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NUTRITIONAL VALUE OF SELECTED WILD EDIBLE MUSHROOMS FROM NEPAL

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ABSTRACT

The present study aims to identify and evaluate nutritional properties and mineral content of two wild edible mushrooms *Cantharellus subcibarius*, and *Laccaria proxima.* The samples were collected during the monsoon season in April to August, 2022 from National zoological garden, Suryabinayak, Bhaktapur, Nepal. Following collection, the samples were thoroughly cleaned to remove any debris and properly labeled. Eight parameters were analyzed such as macro nutrients like protein, fat, crude fiber, ash, carbohydrates and minerals including Iron, Calcium, and Phosphorus. All macro and minerals compositions were determined on a dry weight basis. The moisture content of mushrooms varied from 9.43-10.32%, ash 14.01-14.38%, carbohydrate 50.75-56.82%, fat 1.34-1.38%, protein 17.9-21.52% and fiber 33.76-42.53%. The minerals content of mushroom samples ranged whereas Calcium 171-249.97% The examined wild edible mushrooms (*Cantharellus subcibarius* and *Laccaria proxima*) exhibit elevated levels of protein, fibre, and minerals relative to numerous produced varieties, rendering them nutritionally superior in certain respects. The practical consequences encompass their potential as a sustainable, nutrient-dense food source, enhancing food security, bolstering local economies, and fostering additional research on their production and health advantages.

Keywords: wild edible mushrooms, nutrients, *Cantharellus subcibarius*, *Laccaria proxima*

INTRODUCTION

As fungi, mushroom are special and adaptable parts of human diets, exhibiting a wide range of nutrients that support their health-promoting

qualities (Valverde *et al.,* 2015). Mushrooms are heterotrophic and have highly specific nutritional and ecological requirements. In forest ecosystems, mushroom play a variety of roles. In woods, where the deep shadows of the trees provide a humid environment suitable for the germination and growth of mushrooms, they usually grow during the rainy season. This is especially true when it comes to degrading organic waste such as leaf litter (Srivastava, 2021).

Edible mushroom species are a vital natural resource that the majority of people rely on for nutrition. Despite the fact that many varieties can be found all over the world, the majority of mushrooms are wild. Approximately over 38,000 different kinds of mushrooms in over 1,000 species are toxic and about 2,000 species are edible worldwide (Chang, 1995). Among Nepal's 1,291 known mushrooms species, 159 are regarded as edible (Devkota & Aryal, 2020). Although Pandey and Budhathoki (2007), Giri and Rana (2008), Christensen *et al.,* 2008, Jha and Tripathi (2012), Mishra and Mishra (2013), Pandey *et al.,* 2023 and Shrestha *et al.,* 2023) examined the nutritional value of Nepal's wild edible mushrooms, information on key components or chemical composition of Nepal's Wild mushrooms are still insufficient.

Mushrooms contain a high level of dietary fibers that are compared to those in other vegetables. The healthiest, most nutritious foods are mushrooms, which are lower with calories, high in protein, vitamins, minerals, and fiber (Vishwakarma *et al.,* 2016). Mushrooms come in four varieties: edible, toxic, medicinal, and inedible. These mushrooms are easily available worldwide on all parts of the globe and possess been marketed as nutritious foods with additional health benefits, such as preventing cancer, high blood pressure, and high cholesterol (Kabir and Kimura, 1989); To support human health, they are used as powder, pills, capsules, and dried whole (Oei, 2003).

Previous studies on macro fungi have predominantly focused on their taxonomy, and diversity. There has been little research into the nutritional properties of wild edible mushrooms, with only a few studies conducted in this field. (Pandey, 2008), has shown that edible wild mushrooms (*Cantharellus sp., Laccaria sp., Helvella sp., Russula sp.,*) found in Suryabinayak Forest are nutritionally significant, and comparable to other commonly consumed vegetables (cultived mushroom). However, there are still many wild edible mushrooms in the region that remain

unexplored in terms of their nutritional content. Limited consumption of wild edible. Furthermore, there is also lack of knowledge about how chemical composition of wild mushrooms changed with climatic conditions. For this reason, this study focuses on macro and minerals of commonly consumed wild edible mushrooms of the Suryabinayak, Bhaktapur, Nepal.

