



Studies morphological and determination of folic acid, β -glucans, protein and fiber of local edible mushrooms in Loei province

Niramol Srichana ^{a*}, Suwatchai Misuna ^a, Panruethai Putthongsri ^a and Rasamee Nontee ^a

^a Department of Chemistry, Faculty of Science and Technology, Loei Rajabhat University, Loei, 42000, Thailand

Abstract

Article history:

Received: 08-01-2026

Revised : 10-04-2026

Accepted: 28-04-2026

Published: 05-06-2026

Keywords:

Local Edible Mushrooms;

Folic Acid;

β -Glucans;

Protein;

Fiber

This study examined the morphology and quantified folic acid, β -glucans, protein, and fiber contents in 25 species of local edible mushrooms from Loei Province. This study aimed to analyze the functional nutrients of local edible mushrooms collected from Loei Province. Morphological characteristics of the cap/pileus, stem, gills, flesh, and special features like veils and rings were analyzed, revealing distinct differences useful for effective species classification and reflecting the biodiversity adapted to varied local environments. Quantitative analysis was performed in triplicate using high-performance liquid chromatography for folic acid and beta-glucan. The results showed that folic acid ranged from 0.09 to 1.52 mg 100g⁻¹, β -glucans from 183.46 to 1197.41 mg 100g⁻¹, protein from 1.31 to 35.88%, and fiber from 0.15 to 9.49%. The highest folic acid content was found in *Coprinus fimetarius*, β -glucans in *Lentinus squarrosulus*, protein in *Tylopilus plumbeoviolaceus*, and fiber in *Phallus indusiatus*. Variations in these nutritional components were influenced by physiological traits, nutrient accumulation capacity, environmental conditions, soil fertility, temperature, and rainfall in the collection areas. The research findings hold scientific value and can serve as baseline data for further development into products that enhance the economic value of local edible mushrooms while expanding consumer choices for diverse mushroom consumption.

* Corresponding author : niramol.sri@lru.ac.th

DOI: <https://doi.org/10.55674/cs.v18i3.265617>

© [2026] The Author(s).

1. Introduction

Mushrooms are living organisms classified in the Kingdom Fungi [1]. In Thailand, mushrooms are used in a variety of culinary dishes, and their consumption has increased significantly in recent times, according to research findings, the consumption of mushrooms has been associated with a wide range of health benefits, and numerous studies have confirmed their disease-preventive properties [2]. Mushrooms can be cultivated domestically or purchased in markets, and they are also foraged from natural habitats in different local regions. Numerous studies have confirmed that mushrooms possess disease-preventive properties, as certain compounds in mushrooms can stimulate immune function and reduce the risk of cardiovascular diseases. Research has also reported that mushrooms contain a wide range of biomolecules with both nutritional and medicinal value. Different functional compounds, including terpenes, sterols, ceramides and phenolic compounds have been found in mushrooms [3].

Each mushroom species exhibits distinct morphological characteristics, including the cap, stipe, gills, flesh, and special features such as the volva and annulus. These differences serve as effective criteria for mushroom

identification and also reflect the biodiversity of local mushrooms in Loei Province, which have adapted to various environments and landscapes. Morphological studies on native mushrooms in Loei Province exist, such as Biodiversity of Mushrooms in Conservative Forest in Dansai District [4] of Loei Province, which identified 56 species from 26 families, with 52 species in Basidiomycota, collected from hand-made forests in Dansai District. β -glucans, protein, and fiber content have been analyzed in wild mushrooms from Northeastern Thailand, such as no prior research combines detailed morphological identification with quantification of folic acid, β -glucans, protein, and fiber specifically in Loei Province's native edible mushrooms. While Northeastern mushrooms have been examined for general nutrients or β -glucans, Loei-specific folic acid data and integrated morphological-nutritional profiling remain unstudied. Folic acid (or folate in its natural form) is a type of B vitamin (vitamin B9) that is essential for the production of new cells, especially red blood cells and DNA synthesis. It is therefore particularly important during periods of rapid growth, such as pregnancy. According to structural formula of folic acid as shown in Fig. 1 [5, 6].

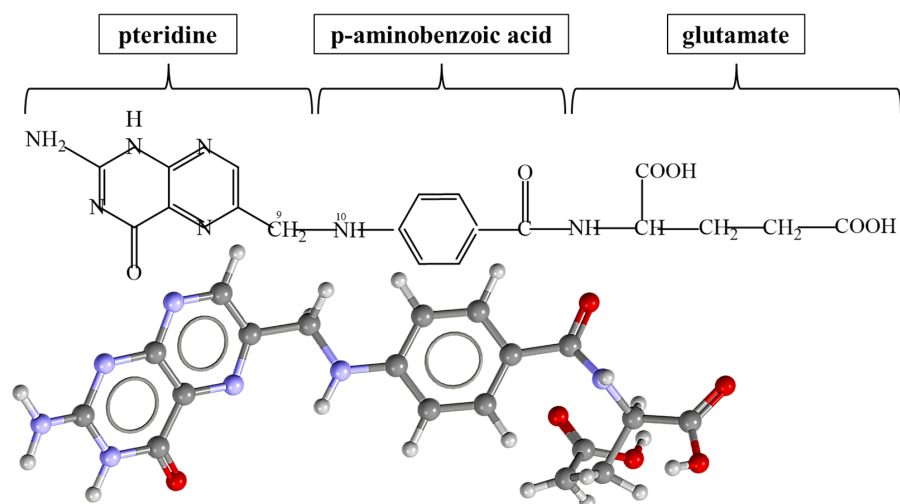


Fig. 1 The chemical structure of folic acid consists of three main components: a pteridine ring, a para-aminobenzoic acid (PABA) group, and a glutamic acid group, which are connected by a methylene bridge.

Beta-glucan (β -glucan) is a type of polysaccharide composed of D-glucose units linked together in chains by beta-glycosidic bonds. Polysaccharide-peptide (PSP) β -glucan, composed of β -D-glucose monomer units connected by glycosidic linkages at positions β (1 $\rightarrow$ 3), (1 $\rightarrow$ 4), and/or (1 $\rightarrow$ 6), either in branched or unbranched configurations, holds significant promise in promoting health. Its beneficial effects include reducing the glycemic index, managing cholesterol levels, and mitigating various cardiovascular diseases. Beta-glucan is found in bacteria, mushrooms, yeast, oats, and barley and schematic model representing

structure and branching degree of β -glucan from different sources as presented in Fig. 2 [7].

Beta-glucan provides various health benefits, such as working synergistically with macrophages, which are the body's most potent immune cells. When combined with beta-glucan, macrophages function more effectively and efficiently. Beta-glucan also helps inhibit the growth of cancer cells and tumors, stimulates the immune system, and regulates other bodily conditions, such as blood pressure, blood glucose, and blood lipid levels [8, 9]. Additionally, it exhibits anti-inflammatory and antioxidant.

