

## Research article

# Nutrient content of selected wild edible mushrooms from Braha-kshetra Community Forest, Dang, West Nepal

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Submitted 25 February 2024; revised 30 November 2024; accepted 10 December 2024; published 2 June 2025

## Abstract

In the present study, four wild edible mushrooms (*Laccaria laccata*, *Lactarius volemus*, *Russula delica*, and *R. poichilochroa*) from Ghorahi, Dang, west Nepal were analyzed for nutrient and mineral contents. The moisture content of mushrooms varied from 5.16 to 5.51%, protein from 24.28 to 24.59%, fat from 2.20 to 2.97%, ash from 11.25 to 15.16%, and carbohydrates from 58.37 to 61.28%. The mineral content ranged 1.13–2.00 µg/g for Ca, 0.90–1.03 µg/g for Mg, 25.99–100.37 µg/g for Mn, 1.03–2.87 µg/g for Fe, 3.06–3.36 µg/g for K, 16.83–17.94 µg/g for Cu, 2.17–4.87 µg/g for P, and 39.61–60.56 µg/g for Zn. *Russula delica* exhibited a high amount of moisture, carbohydrates, and protein, followed by *Lactarius volemus* and *Russula poichilochroa*, whereas *Laccaria laccata* had the least amount of nutrients, except for ash. However, minerals such as Mn, Zn, Fe, P, Mg, and K were present in higher amounts in *L. laccata*, followed by *Lactarius volemus*, *Russula poichilochroa*, and *R. delica*. In conclusion, the four species of mushrooms can be used as important nutrient sources due to their high protein, carbohydrate, and mineral contents.