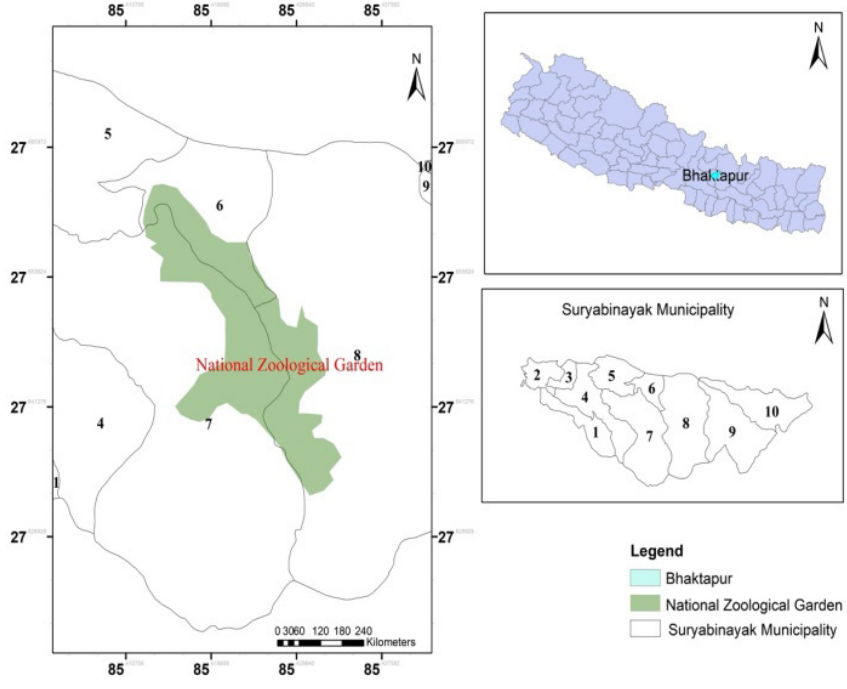
MATERIALS AND METHODS

Study Area

The research was completed in National zoological garden from Suryavinayak, Bhaktapur. It is located in ward no. 6, 7 and 8 in Suryabinayak Municipality between 27°38 "00" and 27°39 "46" North latitude and 85°24 "57" and 85°25 "56" East longitudes. Lying in the middle mountain physiographic zone, its elevation ranges from 1390 to 1700 meters above sea level. The dominant species of this woodland are *Schima wallichii, Castanopsis indica, Rhododendron arboreum, Myrica esculeta, Quercus sp* etc.

Figure 1

Map Showing National Zoological Garden, Suryabinayak, Bhaktapur (Prepared Using Arc GIS 10.8)

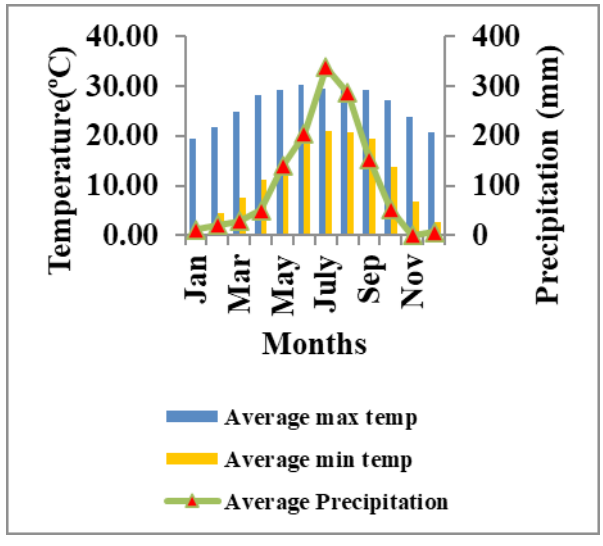


Climatic Data

In these research areas, June 30.33 ˚C the highest monthly average temperature ever recorded. In comparison, 1.87 ˚C was the lowest recorded temperature for January, July had the highest recorded rainfall (338.25mm), while November had the lowest rainfall (0.26 mm). The Department of Hydrology and Meteorology, Government of Nepal, provided the climate data of the closest metrological station for the years 2013 to 2022. The meteorological information was obtained from a weather station. i.e. Bhaktapur.

Figure *2*

Monthly Averages for Precipitation and Temperature at the Closest Station (Bhaktapur).



Collection, Identification and Samples Preparation

On the basis of most dominant and popularly known species two wild edible mushrooms namely *Cantharellus subcibarius* and *Laccaria proxima* were taken for nutrient analysis to determine their macronutrients (moisture content, ash, fat, fiber, protein and carbohydrate) and, minerals (iron, calcium and phosphorus) contents. The sample was collected during monsoon season in April to August, 2022, and photographs were also taken (figure 3 and 4) from its natural habitat. Using a digger, the species found in the soil were carefully taken up. Other specimens were found to grow

on wooden logs, dead plants, and trunks of living trees were also gathered. The fleshy mushroom samples were preserved in bottles with a mixture of formalin, alcohol, with distilled water at a ratio of 70:25:5 (Ainsworth, 1971). Some of the samples were also preserved by drying.