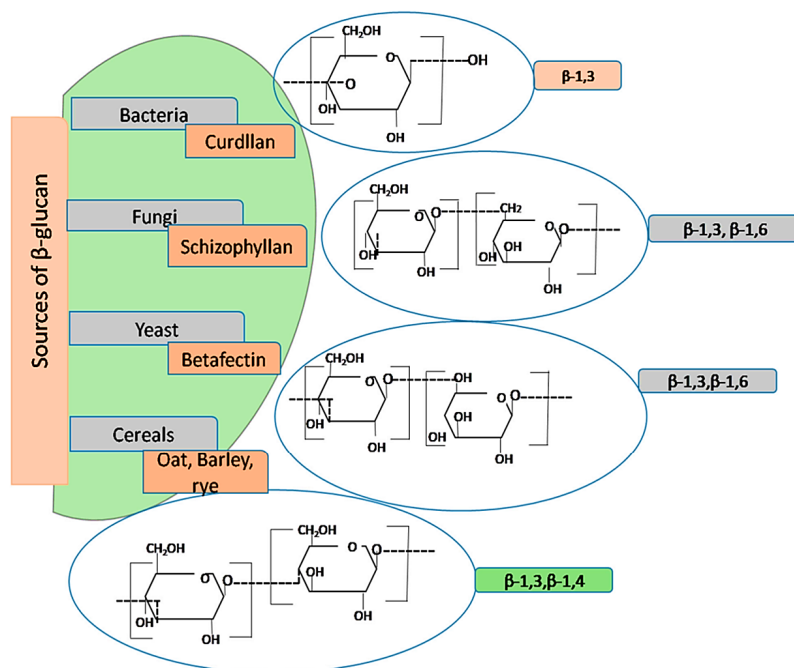


Fig. 2 The chemical structure of beta-glucan consists of glucose (D-glucose) units linked together in chains by β -glycosidic bonds. It shows the structure and branching level of β -glucan from different sources.

Protein is a macronutrient composed of amino acids linked together in long chains, playing essential roles in the body such as forming the structure of muscles, blood, skin, hair and enzymes as well as repairing tissues and building cells. In addition, proteins are high molecular-weight polypeptides composed of more than 50 amino acid residues covalently linked through various types of chemical bonds. Each protein is characterized by a specific composition and sequence of amino acids, which fold into unique three-dimensional conformations that ultimately determine their biological functions. Fiber on the other hand, is a complex carbohydrate that the body cannot digest or absorb in the small intestine. It is classified into two main types: soluble fiber, which helps lower cholesterol, control blood sugar levels and promote satiety; and insoluble fiber which increases stool bulk stimulates bowel movements and prevents constipation.

Dietary fiber is a group of complex carbohydrates that cannot be digested or absorbed in the human small intestine. The chemical structure of dietary fiber is diverse and complex, consisting of various polysaccharides such as cellulose, hemicellulose, pectin, beta-glucan, arabinoxylan, xyloglucan and lignin which is a non-carbohydrate polymer. Each type of dietary fiber has a distinct molecular structure. For example cellulose is a linear polymer of glucose units linked by β -1,4-glycosidic bonds; hemicellulose is composed of various sugars such as xylose, arabinose, mannose and glucose, and has a short branched structure; pectin is a polymer of galacturonic acid with arabinose and galactose side chains; beta-glucan is a polymer of glucose linked by β -1,3 and/or β -1,4 bonds; and lignin is a complex polymer of phenylpropane units.

Loei is one of the provinces in Northeastern Thailand with rich biodiversity. The general landscape consists of high, complex mountains and fertile forests. During the rainy season, a wide variety of plants and crops, such as wild mushrooms, flourish. Local people go into the forest to collect mushrooms for cooking, as mushrooms are nutritious, inexpensive, easily accessible, and have a unique taste. Additionally, mushrooms can be sold to generate extra income and reduce household expenses.

Therefore, the objective of this research is to study the morphology and analyze the content of folic acid, β -glucan, protein, and fiber in 25 species of local mushrooms found in Loei Province: 1) *Amanita hemibapha* 2) *Amanita princeps* 3) *Astraeus hygrometricus* 4) *Boletellus emodensis* 5) *Boletus edulis* 6) *Chiua virens* 7) *Coprinus fimetarius* 8) *Craterellus odoratus* 9) *Galiella rufa* 10) *Gyroporus cyanescens* 11) *Heimioporus japonicus* 12) *Lentinus polychrous* 13) *Lentinus squarrosulus* 14) *Phallus indusiatus* 15) *Russula alboareolata* 16) *Russula cyanoxantha* 17) *Russula emetic* 18) *Russula foetens* 19) *Russula nigricans* 20) *Russula rosacea* 21) *Russula virescens* 22) *Schizophyllum commune* 23) *Termitomyces fuliginosus* 24) *Thaeogyroporus porentosus* and 25) *Tylopilus plumbeoviolaceus*, as shown in Fig. 3. The findings will provide information for mushroom species identification, conservation, and utilization, as well as the development of a nutritional database for local mushrooms. This will benefit science, enhance the economic value of

local mushrooms, and offer more diverse options for mushroom consumption.

2. Materials and Methods

Chemicals used in the study

Folic acid standard (pteroylglutamic acid; $\geq 98\%$ purity; Sigma-Aldrich, Germany), Cobalamin (vitamin B12) standard (Sigma-Aldrich, Germany), β -Glucan standard (from yeast; Sigma-Aldrich, Germany), formic acid, potassium dihydrogen phosphate, sodium carbonate (Gammaco, Thailand), Hydrochloric acid, AR/RPE for analysis (Carlo Erba, Italy), ethanol AR grade, boric acid, sulfuric acid, sodium sulfate, copper sulfate, selenium dioxide, methyl red (New Zealand), methanol for HPLC (USA) and acetonitrile for HPLC (USA).

Preparation of Standard Solution of Vitamin B9 (Folic Acid)

Weigh 1.00 g of vitamin B9 (folic acid standard) using an analytical balance (AG 240, Mettler Toledo, Switzerland). Dissolve the folic acid in 0.1 N sodium hydroxide and transfer the solution quantitatively into a 1,000 mL volumetric flask. Dilute to volume with 0.1 N sodium hydroxide to obtain a stock standard solution of vitamin B9 (folic acid) at a concentration of 1,000 mg L⁻¹.

To prepare working standard solutions of vitamin B9 (folic acid) at concentrations of 0.05, 0.10, 0.50, 1.0, 2.0, 3.0, 4.0 and 5.0 mg L⁻¹, pipette 0.125, 0.25, 1.25, 2.50, 5.00, 7.50, 10.00 and 12.50 mL, respectively, of the stock standard solution (10 mg L⁻¹) into separate 25 mL volumetric flasks and dilute to volume with 0.1 N sodium hydroxide.