**Keywords:** Edible mushrooms, minerals content, samples, species, wild mushroom.

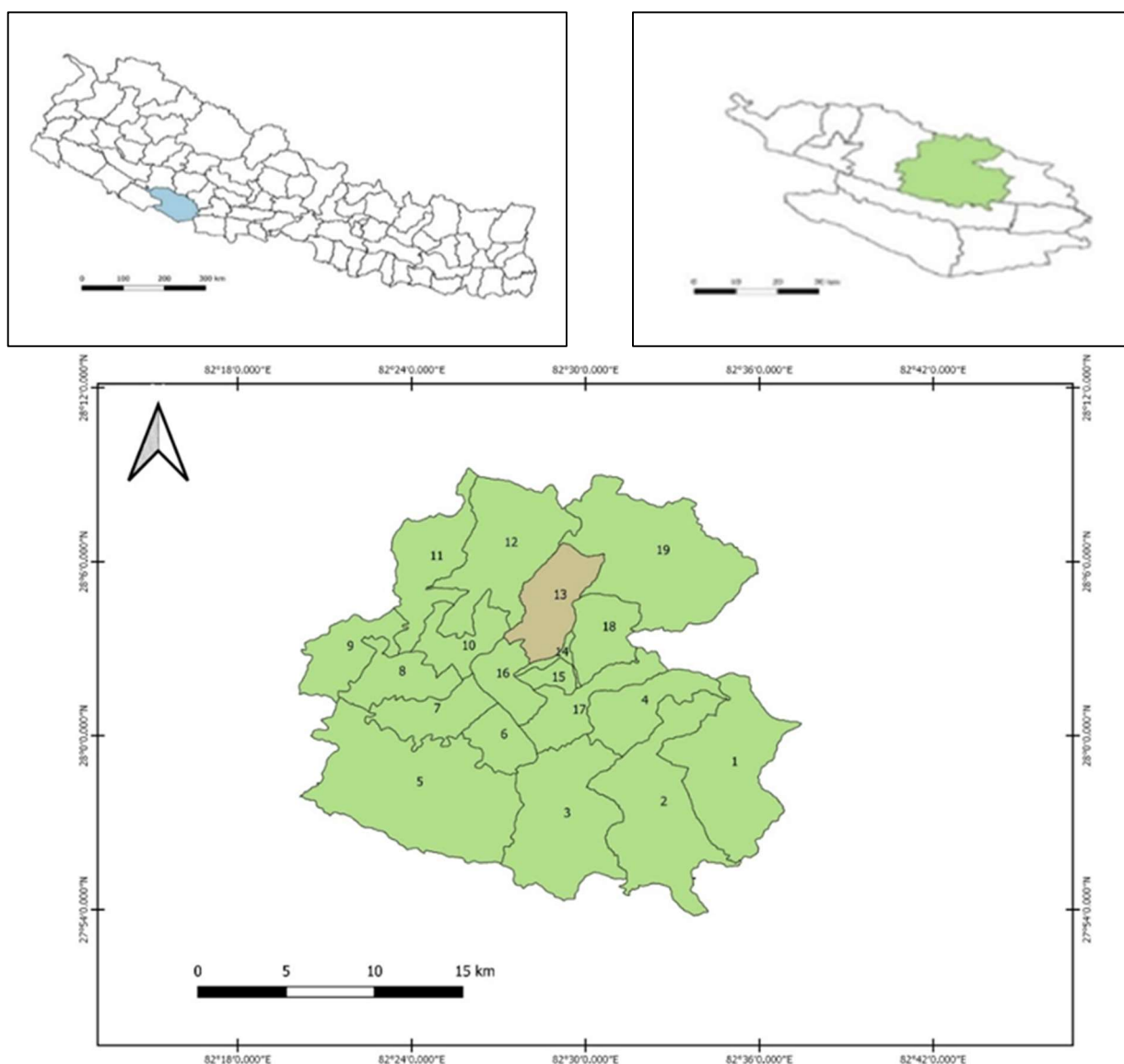
## Introduction

Mushrooms have long been associated with humankind, offering significant biological, sociocultural, and economic benefits (Singh and Manhas 2022). Mushrooms are highly acclaimed for their nutraceutical potential (Chang *et al.* 1981; Téllez-Téllez 2024). They are rich in proteins, carbohydrates, vitamins, minerals, amino acids, and fibers, but are poor in fat (Barros *et al.* 2008; Heleno *et al.* 2009). Due to their nutritional contents, mushrooms typically exhibit the characteristics of a nutrient-rich diet and are most importantly used as food to address the problem of malnutrition (Singh 2023). In general, mushroom fruiting bodies contain 33.9% carbohydrate, 17.5% protein, and 2.9% fats on a dry weight basis, with the remaining portion being minerals (Demirbas 2001). Because of their unique flavor and texture, the consumption of wild edible mushrooms is well established (Kalač 2009; Kumar *et al.* 2021).

Numerous mushroom species are found in nature, but only a few are used as food. They have even been reported as therapeutic food, useful in preventing diseases such as hypertension, hypercholesterolemia, and even cancer (Bobek and Galbavy 1999). Proteins in edible mushrooms have been reported to have a complete profile of essential amino acids (Ayimbila and Keawsonpong 2023). Mushrooms contain high levels of minerals that are essential for the human body. The ash content that gives a

general idea of the mineral content of mushrooms ranges from 6 to 10.9% on a dry weight basis (Cheung 2010). The macro-minerals such as calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), and sodium (Na), as well as micro-minerals such as copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) have been reported in wild edible mushrooms (Genççelep *et al.* 2009; Haro *et al.* 2020). However, mushrooms even contain large concentrations of some heavy metals (e.g., lead, cadmium, and mercury), and great efforts have been made to evaluate the possible dangers to human health from the ingestion of mushrooms containing such metals (Soylak 2005). Iron, copper, manganese, and zinc are essential metals because they play key roles in the biological system; whereas, lead and cadmium are non-essential metals as they are toxic even in trace amounts (Jomova *et al.* 2022). The mineral proportions vary greatly based on mushroom species, types of substrates, and environmental factors (Genççelep 2009). Factors such as growing habitat, types of substrates, mushroom types, and developmental stage may influence the nutritional composition of wild edible mushrooms (Diez and Alvarez 2001).

Despite their importance in human health and economy, wild edible mushrooms are less studied in Nepal, with only a few studies on their nutritional value. In the present study, we aim to explore the nutritional quality of some wild edible mushrooms naturally growing in the Brahakshetra Community Forest, Dang, southwestern Nepal.



**Figure 1.** Map showing study area.

## Materials and methods

### STUDY AREA

The Brahakshetra Community Forest lies in Gorahi-13 in the Dang District of Lumbini Province in southwestern Nepal. The elevation ranges from 710 to 1240 m above sea level. The study area occupies nearly 491.18 hectares of land with planted forest occupying 5.86 hectares. The summer monsoon influences the climate of the study area. The area receives an average annual rainfall of 1665 mm with an average minimum and maximum temperature of 17° and 29° C respectively (GoN 2019, 2024). *Shorea robusta* is the dominant tree species in the community forest, along with other components, including *Dalbergia sissoo*, *Diploknema butyracea*, *Syzygium cumini*, and *Pinus roxburghii*.