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. The specimens were identified using various standard literatures like, (Adhikari, (2000); Corner, (1970); Phillips, (1981); Singer, (1986). We looked through mushroom field guides and visited mycological website (http://www.mycoweb.com; www.mushroomexpert.com).

The species were identified by comparing with the specimens stored in the National Herbarium (KATH) and Tribhuvan University Central Herbarium (TUCH), were also consulted to verify the species identification. Estimation of protein, fat, crude fiber, ash, carbohydrates, calcium, and iron was done in the National Food Research Center, Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur, following the protocols described in Ranganna. (2011). The estimation of moisture content was done at the Central Department of Botany, Tribhuvan University.

Moistures Content

Moistures in the mushroom sample were used by hot air oven technique. Using these techniques, 2 grams of a prepared sample was held in an oven-dried crucible and heated to 110°C for 4hours. The process of heating and cooling was carried out repeatedly until the constant weight was reached. Once the oven-dried sample had properly cooled, it was placed in a desiccator to determine its ultimate weight. The moisture content was calculated using the following formula:

$$\text{Moisture Content\%:} \frac{\text{Fresh weight–Dry weight}}{\text{Fresh weight}} \times 100$$

Total Ash Content

One gram of air-dried sample was taken in a dry, clean porcelain crucible. The sample was ignited at 525ºC keeping inside the muffle furnace until it give constant weight. During complete ignition, the ash-contenting crucible was cooled in a desiccator and its final weight was noted. From the increased weight of the crucible, the ash content of the sample was calculated. The ash contents (%) were calculated by the following formula:

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

Fat Content

Fat in the mushroom sample was ascertained using the Soxhlet extraction method. In this method, ten grams of oven-dried powdered sample was kept in the thimble, weighed, 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 weighted and noted its weight. After that, the thimble and sample were put into the soxhlet apparatus and extracted by petroleum spirit for four to five hours in soxhlet apparatus extraction had been done for seven 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:

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

Where, M1= Initial weight (in gm.) of the dry round bottom flask, M2= Final weight (in gm.) of the dry empty round bottom flask with fat, E= Weight of the sample in grams.

Fiber Content

The amount of crude fiber was measured using the acid base digestion techniques. First, a 0.5-1 gram sample was taken in a crucible and assembled in Fibrotron() that was coupled to a condenser. Following that, digestion with 1.25% v/v. H2SO4 was performed for 30 minutes. To get rid of extra H2SO4, the sample was then washed with warm water.It was then followed digestion with 1.25% NaOH for another 30 minutes. The excess NaOH was then washed away with warm water. Then the sample was washed with alcohol. After being heated at 110º c until constant weight, the crucible with sample was allowed to cool in a desiccator. The crucible was weighed once again. The crucible's contents were lit inside a muffle furnace for 20 minutes, allowed to cool and weighted again. The amount of crude fiber was determined using following formula:

$$\text{Fiber Content\%} = \frac{\text{Wt. after drying} - \text{wt. after ashing}}{\text{Wt. of sample taken}} \times 100$$

Protein Content

The Kjeldal Digestion technique was used to quantify the protein. Using this approach, 3 grams of powdered material and roughly 10 grams of digestion mixture (a copper and sodium sulfate mixture) were combined with 10 milliliters of concentrated sulfuric acid. It was heated to a moderate

temperature and then to a high temperature till the solution turned pellucid blue and white fumes appeared, unless the fourth stopped. Then after the digestion flask was cooled at room temperature for 20 to 30 minutes. Then, the processed sample was carried into the volumetric flask with the help of a pipette and filled distill water to make its volume and closed. The apparatus used for distillation was set up so that cold water would continuously pass through the unit. The distilled was then gathered in 4% boric acid (H3 BO4) solutions that absorb the produced nitrogen content in a beaker. In completing distillation, the distilled sample was titrated with hydrochloric acid (HCl). The given formula was used to calculate the total nitrogen content and the protein content was calculated by multiplying by 6.25:

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

Protein % = X × 6.25

Where, the molecular weight of nitrogen is 14, 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, X = Total nitrogen %, W = Weight of sample taken for digestion, 6.25 is conversion factor.

