Journal of Plant Resources (2023) **21**(1), 13-20
https://doi.org/10.3126/bdpr.v21i1.57197

# Nutrient Analysis of Selected Wild Edible Mushrooms Collected from Thulo Ban Community Forest, Myagdi District, Nepal

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**Abstract**

The study analyzes the nutrient content of three wild edible mushrooms *Cantharellus cibarius, Laccaria laccata* and *Scleroderma cepa* commonly consumed by the local people of Arjam, Myagdi district. Thirteen parameters were analyzed such as ash, carbohydrate, fat, moisture, protein, manganese, zinc, magnesium, potassium, iron, copper, phosphorus and calcium. The test methods used for ash, fat, moisture, protein and phosphorous content were ignition, soxhlet extraction, oven dry method, kjeldahl digestion method and spectrophotometric method respectively. Carbohydrate content was determined by calculation method and iron, manganese, copper, zinc, calcium, magnesium and potassium content estimation were done by AAS method. All macro and micronutrient compositions were determined on a dry weight basis. Ash, carbohydrate, fat, moisture and protein are ranges from 7.05-13.38%, 61.89-71.37%, 0.78-1.94%, 12.37-13.66% and 16.18-24.47% respectively, whereas calcium, magnesium, phosphorus and potassium ranges from 0.13-0.15 µg/g, 0.09-0.11µg/g, 0.25-0.37 µg/g and 1.41-3.40 µg/g respectively. Similarly copper, iron, manganese and zinc ranges from 2.40-30.94 µg/g, 0.08-0.20 µg/g, 7.22-16.06 µg/g and 45.70-77.35 µg/g respectively.

**Keywords:** *Cantharellus cibarius*, *Laccaria laccata*, Parameters, *Scleroderma cepa*

## Introduction

Fungi are significant organisms in nature and can be found almost anywhere (Rudawska & Leski, 2005). They play important role in ecosystem processes and usually reside underground or under tree barks (Iwabuchi et al., 1994; Keizer, 1998; Seen- Irlet et al., 2008). Mushrooms are fruiting bodies of fungi that are seeable to the naked eye and are generally ≥1 cm in size (Arnolds 1992; Redhead & Berch, 1997).

Mushrooms are valuable not only for their ability to biodegrade the substrate, but also for their chemical and nutritional properties (Turkekul et al., 2004). They have a high protein content, carbohydrate, fibers, minerals, trace elements and low fat content (Agahar-Murugkar & Subbulakshmi, 2005; Wani et al., 2010). According to some studies, the amino acid compositions of mushrooms are comparable to those of animal protein (Kalac, 2009; Ogundana & Fagade, 1982). Generally, the fruiting bodies of mushrooms contain approximately 56.8% carbohydrate, 25% protein, 5.7% fats and 12.5% ash by dry weight basis (Demirbas, 2002; Mendil et al., 2004).

The archaeological record reveals edible species associated with people living 13000 years ago in Chile (Rojas & Mansur, 1995) but the eating of wild edible fungi first reliably noted in China, several hundred years before Christ's birth (Aaronson, 2000). Among 1,291 recorded mushrooms species in Nepal 159 mushroom species are considered as edible (Devkota & Aryal, 2020). Although Pandey & Budhathoki (2007), Giri & Rana (2008) and Jha & Tripathi (2012) examined the nutritional value of Nepal's wild edible mushrooms, information on essential elements or chemical composition of Nepal's wild mushrooms are still inadequate. Further, there is also lack of knowledge about how chemical composition of wild mushrooms varied with climatic conditions. For this reason, this study focuses on macro and micronutrients of commonly consumed wild edible mushrooms of the subtropical region of Nepal.