### SAMPLE COLLECTION AND PREPARATION

Fruiting bodies of four wild edible mushroom species [*Laccaria laccata* (Scop.) Cooke, *Lactarius volemus* (Fr.) Kuntze, *Russula*

*delica* Fr. and *Russula poichilochroa* Sarnari) belonging to two families (Hydnangiaceae and Russulaceae) were collected from the community forest during July and August 2020. The species were selected based on their edibility quality and availability (frequency) in the study area. The mushroom samples were cleaned of forest debris using a brush and knife, cut into small pieces, and dried in the shade for two weeks. After completely drying, the samples (*ca.* 100 g of each species) were packed in a zip-lock plastic bag and transported to the laboratory for nutrient analysis.

The collected samples were analyzed for nutrient contents according to the methods given by the Association of Official Analytical Chemists (AOAC 2005). The analyses included the determination of moisture, ash, fat, protein, carbohydrates, ash, and minerals.

### MOISTURE CONTENT

Moisture content was measured using the oven-dry method. A sample (2 g) of the air-dry mushroom was placed in a dry crucible and heated in a hot-air oven at 110°C for 4 hours. The heating

process was repeated until a constant weight was obtained. The oven-dried sample was then cooled in a desecrator, and its final weight was recorded after complete cooling. The moisture content was determined based on the weight loss during drying, using the following formula (Aryal 2015):

$$\text{Moisture \%} = \frac{\text{Loss of weight after drying}}{\text{Sample weight taken for analysis}} \times 100$$

#### ASH CONTENT

An air-dried sample (1 g), placed in a clean and dry porcelain crucible, was ignited in a muffle furnace at 550°C for four hours until all carbonaceous material was removed, causing the ash to change from white to grayish-white (Shrestha *et al.* 2023). After complete ignition, the crucible containing the ash was cooled in a desecrator, and its final weight was recorded. The ash content of the sample was calculated using the following formula:

$$\text{Total ash \%} = \frac{\text{Weight of ash after incineration}}{\text{Sample weight taken for analysis}} \times 100$$

#### FAT CONTENT

Soxhlet extraction procedure was used to determine the fat content. About 10 g of oven-dried powdered sample was placed in a thimble, with its opening plugged using cotton. A clean and dry round-bottom flask was weighed. The thimble with the sample was inserted into the extractor of the Soxhlet apparatus and attached to a pre-weighed flat-bottomed flask and a condenser. The sample was extracted using petroleum spirit. The apparatus was positioned on a heating mantle at 60°C, with continuous cold-water circulation. The solvent-containing flask was continuously heated and all the solvent evaporated and condensed in the Soxhlet extractor. The extraction process continued for seven hours. After extraction, the thimble was removed, and the flask containing the fat residue was dried in an oven at 103 ± 2°C for one hour. The fat content was determined by measuring the weight difference of the flat-bottomed flask before and after extraction. The increase in weight was used to calculate the fat content (%) in the sample.

$$\text{Fat \%} = \frac{M2 - M1}{E} \times 100$$

where, M1 = initial weight of the dry empty flat-bottomed flask, M2 = final weight of the dry empty flat-bottomed flask, and E = weight of the sample.

#### PROTEIN CONTENT

Kjeldahl digestion method was used to determine the protein content. About 2 g of the sample (in powdered form) was digested with 10 g of the digestion mixture (copper sulfate and sodium sulfate) and 10 ml of concentrated sulfuric acid, initially with gentle heating at a low temperature until the white mineral content was obtained. It was then heated at a higher temperature until the solution turned transparent blue and emitted white fumes. The mixture was heated further for 30 minutes and then cooled to room temperature. Once the digestion process was complete, the digestion flask was connected to a receiving flask via a tube. The solution in the digestion flask was distilled under a

strongly alkaline condition by adding sodium hydroxide, which converted ammonium sulfate into ammonia gas. The released ammonia gas excited the digestion flask and entered the receiving flask containing excess boric acid. After complete distillation, the collected distillate in the beaker was titrated against standard sulfuric or hydrochloric acid. The total nitrogen content was then calculated using the following formula, and the protein content was determined by multiplying the result by 6.25.

$$\text{Total nitrogen \%} = \frac{14 \times (V - V1) \times 100 \times S}{W \times 1000}$$

$$\text{Protein \%} = \text{Total nitrogen \%} \times 6.25$$

where, V = volume of standard acid used to neutralize the distillate, V1 = volume of standard acid used to neutralize the blank, S = normality of standard acid (strength), and W = weight of sample taken for digestion.

