifically ganoderic acids), sterols, peptides, phenolics, and trace elements. These compounds endow Ganoderma with many beneficial properties (including antioxidant, immunomodulatory, anti- inflammatory, liver-protective, and anticancer effects) and underpin our knowledge base of nutraceutical research [4,5]. Ganoderma-type fungi are all relatively low in caloric content but contain high levels of bioactive compounds. Many of the bioactive components from these fungi (polysaccharides) augment both the innate and adaptive immune response by activating macrophages, natural killer cells, and the release of cytokine-regulating substances. Similarly, triterpenes isolated from Ganoderma have demonstrated a considerable amount of pharmacologic significance and can modify oxidative stress and inhibit the growth of tumors in humans. There is increasing scientific interest today in using Ganoderma-derived extracts as functional foods, dietary supplements, and adjunct therapies for chronic disease, which reflects an overall shift toward evidence-based integration of medicinal mushrooms into healthcare systems for disease prevention [6]

B vitamins are a group of vitamins that have important functions related to metabolism. They are not stored in your body, so must be consumed daily. Some examples of B vitamins include thiamine, riboflavin, niacin, and cobalamin. Thiamine (Vitamin B_1) helps convert carbohydrates into energy during metabolism, as well connecting glycolysis (the first step of carbohydrate metabolism) with the TCA cycle (the next step). Riboflavin (Vitamin B_2) serves as the basis for both flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are used to perform oxidation-reduction reactions and produce ATP during the electron transport process [7]. Niacin (Vitamin B_3) serves as the precursor for both NAD^+ and $NADP^+$, which are used in a variety of ways by the cell, such as producing ATP from the breakdown of glucose, processing lipids, and repairing DNA. Cobalamin (Vitamin B_{12}) is needed for producing DNA, performing methylation reactions, and maintaining a healthy nervous system as involved in one-carbon metabolism [8]. Recently, edible and medicinal fungi have been identified as a potential source of water-soluble vitamins, particularly B vitamins, for supporting normal metabolic and physiological functions. In contrast to plant sources, fungi naturally accumulate B vitamins through their physiological processes and from contact with their substrates [9].

This study aims to determine the total amount of thiamine (Vitamin B1) that may be extractable from the cultivated mushrooms of the species Ganoderma resinaceus distinguished from cultivated G. lucidum through different extraction methods; chemistries and/or enzymes to extract the maximum amounts of thiamine and its ability to be purified with high purities for utilization as a candidate for biochemical assay and/or potential applications in pharmaceuticals and nutrition. In addition, the scope of the study will highlight Ganoderma resinaceum as a source of bioactive compounds that are it and may provide health benefits when consumed and incorporated as ingredients in functional food products and developed into medicines or therapeutic preparations in the future.

Materials and methods

Extraction of Vitamins from *Ganoderma resinaceum*

In this study, Ganoderma resinaceum dried fruiting bodies provided the biological substrate. Each sample of dried fruiting bodies was physically cleaned, dried and ground into a fine uniform powder to provide a larger surface area for improved extraction of water-soluble vitamins.

Extraction of Thiamine (Vitamin B_1)

Esteve *et al*. [10] described the methods used for thiamine extraction with some basic modifications. 2g of dried powdered mushroom samples were mixed with a 0.1 M HCl solution (40 mL), thoroughly blended, and placed in a 96°C water bath for 30 min to release thiamine from the fungal matrix. Once the solutions were at room temperature, the pH was adjusted to 4.5 with sodium acetate buffer. To hydrolyze polysaccharides, 500 mg of amylase was added to the solution, and the mixture was then incubated for 3 h at 50°C under controlled conditions to release the vitamin content. Following enzymatic digestion, the solution was cooled and precipitated with 2 mL of 50% trichloroacetic acid (TCA) and heated at 100°C for 5 min and then centrifuged at 4000 r.p.m. for 5 min. The supernatant was collected and filtered through a 0.45 µm membrane filter to remove any leftover particulates. 1 mL of the filtered sample was transferred to an Eppendorf tube with 1 mL of solvent consisting of acetonitrile:glacial acetic acid:water (5:1:94 v/v/v) and mixed thoroughly using a vortex mixer before being stored in amber colored glass vials at low temperature until ready for analytical processing.