Preparation of β -Glucan standard Solution

Weigh 0.05 g of β -Glucan standard and dissolve it in deionized water. Adjust the volume to 100 mL with deionized water. Heat the solution at 80 °C for 30 min to obtain a β -Glucan standard solution with a concentration of 500 mg L⁻¹ in a volume of 100 mL. Preparation of working standard solutions prepare β -Glucan standard solutions at concentrations of 70, 80, 90, 100, 250, 300, and 400 mg L⁻¹ by pipetting 0.7, 0.8, 0.9, 1.0, 2.5, 3.0, and 4.0 mL of the 500 mg L⁻¹ β -Glucan standard solution, respectively adjust each volume to 5 mL with deionized water.

Sample Preparation for Folic Acid Analysis

Mushroom samples were thoroughly cleaned and finely chopped. The samples were first sun-dried until completely dry and then oven-dried at 60 °C. The dried samples were stored in a desiccator until cooled to room temperature and subsequently ground to a fine powder using a mortar and pestle. The powdered samples were stored in a desiccator until further analysis.

Sample Preparation for β -Glucan Analysis

Collect samples of each mushroom species, retaining only the edible portions. Wash the samples with clean water and cut them into small pieces. Dry the samples in sunlight until partially dried, then further dry them in a hot air oven

at 45 °C until completely dry. Transfer the dried samples to a desiccator to cool, then grind them finely using a mortar

and pestle. Place the powdered mushroom samples in zip-lock bags and store them in a desiccator until analysis.

Table 1 High-Performance Liquid Chromatography (HPLC) operating conditions for folic acid and β -glucans analysis.

Type	Condition of the High-Performance Liquid Chromatography	
	Folic Acid	β -glucans
HPLC	LC10AvP, Shimadzu	LC10Av, Shimadzu
Mobile phase	1% Acetic acid: Acetonitrile (80 : 20)	Deionized water (DI water): Methanol and Acetonitrile 1:1 (60:40)
Column	ODS C18 (250 mm×4.6 mm, 5 μ m Particle size)	ODS C18 (250 mm × 4.6 mm, 5 μ m Particle size)
Flow rate	0.5 mL min ⁻¹	1.0 mL min ⁻¹
Detector	UV Detector, wavelength 280 nm	UV Detector, wavelength 320 nm
Injection volume	20 μ L	20 μ L
Post running	20 min	5 min

Extraction and Analytical Method for Folic Acid Determination

A finely ground sample (1.00 g) was mixed with 5 mL of phosphate buffer (pH 7.0), prepared from 0.25 mol L⁻¹ sodium phosphate and 0.37 mol L⁻¹ potassium phosphate. The mixture was shaken on a rotary shaker for 30 min and then centrifuged at 3,000 rpm for 15 min. The supernatant was filtered through a 0.45 μ m membrane filter and analyzed by high-performance liquid chromatography (HPLC) in Table 1 with analyzed in three replicates (n = 3) [10].

Extraction and Analytical Method for β -Glucan Determination

A finely ground sample (1.00 g) was mixed with 25 mL of distilled water. The pH was adjusted to 7.0 using 20% (w v⁻¹) Na₂CO₃, and the mixture was heated at 90 °C for 1 h. The extract was centrifuged at 8,000 rpm for 10 min, and the clear supernatant was collected. The pH of the supernatant was then adjusted to 4.5 with 2 M HCl and centrifuged again at 8,000 rpm for 10 min. Ethanol was added to the resulting clear supernatant at a 1:1 (v v⁻¹) ratio, and the mixture was stored at 4 °C for 12 h. Centrifuge at 3,000 rpm for 10 min, then dry the precipitate in a hot-air oven at 60 °C and cool to room temperature. Redisperse the residue in 25 mL of deionized water and filter successively through Whatman No. 1 filter paper and a cellulose membrane filter. Analyze the filtrate by high-performance liquid chromatography (HPLC) [11]. Before HPLC analysis, degas the sample using an ultrasonic bath with analyzed in three replicates (n = 3).

Protein Analysis Method

Mushroom samples (1.00 g) were digested using a Buchi Digestion Unit K with a selenium catalyst mixture (Na₂SO₄ 3.5% + CuSO₄ 3.5% + SeO₂ 0.5%) and 15 mL of concentrated H₂SO₄ in Buchi tubes. The tubes were placed in the digestion unit and heated for approximately 45 min, or until the digest became clear. The digested samples were cooled, transferred to PE bottles, and kept for subsequent

distillation, following the general Kjeldahl principle for crude protein determination.

For distillation (Buchi Unit K), 50 mL of 2% boric acid solution containing methyl red indicator was prepared as the receiving solution, in line with boric acid trapping used in standard Kjeldahl procedures. The digested sample was diluted with 50 mL of distilled water, and 100 mL of 32% NaOH was then added (or until the solution turned alkaline and changed color). Steam distillation was carried out for 3–4 min. The released ammonia was collected in the boric acid solution and titrated with standardized 0.1 N HCl to the indicator end point as shown in equation (1).

Nitrogen content was calculated from the volume of HCl consumed, and crude protein was obtained by multiplying nitrogen by a conversion factor of 6.25 [12, 13]. Each sample was analyzed in triplicate (n = 3); only data with a relative deviation below 5% were used to calculate the mean protein content (%) of the mushroom samples as shown in equation (2).

$$N \text{ (g\%)} = \frac{(0.1N \text{ HCl sample} - 0.1N \text{ HCl blank}) \times 0.0014 \times N \text{ HCl} \times 100}{\text{Weight of sample}} \quad (1)$$

$$\text{Protein (g per 100g)} = \frac{\% \text{ total nitrogen} \times \text{appropriate nitrogen conversion factor}}{\text{factor}} \quad (2)$$

Fiber Analysis Method

Place the defatted sample in a beaker and add 200 mL of 0.1275 M concentrated H₂SO₄. Boil for 30 min, then filter using a vacuum apparatus. Return the residue to the beaker and add 200 mL of 0.313 M concentrated NaOH. Boil for 30 min, then filter using a vacuum apparatus and return the residue to the beaker. Wash the residue with 1% HCl, followed by hot water until acid-free. Wash the residue twice with 20 mL of 95% C₂H₅OH. Place the residue on pre-dried filter paper and dry in an oven at 105 °C until constant weight. Incinerate the residue at 550 °C until white ash is obtained allow to cool and weigh [14] with analyzed in three replicates (n = 3).

Statistical analysis

Statistical tests used in this evaluation and study include accuracy, precision, linearity, range, limit of detection (LOD), and limit of quantitation (LOQ), along with related statistics for method validation, such as the mean ($\bar{x}$) and standard deviation (SD), based on five replicate determinations.