#### CARBOHYDRATE CONTENT

Total carbohydrate content (%) was determined as 100 minus the sum amount of all other elements (ash %, fat %, and protein %).

#### MINERALS

Mineral content was determined using atomic absorption spectrophotometer (AAS). Five grams of the air-dry mushroom sample was placed into a porcelain crucible and dried in a hot-air oven at 105°C for 3 hours. The dried sample was then ashed in a muffle furnace at 550°C until a white or grey ash residue was obtained. The residue was dissolved in 5 ml of concentrated nitric acid, followed by hydrochloric acid, and the mixture was heated slowly to ensure complete dissolution. The resulting solution was transferred to a volumetric flask and diluted to 50 ml. Elemental composition was then analyzed using a flame atomic absorption spectrometer (Perkin-Elmer model 175).

#### PHOSPHORUS

Phosphorus was determined by the spectrophotometric method. The ashed sample was treated with a 1:1 ratio of distilled water and HCl and filtered through medium-textured filter paper. About 5 ml of aliquot ash solution was treated with molybdovanadate reagent to develop a yellow molybdovanado-phosphoric acid complex. Finally, the absorbance of the sample color was measured by a spectrophotometer at 400 nm. The phosphorus content was calculated based on the observed absorbance.

#### DATA ANALYSIS

Tests of normality and equality of variance for the proximate composition and mineral content were performed. The variation in the mean values of the proximate composition and mineral content in the four edible mushrooms was assessed using one-way ANOVA (in case of normal data) or non-parametric Kruskal Wallis test (in case of non-normal data). The Tukey HSD test was used, in the case of normal data, for multiple comparisons. The mean difference was considered significant at the 0.05 level. All analyses were performed using SPSS version 25 (IBM Corporation 2017).

## Results

### IDENTIFYING CHARACTERISTICS

Identifying characteristics of the selected wild edible mushrooms (Plate 1) are presented below.

**Laccaria laccata** (Scoop.) Cooke [Hydnangiaceae]: Cap orange-brown, color often changing markedly as it dries out, convex to flat with a central depression, 1–4.5 cm diameter, margin smooth; gills attached to the stem, widely spaced and decurrent, pinkish flesh color; stipe fibrous, colored like the cap, 2–10 cm long, up to 1 cm thick, smooth to finely hairy, hollow; spore print white; spores globose, ornamented with spines, 7–11  $\mu\text{m}$ . *Habitat*: grows on soil.

**Lactarius volemus** (Fr.) Fr. [Russulaceae]: Cap brown to orangish-brown or reddish-brown, convex to flat, with a central depression, finely velvety to touch, no concentric zones but darker towards the center, up to 3–8 cm diameter; gills attached, close, creamy white or light brown, turn into brown if injured; stipe 2–6 cm long, 0.6–1 cm thick, colored like the cap or paler, equal or tapering towards the base, solid or hollow; latex or milk produced where injured, turning brownish after some time; spore print white; spores globose or subglobose, with warts, 9–13  $\times$  8–10  $\mu\text{m}$ . *Habitat*: grows on soil.

**Russula delica** Fr. [Russulaceae]: Cap white, convex to flat with a central depression, 6–12 cm diameter; stipe white, short and stout, more or less equal, 2–6 cm tall and 2–4 cm wide, smooth; gills slightly decurrent; spore print white; spores ellipsoidal, 8–11  $\times$  6–8  $\mu\text{m}$ . *Habitat*: grows on soil.