Extraction of Riboflavin (Vitamin B_2)

A mixture consisting of 0.3 g of powdered mushroom (P. ostreatus), 15 ml of 0.1 M hydrochloric acid (HCl) and submitted to 60 minutes of heat in boiling water to extract riboflavin from the substrate. Following the boiling period, the sample was neutralised to a final buffered pH of 4.5 by adding 2.5 M sodium acetate solution. Next, 5 mg of α-amylase and 50 mg of β-glucanase were added to the neutralised sample to assist in the degradation of carbohydrate matrices and thus enhance the availability of riboflavin. The sample was incubated for 24 hours in a controlled environment at 37 °C. Following enzymatic treatment of the extract, the extract was diluted to a final volume of 25 ml with 0.02 M weak acetic acid, followed by filtration through a 0.2 µm filter, and stored in amber bottles under refrigeration until analysis was performed [11].

Extraction of Niacin (Vitamin B_3)

A powdered mushroom sample weighing 0.2 grams was heated with 15 milliliters of HCl acid having a concentration

of 0.1 molarity in a boiling water bath for one hour and then brought rapidly to an ice bath after heating to prevent decomposition of the samples. The pH of the resulting mixture was adjusted to 4.5 with a solution of 2.5 M sodium acetate prior to centrifugation at 10,000 rpm for 10 minutes; the resulting supernatant was collected carefully. The resulting extract was filtered using a 0.2 µm membrane filter and stored in amber glass vials at low temperature until chromatography [11].

Extraction of Cobalamin (Vitamin B_{12})

A buffer of 8.3 mM sodium hydroxide and 20.7 mM acetic acid (pH 4.5) was prepared, and 10 mL was added to 0.2 g of mushroom powder along with 100 µL of 1% sodium cyanide (NaCN) to convert all corrinoid forms to stable cyanocobalamin [12]. The mixture was incubated at 100 °C for 30 minutes, then cooled and centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected and pH adjusted to 6.2, then filtered through a 0.2 µm membrane filter and stored in amber bottles at 4 °C until HPLC analysis.

Identification and Quantification of Vitamins Using HPLC

Extraction of vitamins was performed using High Performance Liquid Chromatography (HPLC) (model number SYKAM, Germany). The separation process took place on a reverse-phase C18 ODS (25 cm x 4.6 mm) column utilizing an acetonitrile/distilled water (75:25) mobile phase at a total flow rate of 0.7 mL/min and detected with a UV Detector set at 280 nm for maximum sensitivity in determining water-soluble vitamins [13]. Standards for thiamine, riboflavin, niacin and cobalamin were used to identify compounds based upon retention times relative to the standards and calibration curves were developed to enable quantitative determination. By comparing the peak area of the sample analyzed to the respective standard, concentrations of vitamins in the samples analyzed were calculated.

Determination of Antioxidant Activity by DPPH Radical Scavenging Assay

The 2,2-dipheny-1-triazene hydrazyl (DPPH) radical scavenging assay was used to assess the antioxidant activities of vitamins B1, B2, B3, & B12 extracted from Ganoderma resinaceum. This assay was performed according to an altered method found in prior studies, where vitamin extracts were made at concentrations of 12.5, 25, 50, 100, and 200 µg/mL in methanol. The positive control, ascorbic acid, was used under the same experimental conditions. In brief, each concentration of the vitamin extracts was combined (1 mL each) with 1 mL of DPPH solution (0.1 mM dissolved in methanol) that was freshly prepared. The mixture of the samples and DPPH solution was vortexed until uniform and incubated for 30 minutes at room temperature in the dark to provide for maximum reaction between the antioxidants and DPPH radicals. After the 30-minute incubation, the absorbance of each sample was measured at 517 nm using a UV-vis spectrophotometer with a blank consisting of methanol instead of the vitamin extract [14].

The radical scavenging activity was calculated based on the following formula:

DPPH scavenging activity (%)= (Ac−As)⁄Ac×100

Where:

- AcA_cAc = absorbance of the control reaction
- AsA_sAs = absorbance of the sample or standard

Results

Identification, Separation and Retention Times of B Vitamins in Ganoderma resinaceum

HPLC analysis detected four B-complex vitamins in the mushroom extract as demonstrated in figure 1: thiamine (B1), riboflavin (B2), niacin (B3), and cobalamin (B12). All four were well-resolved under the applied RP-HPLC conditions, eluting at 3.804 min (B1), 4.824 min (B3), 11.932 min (B2), and 15.076 min (B12), as elucidated in table 1.