Accuracy (%Recovery)

Recovery was used to evaluate the accuracy of the method. A sample of known type and volume is added to a standard solution of known concentration and volume, and 3 replicate analyses were conducted. The percent recovery was then calculated as shown in equation (3);

$$\% \text{Recovery} = \frac{a \times 100}{b} \quad (3)$$

Where a is experimental value and b is Theoretical value.

Precision

The precision of the method was evaluated by replicate analysis of the sample. Analyzed samples ($n = 3$) were used to calculate the standard deviation (SD) and the percentage relative standard deviation (%RSD) according to the following equations as shown in equations (4–6);

$$\text{Mean } \bar{x} = \frac{\sum x_i}{n} \quad (4)$$

$$\text{SD} = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}} \quad (5)$$

$$\% \text{RSD} = \frac{\text{SD}}{\bar{x}} \times 100 \quad (6)$$

Where SD is standard deviation and $\bar{x}$ is Theoretical value.

Determination of the lowest analytical concentration in the limit of detection (LOD) and the analytical minimum concentration in the limit of quantitation (LOQ).

LOD and LOQ under the proposed chromatographic conditions were determined by serially diluting the working standard solution to reach analyte concentrations corresponding to signal-to-noise ratios of approximately 3:1 for LOD and 10:1 for LOQ, respectively. The precision of the method was evaluated by repeated analysis of samples, calculating the standard deviation (SD) and relative standard deviation (%RSD) to assess variability as shown in equations (7–8);

$$\text{Limit of Detection (LOD)} = 3 \times \text{SD} \quad (7)$$

$$\text{Limit of Quantification (LOQ)} = 10 \times \text{SD} \quad (8)$$

Where SD is standard deviation.

3. Results and Discussion

The study investigated the morphology and analyzed the content of folic acid, β -glucans, protein, and fiber in 25 species of local mushrooms from Loei Province Table 2.

Using a High-Performance Liquid Chromatograph. The results showed that each mushroom species had distinct morphological characteristics, such as differences in the cap, stem, gills, flesh, and special features like the volva and annulus, which can be used as effective criteria for species identification and reflect the biodiversity and adaptation of local mushrooms to different environments.

The analysis found folic acid content ranging from 0.01–0.15 mg 100 g⁻¹ ($R^2=0.9968$, %RSD=0.0018%, LOD=2.0371, LOQ=5.2389, 89.31% recovery), β -glucans from 1.83–11.97 mg g⁻¹ ($R^2=0.9970$, %RSD=0.51%, LOD=4.8294, LOQ=16.0980, 109.29% recovery), protein from 1.31–35.88%, and fiber from 0.15–9.49%. The highest folic acid was found in the *C. fimetarius*, the highest β -glucans in the *L. squarrosulus*, the highest protein in the *T. plumbeoviolaceus*, and the highest fiber in the *P. Indusiatus* as shown in (Fig. 4). This statement aligns with international research reporting that wild and cultivated mushrooms exhibit nutritional composition diversity depending on species and environmental factors [15, 16].

The findings are consistent with international studies, which report that wild and cultivated mushrooms exhibit significant variation in nutritional composition, including protein, fiber, and bioactive compounds, depending on species and environmental factors for example, protein content in mushrooms can range widely, and fiber and beta-glucan are prominent components that contribute to their nutritional value. The diversity in morphology and composition among local mushrooms highlights their potential as sources of nutrients and functional food ingredients, supporting their use in developing new food products and dietary supplements. Studies show that protein and amino acid contents in wild mushrooms depend primarily on species, while environmental factors such as soil type, C/N ratio, and soil pH exert lesser influence. Protein in some species remains largely unaffected by these conditions [17] supporting the idea that morphological and nutritional diversity reflects mushrooms environmental adaptations.

This study stands out as the first in Loei Province to integrate taxonomy and morphological characteristics of native mushrooms with chemical and nutritional composition analysis using the same sample set, allowing for species-specific data presentation rather than aggregated averages across mushroom groups. Folic acid quantification in wild mushrooms from Thailand's Northeastern region, particularly tropical forest species, remains scarce in national nutritional databases. Thus, this research establishes baseline data for folic acid levels in Loei native mushrooms, which is valuable for assessing nutritional value in populations relying on wild mushrooms as primary food sources. Moreover, β -glucan content in mushrooms is influenced by environmental factors such as temperature, humidity, and host plant species. By focusing on Loei high-elevation mountainous areas with cooler temperatures and unique dry evergreen and dipterocarp forest ecosystems this study may reveal distinct β -glucan levels compared to research from other regions, providing novel insights relevant to pharmacology and the nutraceutical industry.

Finally, clear species-specific reporting of protein and fiber content supports the development of functional foods and promotes food security in Loei communities and other Northeastern highland areas in tangible ways.

Table 2 25 species of indigenous mushrooms in Loei Province.

No.	Samples	Scientific name	Family	Morphological characteristics	
				External	Internal
1		<i>Amanita hemibapha</i>	Amanitaceae	Pileus pale yellow; stipe whitish yellow, with a volva present at the basal portion; 9–11 cm in length.	Context creamy white; stipe hollow and elongated.
2		<i>Amanita princeps</i>	Amanitaceae	Pileus white; basidioma larger than that of Amanita (yellow form); 10–14 cm in length.	Context white; stipe hollow and elongated.
3		<i>Astraeus hygrometricus</i>	Diplocystaceae	Basidioma globose, black, brown, or white depending on soil coloration; 1–3 cm in length.	Interior with a two-layered peridium; context white in immature basidiomata, turning black at maturity.
4		<i>Boletus edulis</i>	Boletaceae	Pileus yellowish brown, surface rough; stipe robust, yellow; 8–10 cm in length.	Context white, coarse; stipe solid; spore print yellow.
5		<i>Boletellus emodensis</i>	Boletaceae	Basidioma reddish brown, turning greenish brown upon handling; pileus globose; stipe elongated, 10–12 cm in length.	Context bluish green; flesh coarse; stipe solid; spore print yellowish brown.
6		<i>Chiua virens</i>	Boletaceae	Pileus large, brown, with a rough surface; stipe reddish brown; 8–11 cm in length.	Context of the pileus pale yellowish white; stipe context whitish brown; flesh coarse in texture.

Table 2 25 species of indigenous mushrooms in Loei Province (continues).