**Russula poichilochroa** Sarnari [Russulaceae]: Cap cream to light yellow, convex to flat, up to 10 cm diameter, smooth; gills

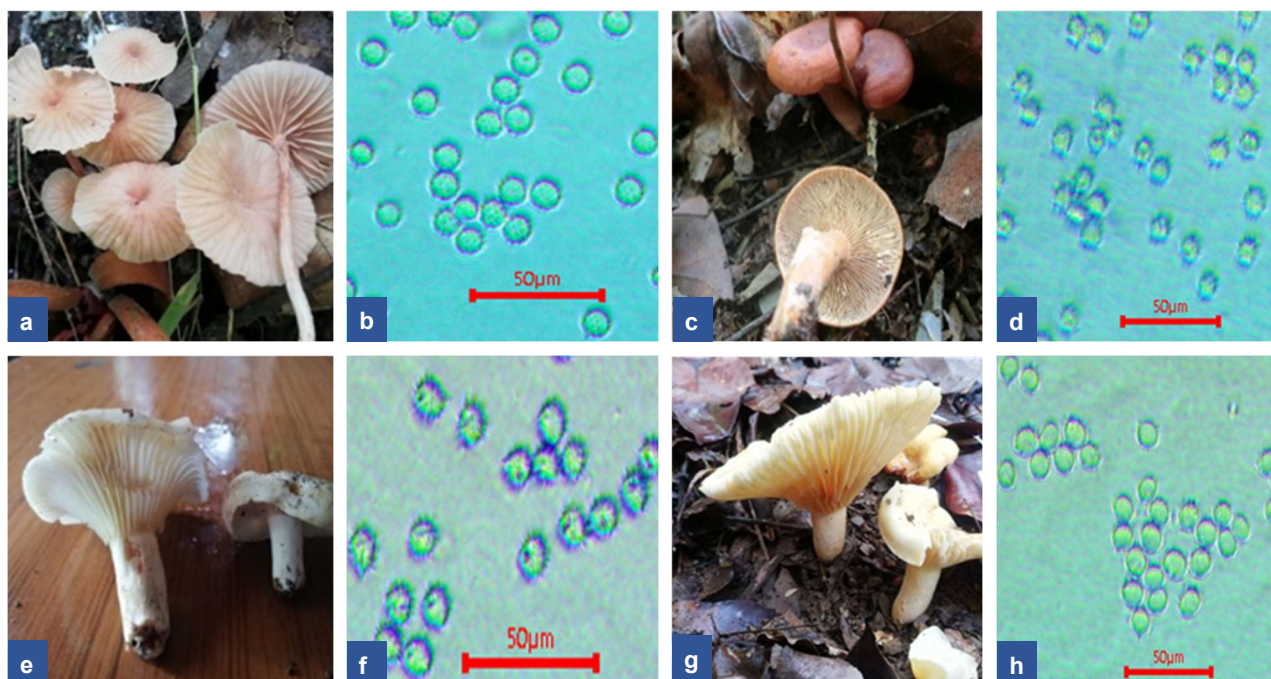
decurrent or closely spaced; stipe same color as the cap or white, smooth, stuffed, 2–4 cm long, 1–2 cm wide; spore print white or cream; spores globose or sub-globose, with warts, 8–13  $\mu\text{m}$ . *Habitat*: grows on soil.

### NUTRITIONAL QUALITY

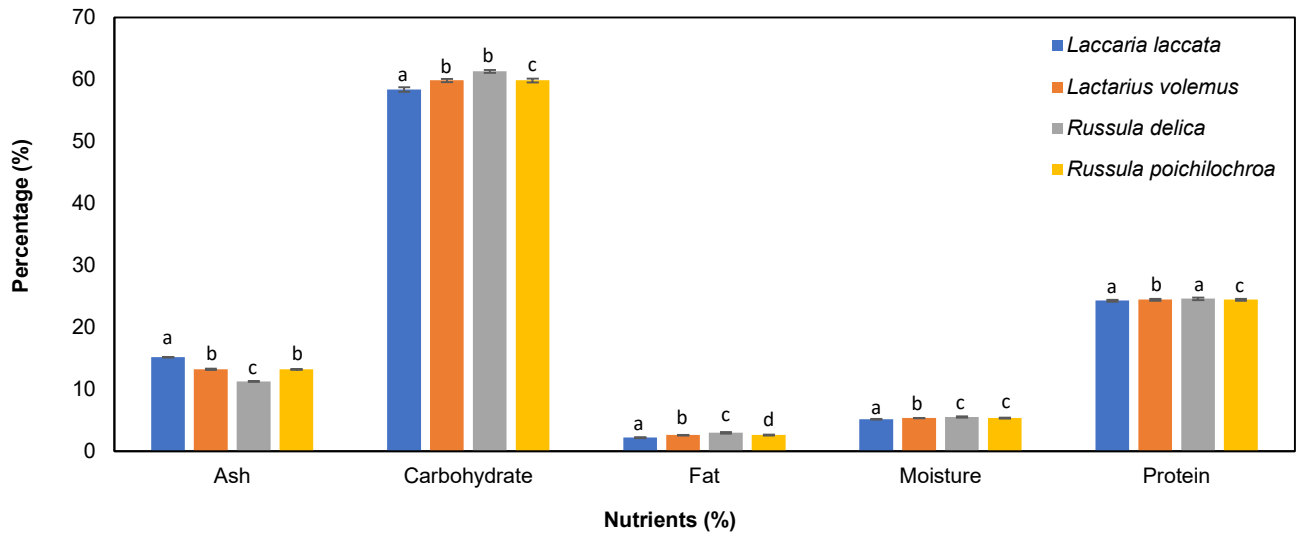
The results of nutritional composition are presented in Figure 2. *Laccaria laccata* had the highest concentration of ash (15.16%), followed by *Lactarius volemus* and *Russula poichilochroa*, while *R. delica* had the least ash content (11.25%). The moisture content of *R. delica* was the highest (5.51%), and *Laccaria laccata* had the lowest (5.16).