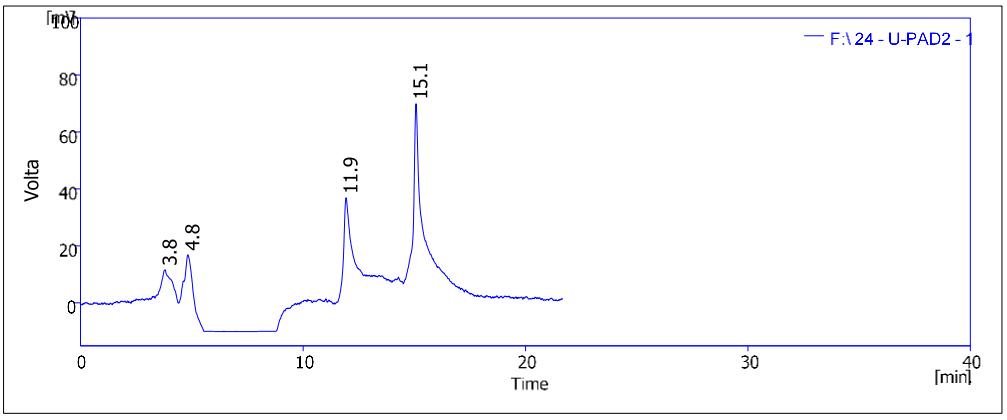

Fig 1: HPLC analysis of mushroom extract

Table 1: HPLC analysis of Ganoderma extract including the 4 B vitamins

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.804	148.666	7.195	9.3	7.4	0.41	B1
2	4.824	220.555	13.277	13.9	13.6	0.26	B3
3	11.932	381.699	23.807	24.0	24.4	0.24	B2
4	15.076	839.352	53.300	52.8	54.6	0.20	B12
	Total	1590.272	97.580	100.0	100.0		

Quantification of B Vitamins in Ganoderma resinaceum

Thiamine (B1) was the most abundant vitamin in the extract at 30.6 mg/L, followed by riboflavin (26.9 mg/L) and niacin (24.8 mg/L). Cobalamin (B12) was present at markedly lower levels (1.8 mg/L).

Table 2: B Vitamins in Ganoderma resinaceum quantification

Vitamin	Concentration (mg/L)
B1 (Thiamine)	30.6
B2 (Riboflavin)	26.9
B3 (Niacin)	24.8
B12 (Cobalamin)	1.8

HPLC Method Validation Using Standard Compounds

Vitamins were identified by comparing sample retention times against analytical standards under the same RP-HPLC conditions. The elution times for thiamine (B1), niacin (B3), riboflavin (B2), and cobalamin (B12) were 3.820, 4.852, 11.903, and 15.053 minutes, respectively, and had minimal deviation from their corresponding sample peaks. All of the peaks were well-resolved and there was no significant overlap between them, which demonstrates that both the C18 column and the mobile phase were appropriate for this application with respect to selectivity. Finally, there is a high degree of correlation between the retention times of the standards and the samples; thus, the method can be considered a reliable technique for the qualitative identification of vitamins in G. resinaceum extracts.

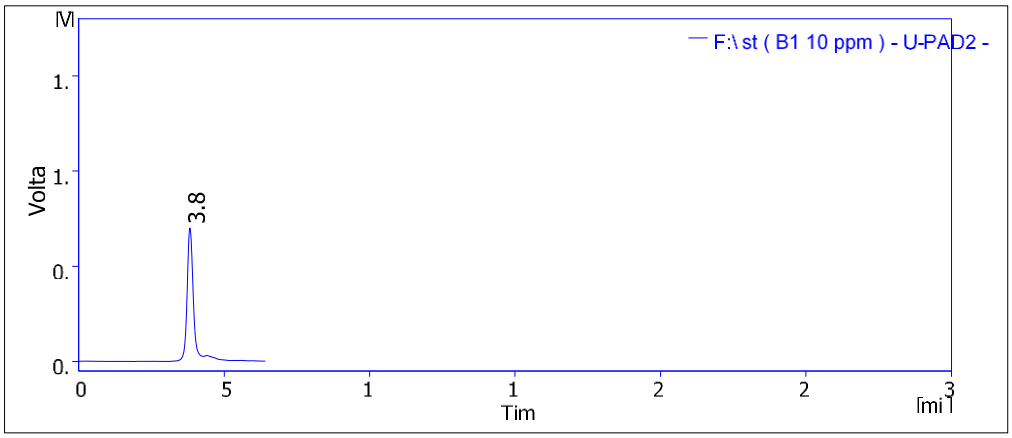

Fig 2: standard curve of B1 vitamin.