No.	Samples	Scientific name	Family	Morphological characteristics	
				External	Internal
7		<i>Coprinus fimetarrius</i>	Psathyrellaceae	Pileus grayish brown, bearing white scales; stipe white; at full maturity the pileus becomes black and water-soaked; 6–9 cm in length.	Context white; stipe hollow and elongated.
8		<i>Craterellus odoratus</i>	Tricholomaceae	Pileus bright yellow, surface smooth; stipe creamy white; 5–7 cm in length.	Young basidioma with pale yellowish white context; stipe white, hollow, and elongated.
9		<i>Galiella rufa</i>	Sarcosomataceae	Young basidiomata gray, with a depressed disc that is brownish orange; mature basidiomata black, bearing fine hairs around the margin; texture soft; 4–6 cm in length.	Context gelatinous; inner surface of the pileus yellow; basidioma containing a higher moisture content than other species.
10		<i>Gyroporus cyanescens</i>	Gyroporaceae	Pileus brownish purple; stipe robust, with a yellow base; 7–10 cm in length.	Context white; flesh coarse; spore print brownish purple.
11		<i>Heimioporus japonicus</i>	Boletaceae	Pileus reddish orange; stipe red, robust, longitudinally striate with red fibers throughout, 14–17 cm in length.	Context pale yellow; stipe context reddish brown; flesh coarse; spore print yellow.
12		<i>Lentinus polychrous</i>	Polyporaceae	Pileus brown, surface with short hairs; basidioma sticky; gills creamy white; 6–9 cm in length.	Context grayish white, surface smooth; stipe sticky.
13		<i>Lentinus squarrosulus</i>	Polyporaceae	Pileus light brown, bearing white or pale brown scales; 6–8 cm in length.	Context yellowish white; stipe solid and sticky.

Table 2 25 species of indigenous mushrooms in Loei Province (continues).

No.	Samples	Scientific name	Family	Morphological characteristics	
				External	Internal
14		<i>Phallus indusiatus</i>	Phallaceae	Pileus yellow, covered with grayish-brown spores over the entire surface; stipe elongated, bearing a volva and a net-like structure surrounding the stipe; basidioma emitting a fetid odor; 12–15 cm in length.	Context white; stipe hollow, elongated, and spongy in texture; volva gelatinous and mucilaginous.
15		<i>Russula alboareolata</i>	Russulaceae	Basidioma white; gills pale yellow; pileus bearing white scales; 5–7 cm in length.	Context white; stipe interior containing soft, white, farinose material.
16		<i>Russula cyanoxantha</i>	Russulaceae	Pileus purple or green, thin; stipe white, 6–8 cm in length.	Context white; stipe hollow and elongated; interior of the stipe containing soft, white, powdery material.
17		<i>Russula emetic</i>	Russulaceae	Pileus reddish pink, with a red depressed center; pileus thin; basidioma small-sized; 4–6 cm in length.	Context white; stipe usually hollow and elongated; interior of the stipe containing soft, white, farinose material.
18		<i>Russula foetens</i>	Russulaceae	Pileus yellowish brown, surface smooth, slightly viscid; 8–10 cm in length.	Context yellowish white; stipe hollow and elongated; cavity containing brown, powdery material.
19		<i>Russula nigricans</i>	Russulaceae	Basidioma grayish white when on the ground, turning black like charcoal upon handling.	Context reddish brown; flesh coarse; stipe solid.

Table 2 25 species of indigenous mushrooms in Loei Province (continues).

No.	Samples	Scientific name	Family	Morphological characteristics	
				External	Internal
20		<i>Russula rosacea</i>	Russulaceae	Pileus bright red; stipe whitish red; basidioma firm and thick; 8–10 cm in length.	Context white, surface coarse; stipe solid.
21		<i>Russula virescens</i>	Russulaceae	Pileus whitish yellow or green; stipe robust; surface smooth; basidioma with a pleasant aroma; 5–7 cm in length.	Context white; stipe solid in immature basidiomata, becoming slightly hollow at maturity; interior containing soft, white, farinose material.
22		<i>Schizophyllum commune</i>	Schizophyllaceae	Basidioma white, small-sized; stipe short; basidioma tough in texture; pileus fan-shaped; 1–2 cm in length.	Lamellae elongated and decurrent, extending from the pileus to the stipe; context white; basidioma tough in texture.
23		<i>Termitomyces fuliginosus</i>	Lyophyllaceae	Pileus light brown, with a dark brown, pointed umbo at the center; stipe elongated, 10–12 cm in length.	Context white; stipe hollow and elongated; surface smooth.
24		<i>Thaeogyroporus porentosus</i>	Boletaceae	Pileus convex to planoconvex, dark brown with pale yellowish tones, surface shiny; context firm, dark brown; stipe robust; immature basidiomata with fine, velvety brown hairs; 12–15 cm in length.	Context pale yellow; spongy in texture.
25		<i>Tylopilus plumbeoviolaceus</i>	Boletaceae	Pileus purplish gray; stipe robust, whitish purple; basidioma firm; 6–9 cm in length.	Context white; flesh coarse; stipe solid; spore print whitish yellow.

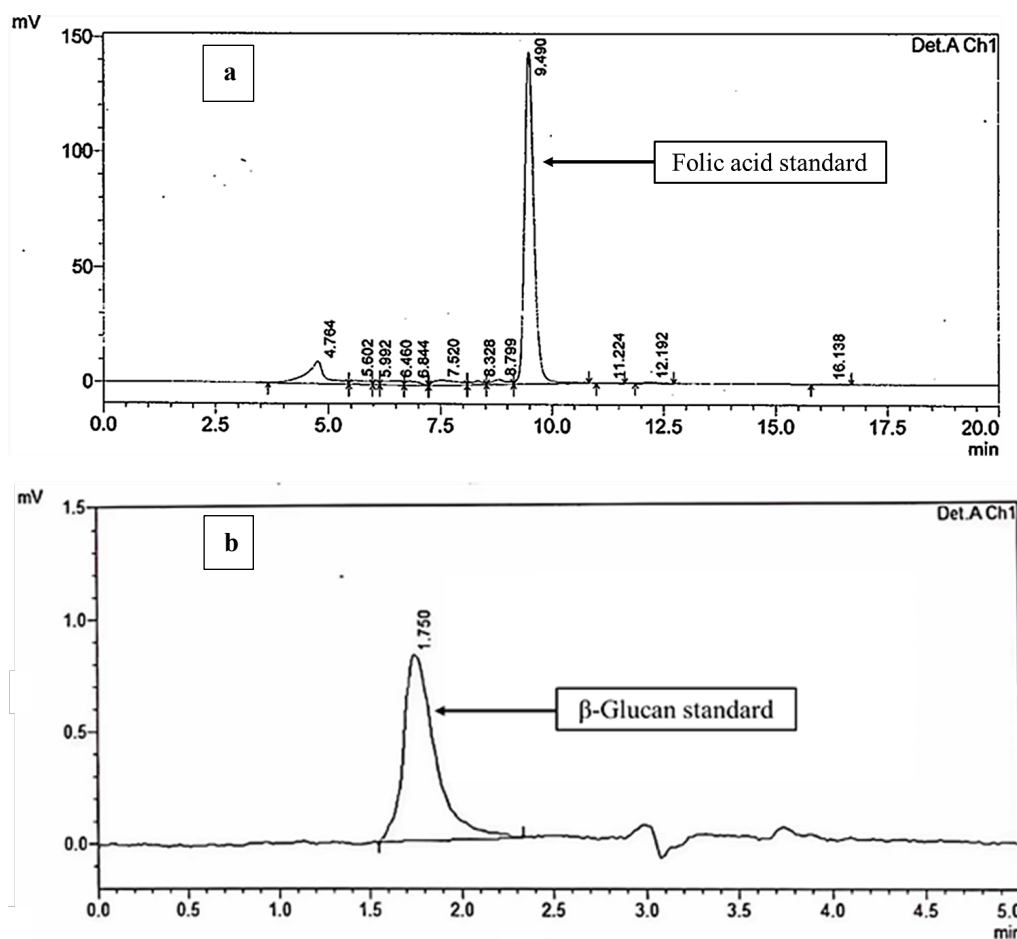


Fig. 3 HPLC chromatogram for (a) folic acid reference standard and (b) β -glucans reference standard.