We found a significant difference in carbohydrate, fat, and protein contents among the four species. For carbohydrates, *Russula delica* had the highest value (61.28%), and *Laccaria laccata* had the lowest value (58.37%). The fat content of *Russula delica* was the highest (2.97%) and lowest in *Laccaria laccata* (2.20%). *Russula delica* had the highest concentration of protein (24.59%), followed by *Lactarius volemus* and *Russula poichilochroa*, while *Laccaria laccata* had the least (24.28%).

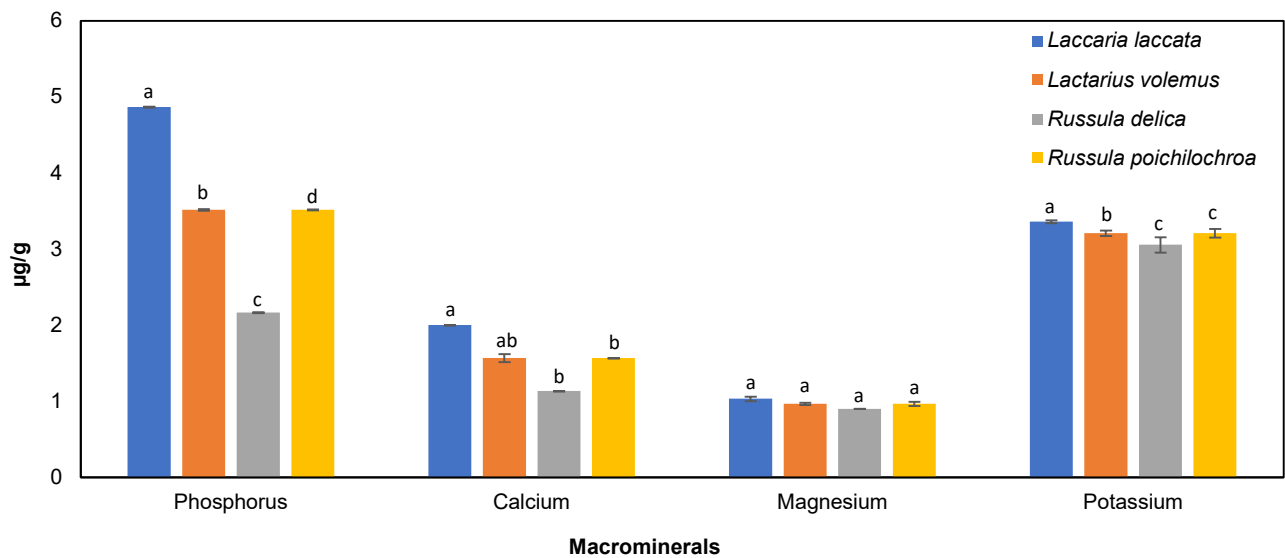
The macro-mineral composition is presented in Figure 3. We found significant difference ( $p < 0.05$ ) in calcium, potassium, and phosphorus content but not in magnesium content among the four species studied. *Laccaria laccata* had the highest phosphorus content (4.87  $\mu\text{g/g}$ ) and *Russula delica* had the lowest content (2.17  $\mu\text{g/g}$ ). Calcium content was 2.00  $\mu\text{g/g}$  dry weight for *Laccaria laccata*, the highest value among the four species, and the lowest value was found in *Russula delica* (1.13  $\mu\text{g/g}$ ). *Laccaria laccata* had the highest magnesium content (1.03  $\mu\text{g/g}$ ), followed by *Russula delica*, *Lactarius volemus*, and *Russula poichilochroa*. The highest potassium content was recorded in *Laccaria laccata* (3.36  $\mu\text{g/g}$ ) and the least in *Russula delica* (3.06  $\mu\text{g/g}$ ).