Table 3: Chromatographic Identification of Vitamin B1 Using RP-HPLC

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.820	1427.272	218.824	100.0	100.0	0.11	B 1
	Total	1427.272	218.824	100.0	100.0		

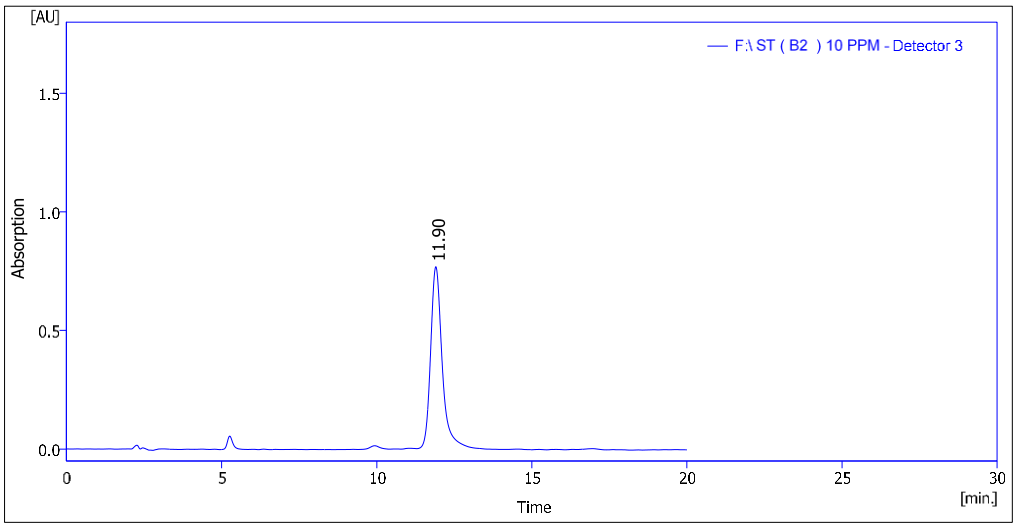

Fig 3: standard curve of B2 vitamin.

Table 4: Chromatographic Identification of Vitamin B2 Using RP-HPLC

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	11.903	399.062	69.335	100.0	100.0	0.10	B2
	Total	399.062	69.335	100.0	100.0		

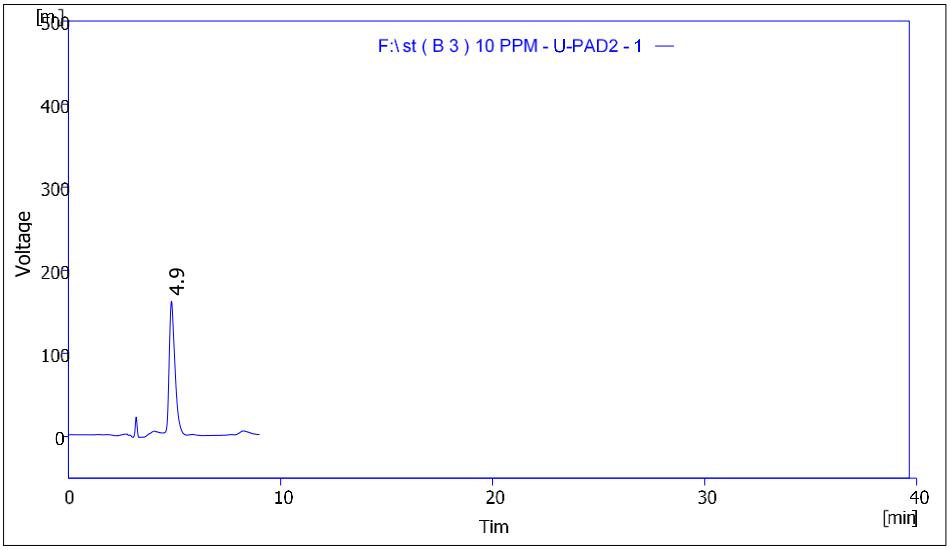

Fig 4: standard curve of B3 vitamin.