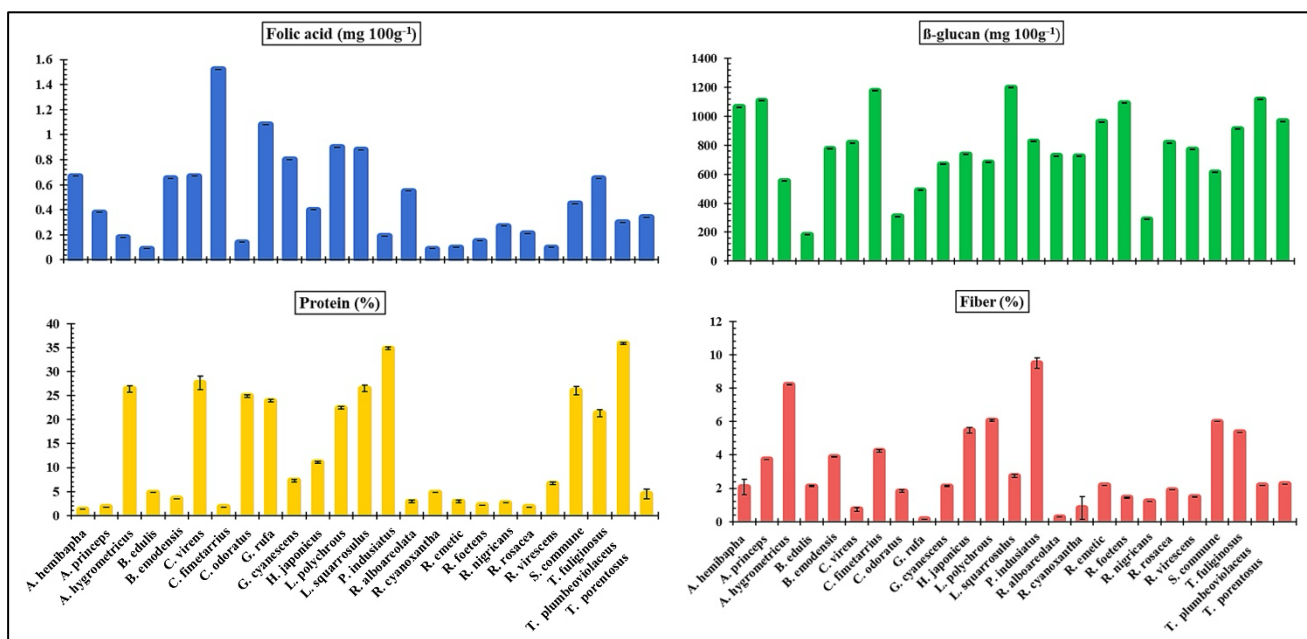
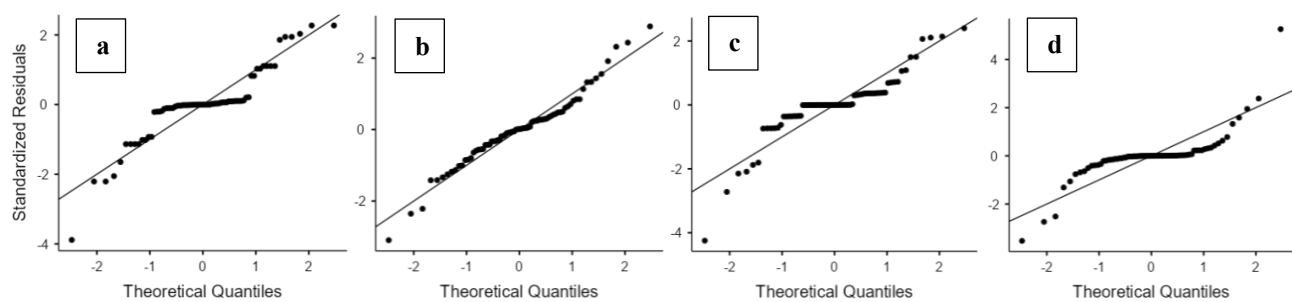


Fig. 4 Diagram illustrating the contents of folic acid, β -glucans, protein, and fiber in local edible mushrooms from Loei Province.

Table 3 Results of the analysis for the amounts of folic acid, β -glucans, protein, and fiber in wild mushrooms from Loei Province and Mean $\pm$ SD of nutritional composition of 25 wild mushroom species (n = 3 replicates per species). Different superscript letters within each column indicate statistically significant differences (Tukey HSD, $p < 0.05$).