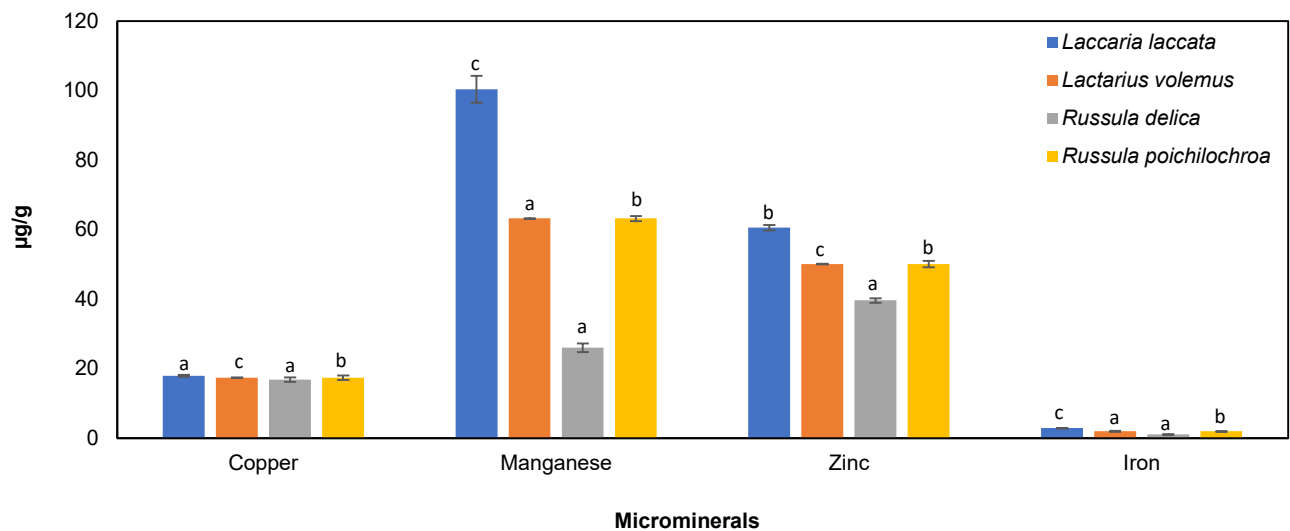
**Plate 1.** Wild edible mushrooms and their spores. a, b, *Laccaria laccata* (a, habit; b, spores); c, d, *Lactarius volemus* (c, habit; d, spores); e, f, *Russula delica* (e, habit; f, spores); g, h, *Russula poichilochroa* (g, habit; h, spores).



**Figure 2.** Nutrient contents in four mushroom species studied.



**Figure 3.** Macro-minerals in four mushroom species studied.



**Figure 4.** Micro-minerals in four mushroom species studied.



Figure 4 shows the micro-mineral composition. There was a significant difference ( $p < 0.05$ ) in copper, manganese, zinc, and phosphorus among the four edible mushroom species. Copper content varied from 16.83 µg/g (in *Russula delica*) to 17.94 µg/g (in *Laccaria laccata*). *L. laccata* had the highest manganese content (100.37 µg/g), and *Russula delica* had the least (25.99 µg/g). The highest zinc content was in *Laccaria laccata* and the least in *Russula delica*. The iron content was 2.87 µg/g for *Laccaria laccata*, the highest among the four species, followed by *Lactarius volemus* and *Russula poichilochroa*.

## Discussion

Mushrooms have great nutritional value since they are rich in proteins with an important content of essential amino acids, and fibers, poor in fat, and high in vitamins and minerals (Adejumo and Awosanya 2005; Barros *et al.* 2008). The proximate composition of four edible mushrooms (*Laccaria laccata*, *Lactarius volemus*, *Russula delica*, and *R. poichilochroa*) was examined for their moisture, ash, carbohydrate, protein, and fat contents. We observed that the moisture content of the four edible mushrooms ranged from 5.16 to 5.51% on a dry weight basis. Fresh mushrooms contain about 90% moisture and 10% dry matter, while dry mushrooms contain about 90% dry matter, and 10% moisture (Chang *et al.* 1981; Chang and Buswell 1996).