Table 5: Chromatographic Identification of Vitamin B3 Using RP-HPLC

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.852	321.164	45.996	100.0	100.0	0.13	B3
	Total	321.164	45.996	100.0	100.0		

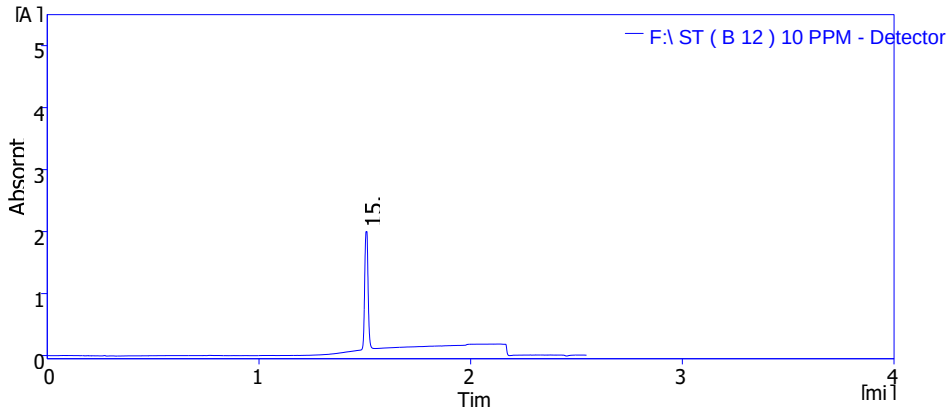

Fig 5: standard curve of B12 vitamin.

Table 6: Chromatographic Identification of Vitamin B12 Using RP-HPLC

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	15.053	5154.145	702.704	100.0	100.0	0.12	B12
	Total	5154.145	702.704	100.0	100.0		

Antioxidant Activity of Ganoderma resinaceum Extract

DPPH free radical scavenging activity was evaluated for vitamins B1, B2, B3, and B12 across concentrations of 12.5–200 µg/mL, with ascorbic acid as a positive control.

B1 showed moderate, concentration-dependent activity. At 12.5 and 25 µg/mL it was significantly weaker than ascorbic acid; the gap narrowed at higher concentrations, with no significant difference at 100 or 200 µg/mL (NS) B2 followed a similar pattern. Activity was significantly lower than ascorbic acid at 12.5 µg/mL and 25 µg/mL, but the difference was no longer significant at 200 µg/mL (NS).

B3 rose gradually with concentration. Significant differences from ascorbic acid were detected at 12.5 µg/mL, 25 µg/mL, and 50 µg/mL, with no difference at 100 µg/mL (NS). At 200 µg/mL, B3 activity fell below ascorbic acid again.

B12 had the lowest activity overall. Scavenging remained well below ascorbic acid at all concentrations ($p < 0.0001$ throughout), with values not exceeding ~15% at 200 µg/mL.