## Materials and Methods

### *Study area*

The research was conducted in the Thulo Ban Community Forest of Arjam, Beni Municipality

1, Myagdi District, Gandaki Province, Nepal. Geographically, it is located between 83°34'35.1" E longitude and 28°19'09.8" N latitude (Figure 1). The forest is situated at an altitude of 1450 m above sea level with subtropical climate. The forest covers an area of 114 ha. The study area comprises subtropical pine mixed forest dominated by tree species such as *Pinus roxburghii, Schima wallichii, Rhododendron arboreum, Egelhardia spicata* and *Lyonia ovalifolia.*

### *Sample collection, processing and identification of mushrooms*

On the basis of most dominant and popularly known species, three wild edible mushrooms namely *Cantharellus cibarius, Laccaria laccata* and *Scleroderma cepa* were taken for nutrient analysis to determine their macronutrients (moisture content, fat, protein, carbohydrate and ash), macrominerals (magnesium, calcium, potassium and phosphorus) and various microminerals (iron, manganese, copper and zinc). The sample was collected during rainy season 2020, and photographs were also taken (Figure 2, 3 and 4). The collected mushrooms species were cleaned thoroughly with the help of brush to free them from mud, dried on blotting paper, sliced without division of pileus and stipe, air- dried and powdered to about 1mm particle size and store at room temperature in polyethylene bottles until analysis (Mallikarjuna et al., 2013).

The spore print papers were peeled off and laid out on a slide, stained with 1-2 drops of lactophenol and cotton blue, covered with a cover slip and examined under a microscope to determine the length and width of each species' spore. Immersion oil was used to magnify small spores when working with them. The specimens were identified using various books and standard literatures (Adhikari, 2000; Corner, 1970; Phillips, 1981; Watling, 1973). Mushroom field guides were consulted and mycological websites (http:/www.mycoweb.com; www.mushroomexpert.com) were accessed.

### *Determination of macronutrient*

The nutrient values of three wild edible mushroom species were determined using AOAC (Association of Official Analytical Chemists), 18th edition official method (Horwitz & Latimer, 2005).