No.	Samples (scientific name)	Folic acid (mg 100 g ⁻¹)	β -glucan (mg 100 g ⁻¹)	Protein (%)	Fiber (%)
1	<i>A. hemibapha</i>	0.67 $\pm$ 0.00 ^f	1064.03 $\pm$ 0.04 ^c	1.31 $\pm$ 0.00 ^j	2.07 $\pm$ 0.46 ^g
2	<i>A. princeps</i>	0.38 $\pm$ 0.00 ^r	1107.92 $\pm$ 0.06 ^k	1.73 $\pm$ 0.00 ^b	3.72 $\pm$ 0.00 ^b
3	<i>A. hygrometricus</i>	0.18 $\pm$ 0.00 ^l	554.23 $\pm$ 0.28 ^b	26.39 $\pm$ 0.67 ⁱ	8.22 $\pm$ 0.01 ^e
4	<i>B. edulis</i>	0.09 $\pm$ 0.00 ^u	183.46 $\pm$ 0.04 ^g	4.81 $\pm$ 0.00 ^h	2.13 $\pm$ 0.03 ^g
5	<i>B. emodensis</i>	0.65 $\pm$ 0.00 ^h	776.93 $\pm$ 0.07 ⁿ	3.49 $\pm$ 0.00 ^h	3.88 $\pm$ 0.01 ^e
6	<i>C. virens</i>	0.67 $\pm$ 0.00 ^a	816.05 $\pm$ 0.08 ^a	27.64 $\pm$ 1.47 ⁱ	0.73 $\pm$ 0.10 ^e
7	<i>C. fimetarius</i>	1.52 $\pm$ 0.00 ^s	1176.56 $\pm$ 0.17 ^m	1.75 $\pm$ 0.00 ^c	4.24 $\pm$ 0.08 ^g
8	<i>C. odoratus</i>	0.14 $\pm$ 0.00 ^f	308.80 $\pm$ 0.10 ^f	24.90 $\pm$ 0.24 ^b	1.83 $\pm$ 0.07 ⁱ
9	<i>G. cyanescens</i>	0.80 $\pm$ 0.00 ^c	668.46 $\pm$ 0.06 ⁱ	7.29 $\pm$ 0.25 ^g	2.12 $\pm$ 0.03 ^g
10	<i>G. rufa</i>	1.08 $\pm$ 0.00 ^b	491.79 $\pm$ 0.39 ^l	23.91 $\pm$ 0.26 ^d	0.15 $\pm$ 0.00 ^j
11	<i>H. japonicus</i>	0.40 $\pm$ 0.00 ^k	737.85 $\pm$ 0.18 ^g	11.09 $\pm$ 0.25 ^f	5.47 $\pm$ 0.17 ^d
12	<i>L. polychrous</i>	0.90 $\pm$ 0.00 ^c	683.27 $\pm$ 0.15 ^h	22.45 $\pm$ 0.22 ^d	6.06 $\pm$ 0.07 ^c
13	<i>L. squarrosulus</i>	0.88 $\pm$ 0.00 ^d	1197.41 $\pm$ 0.03 ^a	26.50 $\pm$ 0.69 ^b	2.74 $\pm$ 0.10 ^f
14	<i>P. indusiatus</i>	0.19 $\pm$ 0.00 ^q	828.05 $\pm$ 0.11 ^f	34.85 $\pm$ 0.25 ^a	9.49 $\pm$ 0.31 ^a
15	<i>R. alboareolata</i>	0.55 $\pm$ 0.00 ⁱ	727.32 $\pm$ 0.06 ^g	2.91 $\pm$ 0.25 ⁱ	0.28 $\pm$ 0.00 ⁱ
16	<i>R. cyanoxantha</i>	0.09 $\pm$ 0.00 ^t	726.26 $\pm$ 0.30 ^h	4.80 $\pm$ 0.00 ^h	0.82 $\pm$ 0.67 ⁱ
17	<i>R. emetic</i>	0.10 $\pm$ 0.00 ^t	961.62 $\pm$ 0.05 ^d	2.91 $\pm$ 0.25 ⁱ	2.17 $\pm$ 0.00 ^f
18	<i>R. foetens</i>	0.15 $\pm$ 0.00 ^s	1092.93 $\pm$ 0.28 ^b	2.17 $\pm$ 0.00 ⁱ	1.43 $\pm$ 0.04 ^h
19	<i>R. nigricans</i>	0.27 $\pm$ 0.00 ^o	290.10 $\pm$ 0.12 ^m	2.62 $\pm$ 0.00 ⁱ	1.21 $\pm$ 0.00 ^h
20	<i>R. rosacea</i>	0.21 $\pm$ 0.00 ^p	816.05 $\pm$ 0.18 ^f	1.75 $\pm$ 0.00 ⁱ	1.90 $\pm$ 0.00 ^g
21	<i>R. virescens</i>	0.10 $\pm$ 0.00 ^t	771.30 $\pm$ 0.06 ^g	6.70 $\pm$ 0.25 ^g	1.48 $\pm$ 0.03 ^h
22	<i>S. commune</i>	0.45 $\pm$ 0.00 ^j	614.10 $\pm$ 0.18 ^j	26.05 $\pm$ 0.83 ^c	5.99 $\pm$ 0.00 ^c
23	<i>T. fuliginosus</i>	0.65 $\pm$ 0.00 ^g	912.18 $\pm$ 0.11 ^e	21.27 $\pm$ 0.74 ^c	5.36 $\pm$ 0.00 ^d
24	<i>T. plumbeoviolaceus</i>	0.30 $\pm$ 0.00 ⁿ	1117.87 $\pm$ 0.07 ^d	35.88 $\pm$ 0.24 ^h	2.18 $\pm$ 0.00 ^f
25	<i>T. porentosus</i>	0.34 $\pm$ 0.00 ^m	965.99 $\pm$ 0.10 ^b	4.50 $\pm$ 1.03 ^a	2.25 $\pm$ 0.00 ^f
F-value		229226.448	894.333	1820.194	521.270
p-value		< 0.001	< 0.001	< 0.001	< 0.001

SD, standard deviation; ANOVA, one-way analysis of variance [18].

**Fig. 5** Assumption Checks plots of (a) folic acid, (b) β -glucans, (c) protein, and (d) fiber

From Table 3, One-way analysis of variance (one-way ANOVA) was used to compare the nutritional components (folic acid, β -glucan, protein, and fiber) among 25 wild mushroom species (n = 3 replicates per species). Post-hoc comparisons were performed using Tukey's honestly

significant difference (Tukey HSD) test, with statistical significance set at $p < 0.05$. Mushroom species had a significant effect on folic acid, β -glucan, protein, and fiber contents ($F = 229226.448, 894.333, 1820.194$, and 521.270 , respectively; $p < 0.001$ for all variables). Values with

different superscript letters within the same column indicate statistically significant differences at $p < 0.05$ according to the Tukey HSD test (Fig 5). Among the investigated species, *P. indusiatus* showed the highest protein and fiber contents (34.85 ± 0.25 mg 100 g^{-1} and 9.49 ± 0.31 mg 100 g^{-1} , respectively), with distinct superscript letters indicating significant differences from many other species.

4. Conclusion

The study of morphology and analysis of folic acid, β -glucans, protein, and fiber content in 25 species of local mushrooms from Loei Province revealed that morphological features such as cap, gills, stipe, and stipe base are fundamental for clear species identification and classification. Morphological characteristics also help communities distinguish between edible and poisonous mushrooms, which is crucial for food safety, as some edible species have toxic relatives within the same genus. The analysis showed that the highest folic acid content was found in the *C. fimetarius*. (1.52 mg 100 g^{-1}), while the lowest was in several species such as *R. virescens.*, *R. emetic.*, *B. edulis.*, *C. odoratus.*, *R. foetens.*, and *R. cyanoxantha* (0.09 mg 100 g^{-1}). The highest β -glucans content was found in the *L. squarrosulus* (1197.41 mg 100 g^{-1}), and the lowest in the *B. edulis* (183.41 mg 100 g^{-1}). The highest protein content was in the *T. plumbeoviolaceus* (35.88%), and the lowest in the *A. hemibapha*. (1.31%) The highest fiber content was in the *P. indusiatus* (9.49%), and the lowest in the *G. rufa* (0.15%). The diversity in morphology and composition among these mushrooms reflects their adaptation to different environments and highlights the ecological diversity of the region. Morphological studies not only aid in species identification but also support conservation and economic utilization, especially for popular species. The variation in folic acid, β -glucans, protein, and fiber content among mushroom species is influenced by genetic factors, physiological composition, nutrient accumulation capacity, environmental temperature, humidity, light, species differences, growth stage, and soil fertility. These findings are consistent with international research, which shows that mushroom morphology and nutritional composition vary significantly by species and environment, and that proper identification is essential for safe consumption and economic development.