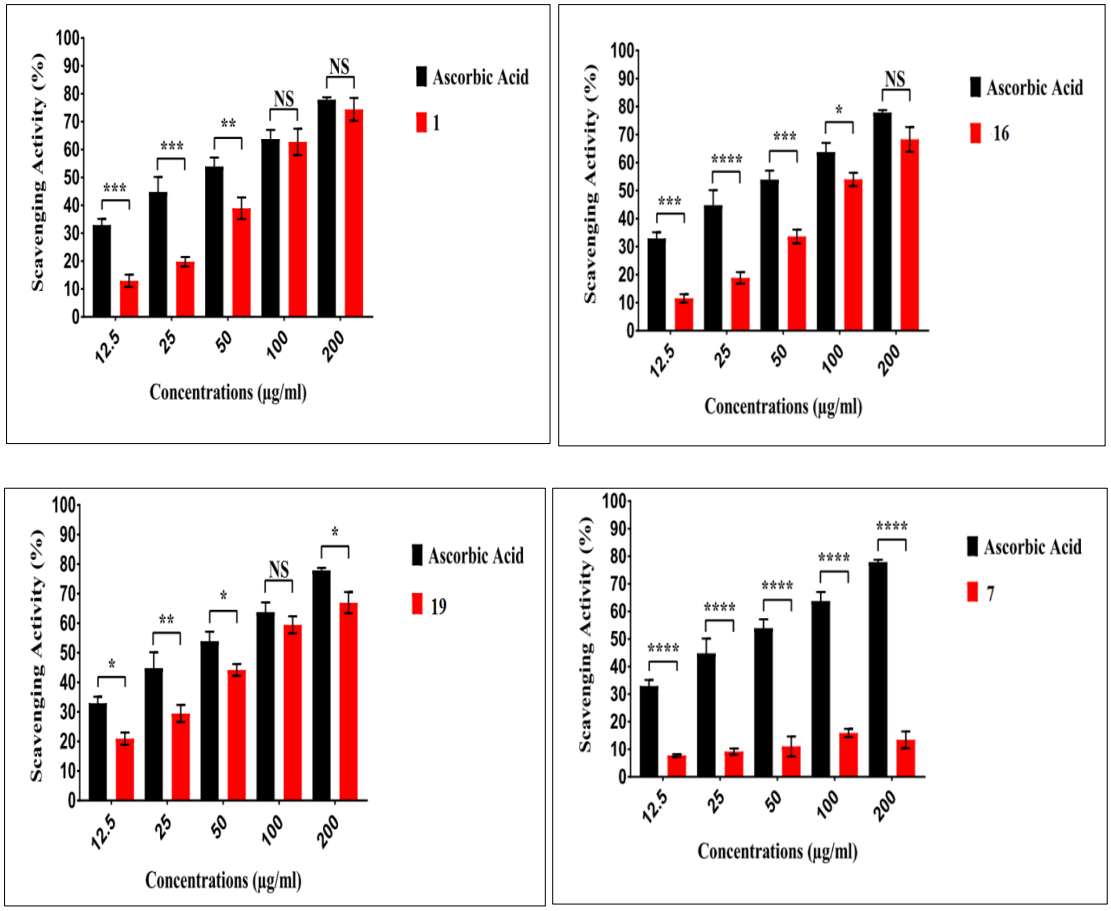

Fig 6: DPPH Antioxidant Activity of Vitamins B1, B2, B3, and B12 Compared with Ascorbic Acid

Discussion

Mushrooms are being recognized for their bioactive compounds as well as B-complex vitamins that may be important for energy metabolism, neurological function and antioxidant activity; specifically, *Ganoderma* has received considerable research attention from this perspective. Furthermore, to quantify the amount of these vitamins in complex biological matrices, RP-HPLC is the conventionally used method for this purpose. This study determines the presence and quantifies the B-complex vitamins present in *G. resinaceum* along with their antioxidant activity.

All four B-complex vitamins were successfully identified and isolated using RP-HPLC. Their retention times were very similar to those found by analytical standards. Given how mushrooms contain multiple metabolites, variations between retention times for both samples and standards can be expected because the different components of the mushroom will interact with the stationary phase in unique ways. There are studies that have shown that such chromatographic behavior occurs with different B vitamins analysed by RP-HPLC methods with C18 type columns [16, 17]. The chromatographic behaviour also agrees well with data from other studies, such as those by Hossain *et al*. (2019), who identified and isolated thiamine and riboflavin with good reproducibility from mushroom samples [15]. The presence of B vitamins (thiamine, riboflavin, niacin and cobalamin) in Ganoderma resinaceum also fits with what has been seen previously for other species of Ganoderma [18]. The concentration of thiamine was the highest at 30.6 mg/L, followed by riboflavin at 26.9 mg/L and niacin at 24.8 mg/L; however, cobalamin was only found to have a concentration of 1.8 mg/L. The distribution of B vitamins in Ganoderma resinaceum are similar to those seen for other species of basidiomycetes [19, 20].

The predominance of thiamine is indicative of the essential role of thiamine pyrophosphate (TPP) as coenzyme in carbohydrate metabolism and the TCA or Krebs cycle, both of which are continuously functional in the growing fungal tissue. Riboflavin is the precursor for FAD and FMN, and both of these are critical for mitochondrial electron transport, as well as for oxidative phosphorylation. Because both of the previous examples of vitamin B2 -riboflavin- are present in high concentration, they also reflect what has been reported for Pleurotus ostreatus and Agaricus bisporus [19, 21]. Similar patterns exist with niacin; given that niacin is a precursor for NAD^+ and $NADP^+$, which are continuously recycled through glycolysis and biosynthetic redox reactions, niacin abundance also correlates with prior reports for Pleurotus spp. [19, 21, 22].