**Moisture:** The oven-dry method was used to determine the amount of moisture in the mushroom

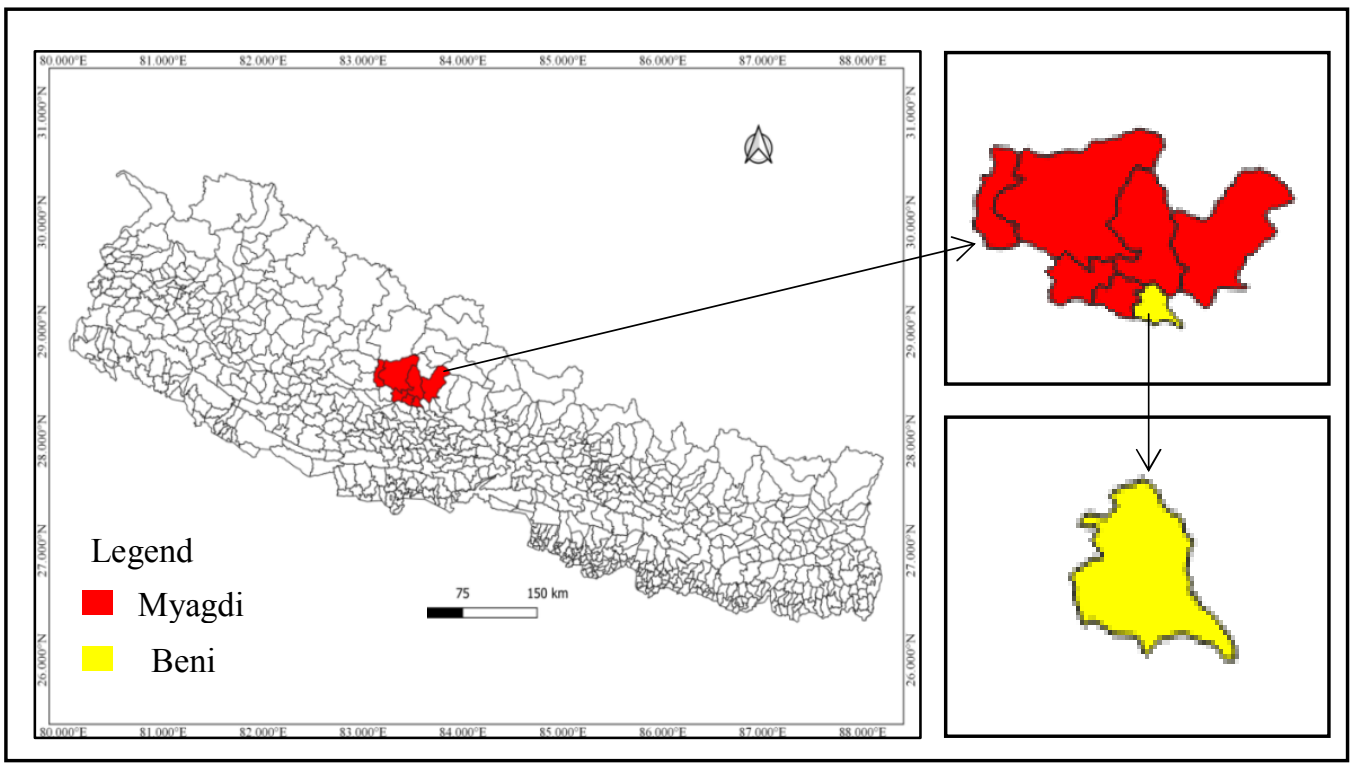


**Figure 1**: Map showing the study area

sample. In this method, two gram sample was taken in a tarred oven-dried crucible and placed into a hot air oven for 110ºC, until it gives constant weight. The oven-dried sample was cooled in a desiccator and the final weight was taken after proper cooling. The lost weight during the drying represents the moisture contents (%) calculated by the following formula;

$$\text{Moisture \%} = \frac{\text{Loss of weight due to drying}}{\text{Weight of a sample taken for analysis}} \times 100\%$$

**Ash:** One gram of air-dried sample was taken qualitatively in a clean and dry porcelain crucible. The sample was ignited at 550ºC keeping inside the muffle furnace until it gives constant weight. After complete ignition, the ash-containing crucible was cooled in a desiccator and its final weight was recorded. From the increased weight of the crucible, the ash content of the sample was calculated. The ash contents (%) are calculated by the following formula;

$$\text{Total ash \%} = \frac{\text{Weight of ash after incineration}}{\text{Weight of the sample taken for ashing}} \times 100\%$$

**Protein:** Protein was determined by the Kjeldahl Digestion method. In this method, three gram powdered sample was mixed with about ten gram digestion mixture (mixture of copper sulfate and sodium sulfate) in presence of 10 ml concentrated sulfuric acid. Unless the forth ceases, it was heated at low temperature and additional heated at high temperature until the solution turns into pellucid blue and white fumes come. Then after digestion flask was cooled at room temperature for 20-30 min. Then the digested sample was transferred into the volumetric flask with the help of a pipette and added distill water to make its volume and closed. For distillation, the apparatus was set in such a way that the cold water continuous flow through the unit. The distilled was then collected in a 4% boric acid ($H_3BO_4$) solution that absorb the liberated nitrogen content in a beaker. 200 ml beaker containing boric acid was then titrated. After completing distillation, the distilled sample was titrated against hydrochloric acid (HCl). The following formula was used to calculate the total nitrogen content and the protein content was calculated by multiplying by 6.25.

$$\text{Total Nitrogen \%: } \frac{14\times(V-V1)\times100\times S}{W\times1000} = X$$

$$\text{Protein \%} = X \times 6.25$$

Where;

14 = Molecular weight of Nitrogen
V = Standard acid volume used to neutralize the distillate
V1 = Standard acid volume used to neutralize the blank
S = Normality of standard acid (strength)
X = Total nitrogen percent
W = Weight of sample taken for digestion
6.25= Conversion factor