5. Acknowledgement

This research work was supported by Thailand Science Research and Innovation (TSRI) (No.6309.FB1.2/160/2567-FRB670034/0214-4695979) and Chemistry program, Faculty of Science and Technology, Loei Rajabhat University.

6. Declaration of Generative AI in Scientific Writing

The authors acknowledge that generative AI tools were used to improve language clarity, grammar, and formatting in this manuscript. No content, data interpretation, or results were generated or altered by AI.

7. CRediT Author Statement

Niramol Srichana: Conceptualization, Validation, Methodology, Formal analysis, Data analysis, Writing Original Draft **Suwatchai Misuna:** Software, Data Curation **Panruethai Putthongsri:** Resources **Rasamee Nontee:** Resources

8. Research Involving Human and Animals Rights

Not applicable

9. Ethics Approval and Consent to Participate

Not applicable

10. Declaration of Competing Interest

The authors declare that they have no conflict of interest.

11. References

- [1] Chanluang, S., Nilatawong, P., Sutheerapongs, P., Cherdkoksri, K., Mitwong, W., & Kaewamatawong, R. (2017). Amount of protein, crude fiber and β -glucan, and anti-oxidative activity in edible wild mushrooms used as food in Ubon Ratchathani Province. *Journal of Science and Technology, Ubon Ratchathani University*, 19(Special Issue), 240-248.
- [2] Zhu, F., Du, B., Bian, Z., & Xu, B. (2015). Beta-glucans from edible and medicinal mushrooms: Characteristics, physicochemical and biological activities. *Journal of Food Composition and Analysis*, 41, 165-173. <https://doi.org/10.1016/j.jfca.2015.01.019>
- [3] Kupradit, C., Ranok, A., Mangkalanon, S., Khongla, C., & Musika, S. (2023). β -glucan and antioxidant activities of four edible mushroom extracts from Thailand. *Asian Journal of Agriculture and Biology*, 1, 202107285. <https://doi.org/10.35495/ajab.2021.07.285>
- [4] Benchawattananon, R. (2016). Biodiversity of mushrooms in conservative forest in Dansai District of Loei Province, Thailand. *Tropical Life Sciences Research*, 27(Supp. 1), 103–109. <https://doi.org/10.21315/tlsr2016.27.3.14>
- [5] Mironczuk-Chodakowska, I., Kujawowicz, K., & Witkowska, A. M. (2021). Beta-glucans from fungi: Biological and health-promoting potential in the COVID-19 pandemic era. *Nutrients*, 13(11), 3960. <https://doi.org/10.3390/nu13113960>
- [6] Cieslik, E., & Cieslik, I. (2018). Occurrence and significance of folic acid. *Pteridines*, 29(1), 187–195. <https://doi.org/10.1515/pteridines-2018-0017>
- [7] Singla, A., Gupta, O. P., Sagwal, V., Kumar, A., Patwa, N., Mohan, N., Ankush, Kumar, D., Vir, O., Singh, J., Kumar, L., Lal, C., & Singh, G. (2024). Beta-glucan as a soluble dietary fiber source: Origins, biosynthesis, extraction, purification, structural characteristics, bioavailability, biofunctional attributes, industrial utilization, and global trade. *Nutrients*, 16(6), 900. <https://doi.org/10.3390/nu16060900>
- [8] Inyod, T., Keawsompong, S., Payapanon, A., Lattirasuvan, T., Chawanoraset, K., Termarom, T., Konee, C., Yatsom, S., Bualoi, S., & Eamprasong, P. (2023). Factors influencing mycelial growth, yield, quality and β -glucan content of Hed Taen Rad (*Macrocybe crassa*). *Journal of Science and Technology, Ubon Ratchathani University*, 25(2), 71–86.

- [9] Suwanno, S., & Phiraporn, C. (2016). 1, 3- β -glucan content of local medicinal mushrooms from the southern region of Thailand. *Walailak Journal of Science and Technology (WJST)*, 15(3), 189–200.
- [10] Paucean, A., Moldovan, O. P., Muresan, V., Socaci, S. A., Dulf, F. V., Alexa, E., Man, S. M., Mureşan, A. E., & Muste, S. (2018). Folic acid, minerals, amino-acids, fatty acids and volatile compounds of green and red lentils. Folic acid content optimization in wheat-lentils composite flours. *Chemistry Central Journal*, 12, 88. <https://doi.org/10.1186/s13065-018-0456-8>
- [11] Al-Saffar, A. Z., Hadi, N. A., & Khalaf, H. M. (2020). Antitumor activity of β -glucan extracted from *Pleurotus eryngii*. *Indian Journal of Forensic Medicine & Toxicology*, 14(4), 2214–2221. <https://doi.org/10.37506/ijfmt.v14i4.11880>
- [12] Puwastien, P., Siong, T. E., Kantasubrata, J., Craven, G., Feliciano, R. R., & Judprasong, K. (2011). *ASEAN manual of food analysis*. Institute of Nutrition, Mahidol University.
- [13] AOAC. (1990). *Official Methods of Analysis of AOAC International* (15th ed.). AOAC, Association of Official Analytical Chemists.
- [14] AOAC. (2000). *Official Methods of Analysis of AOAC International* (17th ed.). AOAC International.
- [15] Kalac, P. (2013). A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. *Journal of the Science of Food and Agriculture*, 93(2), 209–218. <https://doi.org/10.1002/jsfa.5960>
- [16] Dimopoulou, M., Kolonas, A., Mourtakos, S., Androutsos, O., & Gortzi, O. (2022). Nutritional composition and biological properties of sixteen edible mushroom species. *Applied Sciences*, 12(16), 8074. <https://doi.org/10.3390/app12168074>
- [17] Stojek, K., Bobrowska-Korczak, B., Frączek, J., Piotrowski, M., Krosniak, M., & Jaroszewicz, B. (2025). Protein content and amino acid profile of wild mushrooms depend on environmental conditions. *Fungal Biology*, 129(6), 101620. <https://doi.org/10.1016/j.funbio.2025.101620>
- [18] The jamovi project. (2025). *jamovi* (Version 2.7) [Computer Software]. <https://www.jamovi.org>.