Niacin abundances are somewhat less than those of thiamine or riboflavin supports the hypothesis that metabolic processes favor TPP- and FAD-dependent pathways during the breakdown of substrate. Cobalamin levels were quite low, which is expected; fungi are not primary producers of vitamin B12, as it is produced almost exclusively by bacteria and archaea [23]. Therefore, low levels of cobalamin detected in this study likely result from environmental sources, substrate contamination and/or from bacterial associates, and align with similar findings for Pleurotus spp. [24].

The antioxidant characteristics provided by B-complex vitamins obtained from Ganoderma resinaceum result from their distinct biosynthesis properties relative to the determination of oxidation state according to redox biology and not as would normally be determined by a classic scavenger reaction based on phenolics. Although B vitamins do not work as direct antioxidants (like polyphenols), there is increasing evidence that some kinds of B vitamins help to reduce oxidative stress through their oxygen and hydrogen transfer reactions used for redox cycling and coenzyme regeneration. Riboflavin (B2) is of specific interest because it is the primary precursor of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), which are important cofactors involved in the mitochondrial electron transport system and multiple dehydrogenase reactions. Recent research studies, both biochemically and nutritionally, have found that riboflavin will mediate redox reaction mechanisms resulting in the creation of either reactive oxygen species or mitigation of reactive oxygen species depending upon environmental conditions and riboflavin concentration; therefore, demonstrating riboflavin's ability to act either as an antioxidant or pro-oxidant within in vitro systems [25, 26].

The antioxidant function of niacin/vitamin B3 is predominantly via its role as a precursor of NAD+ and NADP+, which are key co-factors to support cellular redox homeostasis. Endogenously, the enzymatic systems that produce NADPH (glutathione and thioredoxin pathways), as well as many others, are the dominant sources of antioxidant protection for aerobic organisms. Recent reviews of the literature note that niacin is helping to regulate oxidative stress primarily by maintaining pools of NAD(P)H for use by enzymatic antioxidant systems, rather than functioning as a direct radical scavenger [26,27,28]. This may explain the relatively gradual and concentration-dependent behaviour of niacin with regard to redox potential observed in chemical assays. Although thiamine (B1) is best known for its role in the metabolism of carbohydrates, where it acts as thiamine pyrophosphate (TPP), thiamine has recently been shown to also have indirect antioxidant effects, in part by maintaining mitochondrial energy efficiency and by lowering the metabolic oxidative burden on the organism. Thiamine deficiency has been associated to increased oxidative stress, and conversely, adequate thiamine levels are necessary for preserving mitochondrial integrity and decreasing reactive oxygen species formation by promoting greater metabolic flux [30, 31, 32]. In contrast, while vitamin B12 is not thought of as a direct source of antioxidants, its biological importance in redox processes is largely attributed to its role in the enzymatic pathways of methionine synthase and methylmalonyl-CoA mutase. Vitamin B12 is most often found in fungi as a result of microbial interactions, rather than from endogenous synthesis, and its antioxidant contribution is considered indirect and secondary [24, 33].

The existing literature indicates that B vitamins found in mushroom extracts do not display true radical scavenging capabilities, but instead release antioxidants by providing secondary redox reactions. They primarily act as cofactors in enzymes, assist in mitochondrial respiration/metabolism, and

provide support for the maintenance of cellular NAD(P)H-dependent antioxidant systems. The most recent studies indicate that mushrooms produce their total antioxidant capacity primarily from phenolic compounds, polysaccharides, and ergothioneine, with vitamins functioning in a supportive role to provide metabolic support as antioxidants rather than directly acting on free radicals [19, 21].

Conclusion

Ganoderma resinaceum fruiting bodies contain four water-soluble B vitamins detectable and quantifiable by RP-HPLC. Thiamine was the most abundant at 30.6 mg/L, followed by riboflavin and niacin at comparable levels, while cobalamin was present only in trace amounts. As a result of utilizing the chromatography technique, the production of clean peaks was achieved with excellent correlation between the retention times of both the sample and standard. The results of the antioxidant studies conducted using the DPPH radical showed that B1, B2, and B3 demonstrate a concentration dependent DPPH scavenging capability and B1, B2, and B3 have equal to that of ascorbic acid at higher concentrations. B12 showed little antioxidant activity, reflecting its functional role biochemically. Based on these data, it may be concluded that the fungi G. resinaceum provides an important dietary source of vitamin B. Future research should focus on examining the bioavailability of these vitamins; the effects of growth conditions and extraction parameters on vitamin yield; and whether the antioxidant characteristics identified in vitro translate into biological systems.

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