**Fat:** Fat in mushrooms sample was determined by the Soxhlet Extraction method. In this method, ten gram of oven-dry powdered sample was kept in the thimble, weighted, noted the sample weight and placed cotton into the thimble in a way that covers the sample and folded. The dried round bottom flask was weighed and noted its weight. After that, the thimble and sample were put into the soxhlet apparatus and extracted by petroleum spirit for 4-5 hrs. in soxhlet apparatus. Extraction had been done for 7 hrs. The solvent was evaporated in a tarred evaporating dish and weighted. From the increased weight of the dish, the fat percentage of the sample was calculated by the following formula;

$$\text{Fat \%} = \frac{M_2-M_1}{E} \times 100\%$$

$M_1$ = Initial weight (in gm.) of the dry empty round bottom flask
$M_2$ = Final weight (in gm.) of the dry empty round bottom flask
E = Weight of the sample in grams

**Carbohydrate:** Carbohydrate was calculated from the observed value of ash, fat and protein.

Carbohydrate (%) = 100- (Ash%+Fat%+Protein).

***Determination of macro and micro minerals***

The mineral contents such as Iron, Manganese, Copper, Zinc, Magnesium, Calcium and Potassium were determined through atomic absorption spectrophotometer (AAS). In this method, five gram

of mushroom sample was placed in a porcelain crucible and dried in a hot air oven set to 105°C for 3 hrs. The samples were then ashed in a muffle furnace at 550°C unless the ash residue was white or grey. The obtained ash was dissolved in 5ml of a mixture of $HNO_3$ and hydrochloric acid and the solution was slowly heated to melt the residue before being transferred to a volumetric flask and diluted to make 50 ml. Then, the sample containing element was determined by atomic absorption spectrometry, by using flame atomic absorption spectrometer.

**Phosphorous:** Ash of the sample was extracted by 1:1 HCl and distilled water was then filtered through medium-textured filter paper to get clear filtrate. An aliquot of the sample was treated with Molybdovanadate reagent to develop yellow color. Finally, the absorbance of the yellow color of the sample solution was measured by a spectrophotometer at 400 nm. From the observed absorbance of the sample, the concentration of phosphorous was calculated.

### *Statistical analysis*

To compare the mean value of nutrients between and within species, one-way ANOVA and the non-parametric Kruskal Wallis test were used at 5% probability level of significance. To ensure accuracy, the analysis was performed three times. The experimental result was given as the mean ± standard error (SE).

## Results and Discussion

All macronutrient, macrominerals and microminerals estimations were determined on a dry weight basis. Each parameter was repeated thrice and the mean of them was considered as the final result.

### *Macronutrient profile*

The highest ash content (13.38%) was found in *Laccaria laccata*, whereas the lowest ash content was found in *Scleroderma cepa* (7.05%). *Cantharellus cibarius* is rich in both carbohydrate (71.37%) and fat (1.94%) in comparison to *Laccaria*


**Figure 2**: *Cantharellus cibarius* with their spores


**Figure 3**: *Laccaria laccata* with their spores

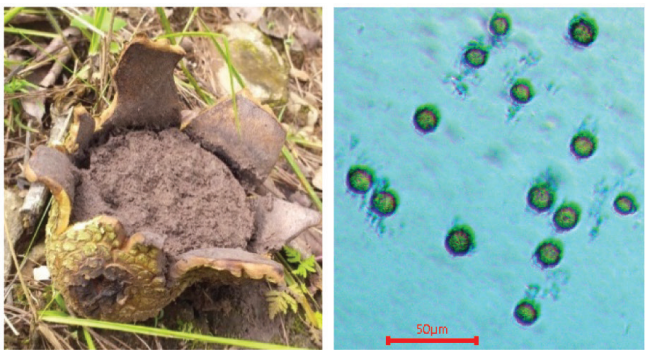


**Figure 4**: *Scleroderma cepa* with their spores

*laccata* (61.89%, 1.41%) and *Scleroderma cepa* (67.68%, 0.78%) (Figure 5). There was a significant difference ($P<0.05$) between these species in ash, carbohydrate, fat, moisture and protein. The moisture content of *Cantharellus cibarius* (13.66%) and *Laccaria laccata* (13.63%) was quite similar whereas *Scleroderma cepa* (12.37%) had a slightly lower value. Among the samples evaluated, protein content was found to be highest in *Scleroderma cepa* (24.47%) compared to the other two species of *Laccaria laccata* (23.3%) and *Cantharellus cibarius* (16.18%) (Figure 5).