The range of ash content (11.25–15.16%) in the wild edible mushrooms recorded in the present study falls within the range described in the literature. For example, Thatoi and Singdevsachan (2014) found that the ash content of wild edible mushrooms from India varied from 1.1 to 17.92%. In another study, Ouzouni *et al.* (2009) reported lower ash contents of some wild edible mushrooms from Greece, the value of which varied from 5.61% (*Russula delica*) to 9.44% (*Cantharellus cibarius*) on a dry matter basis.

Edible mushrooms have been reported to contain high proportions of carbohydrates. The range of carbohydrate content (58.37–61.28%) in the mushroom species recorded in the present study falls within the range reported in the literature. In a study, Ouzouni *et al.* (2009) showed that the total carbohydrate concentration of wild edible mushrooms from Greece varies from 55.33% (*Hygrophorus russula*) to 66.87% (*Armillaria tabesceus*) on a dry matter basis. Adejumo and Awosanya (2005) showed that *Lactarius trivialis* contained 64% of carbohydrates, while *Russula vesca* exhibited up to 71%. Johnsy *et al.* (2011) showed comparatively lower carbohydrate content (ranging from 40.6% to 53.3%) of edible wild mushrooms from the Western Ghats of India. These facts indicate that a significant portion of a dried mushroom's weight comes from carbohydrates.

The protein and fat content in the studied species ranged from 24.28 to 24.59% and 5.16 to 5.51% respectively, and the results are consistent with the findings of other studies (Adejumo and Awosanya 2005; Ouzouni *et al.* 2009). Ouzouni *et al.* (2009) reported a high amount of protein (21.57–34.77%) but poor fat (2.10–6.00%) in the members of some wild edible mushroom genera such as *Amanita*, *Armillaria*, *Boletus*, *Cantharellus*, *Fistulina*, *Hygrophorus*, *Lepista*, *Ramaria*, and *Russula*.

Among minerals, the studied species were rich in manganese, zinc, copper, phosphorus, potassium, and calcium,

but were poor in iron and magnesium. The mineral contents in the studied species ranged 1.13–2.00 µg/g for calcium, 3.06–3.36 µg/g for potassium, 0.90–1.03 µg/g for magnesium, 25.99–100.37 µg/g for manganese, 1.03–2.87 µg/g for iron, 16.83–17.94 µg/g for copper, 2.17–4.87 µg/g for phosphorus, and 39.61–60.56 µg/g for zinc. These results are far lower than the previous findings on wild edible mushrooms. For example, Adejumo and Awosanya (2005) showed the mineral content in four wild edible mushroom species of the genera *Lactarius*, *Lentinus*, *Russula*, and *Termitomyces* from Nigeria to range 31–216 g/kg for calcium, 2.2–5.8 g/kg for potassium, 497–1230 mg/kg for iron, 4–8 mg/kg for copper, and 50–136 mg/kg for manganese. Similarly, Ouzouni *et al.* (2007, 2009) studied the nutritional value of 10 wild edible mushroom species from Greece belonging to the genera *Amanita*, *Armillaria*, *Boletus*, *Cantharellus*, *Fistulina*, *Hygrophorus*, *Lepista*, *Ramaria*, and *Russula*, where they reported the mineral content to range 688.7–1150.7 µg/g for magnesium, 7.19–62.63 µg/g for manganese, 38.9–499.0 µg/g for iron, 7.38–75.06 µg/g for copper, and 34.43–98.99 µg/g for zinc. The difference in mineral concentrations is possibly related to geographical location, age of fruiting bodies, substrate composition, and age of mycelium (Diez and Alvarez 2001).

In conclusion, the wild edible mushrooms analyzed for their nutrient content are rich sources of carbohydrates, protein, and essential minerals while containing low levels of fat, thus making them a valuable and healthy food source for human consumption.

## Acknowledgements

The authors thank the Central Department of Botany, Tribhuvan University, and Aashtha Scientific Research Service Pvt. Ltd. for providing laboratory facilities. We sincerely appreciate the authorities and user group members of Brahakshetra Community Forest for granting permission to conduct the study. Our gratitude also extends to the local people for sharing valuable information regarding various aspects of edible mushrooms. Finally, we are grateful to the reviewers and journal editors for their insightful comments and suggestions on earlier versions of the manuscript.

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