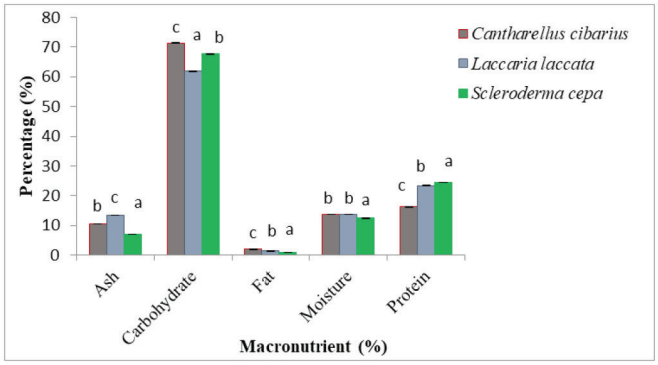


**Figure 5**: Macronutrients profile of three wild edible mushrooms

## *Macrominerals profile*

In all three sample potassium (1.41-3.62 µg/g) was dominant macro element followed by Phosphorous (0.35-0.38 µg/g), calcium (0.13-0.15 µg/g) and magnesium (0.9-0.11µg/g). In terms of potassium, there was a significant difference ($P<0.05$) between the three species, but no significant difference in terms of calcium, magnesium, or phosphorus between the three species (Figure 6).

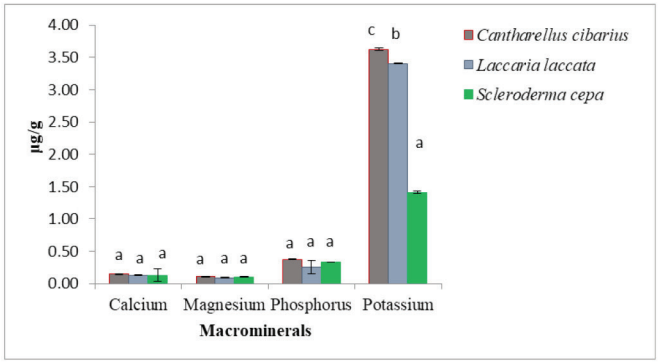


**Figure 6**: Macrominerals profile of three wild edible mushrooms

## *Microminerals profile*

Copper (30.94 µg/g) and manganese (16.06 µg/g) were highest in *Laccaria laccata*, whereas copper (2.40 µg/g) and manganese (7.22 µg/g) were lowest in *Scleroderma cepa*. In case of iron, *Laccaria laccata* dominated over *Scleroderma cepa* (0.16 µg/g) and *Cantharellus cibarius* (0.08 µg/g) with the value of (0.20 µg/g). Similarly, *Scleroderma cepa* (77.35 µg/g) dominated over *Laccaria laccata* (56.67 µg/g) and *Cantharellus cibarius* (45.70 µg/g) in the context of zinc (Figure 7). All three mushroom species showed significant differences ($P<0.05$).

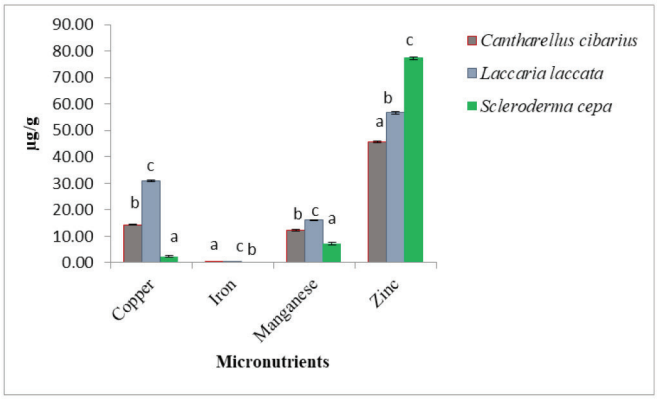


**Figure 7**: Microminerals profile of three wild edible mushrooms

## *Macronutrient and macrominerals profile*

Fresh mushrooms have an average moisture content of 85-95% whereas air-dried specimens have a moisture content of 5-20%, depending on time and storage (Crisan & Sands, 1978). In the present study, the moisture content ranged between 12.37-13.66 %. The mushrooms' moisture content varied depending on the type of mushrooms. (Cuptapun et al., 2010) Studied the moisture content of four edible mushrooms and documented 7.21-7.5% moisture content on a dry weight basis. Because of the high moisture content, fresh mushrooms cannot be stored for a long duration of time. This is because high water activity encourages microbial growth (Bano, 1976). The average crude protein content of edible mushrooms ranges between 19 and 40% (Kurtzman, 1978). The present study found protein content in *Laccaria laccata* was 23.30% which is lower than the values reported by Jha and Tripathi (2012) but higher than the study done by (Egwim et

al., 2011). Similarly, protein content in *Cantharellus cibarius* was 16.18% less than the value given by (Egwim et al., 2011) whereas, *Scleroderma cepa* contained 24.47% protein. The ash content among three wild mushrooms ranges from 7.05-13.38%. These results were similar to the result reported by (Singha et al., 2017). In general, mushrooms are low-calorie foods due to their low fat content. In mushrooms, fat content is very low as compared to carbohydrates and proteins. The fat content in three species of mushroom under study ranges from 0.78-1.94%. *Scleroderma cepa* had low-fat content compared to two other species. The results showed that carbohydrates were abundant in all three species. The obtained value of carbohydrates indicates that the mushrooms are good energy food resources. *C. cibarius, L. laccata* and *S. cepa* are similar in terms of their calcium, magnesium and phosphorous content but differ in terms of potassium content. The nutrition composition of different mushroom species varied; most likely due to their ability to accumulate minerals and other nutrients into their tissue (Teke et al., 2021).

Mushrooms make a crucial contribution to the nutrient provide in our diet. The major compounds of mushrooms are protein, carbohydrate and fat. According to the findings of our study, *Scleroderma cepa* is highly nutritious because of its high protein, carbohydrate and low fat content.

### *Microminerals profile*

The element content of mushrooms is determined by the element content of the soil (Mleczek et al., 2016). Zinc is widely distributed among organisms that exist due to its biological importance. Mushrooms are Zinc accumulators (Mendil et al., 2004). The study revealed a high content of zinc. It may be due to the higher accumulation capacity of zinc by these mushrooms. Copper and manganese contents were higher in *L. laccata* and lower in *S. cepa* but iron content was low in all three species. It might be due to the elemental content varied not only with respect to the regions of the mushrooms where they grow, but also depending on the substratum, atmospheric conditions, age and part of the fructification (Manzi et al., 1999). Many trace minerals are significantly higher in mushrooms than in growing plants, vegetables and fruit. Concentration was found to be based on the species physiology, especially its ecosystem pattern (Duarte et al., 2006).

## Conclusion

The present study concluded that mushrooms contain a small amount of fat and a high amount of carbohydrates and proteins. Hence, this makes it a highly nutritive and good energetic food. These wild edible mushrooms have very good nutritional value so they should be further studied to develop dietary supplements.

## Author Contributions

Shashi Shrestha has done field work, lab work, data analysis and writing of the first draft of manuscript. Sadikshya Thapa did field work, lab work, review and editing of the manuscript. Sanjay Kumar Jha had done research design and conceptualization, contribution for supervision, critically reviewed the results and manuscript finalization.

## Acknowledgements

The authors would like to thank the Central Department of Botany, Tribhuvan University for providing laboratory equipment and the community forest member for granting approval to conduct a study in the Thulo Ban community forest.

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