Carbohydrate Content

The Carbohydrate content was calculated by using the following formula:

Carbohydrate Content (%) = 100- (ash%+ fat%+ protein %)

Mineral Determination

Preparation of Ash Solution

The ash that resulted from ashing one gram of sample was mixed with 25 milliliters of 10% HCl. Whatman filter paper number 1 was used to filter the mixture, and the filtrate volume was adjusted to 100 milliliters.

Phosphorus

About 5 ml of ash solution obtained by dry ashing and 5 ml of molybdate reagent was mixed well. Aminonalphtholsulphonic acid solution of volume 2ml mixed and made the volume to 50 ml. Blank solution was prepared similarly using water in place of the sample. The sample solution was allowed to stand for 15 min and colour had been measured at 650 nm settings the blank at 100% transmission.

Phosphorus content (mg/100 g) = (mg of P in ash solution x vol. of ash solution x 100) / (ml. of ash solution x wt. of sample)

Standard curve of Phosphorus

A 10 ml standard potassium phosphate solution was diluted by using 10 ml water. In a 50 ml of volumetric flask 40 ml of aliquot Pipetted out. Then 5ml of molybdate reagent was added and mixed. After that, 2 ml of aminonaphtholsulphonic acid reagent was added and mixed. The final volume was made 50ml and measured color as in sample. The plot concentration against absorbance was made.

Calcium

An aliquot of 50 ml of the ash solution and 50 ml distilled water was pipetted into 250 ml beaker and 10 ml of saturated ammonium oxalate solution and 2 drops of methyl red indicator was added. To make solution slightly alkaline dil. ammonia was added drop wise until the color turns yellow and few drops of acetic acid was poured until it gets faint pink color to make the solution slightly acidic. Then, solution was heated to the boiling point and left overnight. The solution was filtered using Whatman filter paper no. 42 and precipitate was washed with distilled water. The precipitate was dissolved in dilute Sulphuric acid to make volume of 200ml. Thus, prepared 25 ml solution was pipetted in a conical flask and titrated with 0.01 N KMnO4 until a persistent pink endpoint was achieved.

Calcium content (mg/100 g) = (Titer x N of KMnO4 x 20 x volume of ash x 100) / (volume of ash solution x wt. of sample)

Iron Content

Iron content in mushroom samples was determined by a colorimetric method. A blank solution was prepared by using 0.5 ml of concentrated sulfuric acid (H_2SO_4), 15 ml of distilled water, 1 ml of potassium persulfate solution, and 2 ml of potassium thiocyanate solution. The solution of 10 ml of the mushroom sample extract, 5 ml of distilled water, 0.5 mL of concentrated H_2SO_4, 1 ml of potassium persulfate solution, and 2 ml of potassium thiocyanate solution was made in a separate container and allow the mixture to react for approximately 15 minutes to ensure complete color development. Similarly, a standard solution was prepared by combining 1 ml of a known iron standard solution, 14 ml of distilled water, 0.5 ml of concentrated H_2SO_4, 1 ml of potassium persulfate solution, and 2 ml of potassium thiocyanate solution and let it react for 15 minutes. After the reaction time, the absorbance of the sample, blank, and standard solutions were measured at 480 nm using a colorimeter.

Iron content (mg/100 g) = (Optical density of sample x 0.1 x total volume of ash solution x 100) / (Optical density of standard x 5 x wt. of sample)

Statistical analysis

Excel was used to calculate the mean nutritional value among species and to conduct an independent sample T-test. Significance was recognized at the 5% level of significance.

The analysis was conducted three times to guarantee the accuracy of the findings. The mean ± standard error represented the experimental outcome.

RESULTS AND DISCUSSION

The estimations for minerals and macronutrients were calculated on dry weight. Every parameter was repeated twice and the mean of them was considered as the final result. The Suryabinayak people are familiar (highly consumed) with these two common wild edible mushrooms: *Cantharellus subcibarius*, *Laccaria proxima* (Figure 3and 4), which are widely consumed across various regions of Suryabinayak forest (Pandey, 2008).

Figure 3

Cantharellus subcibarius with their spores

Figure 4

Laccaria proxima with their spores

Table 1

Proximate nutritional analysis of collected wild edible mushrooms

Species	Moisture Content (%)	Ash Content (%)	Carbohydrate Content (%)	Fat Content (%)	Protein Content (%)	Fiber Content (%)
C. subcibarius	9..42±0.31	14.38±0.011	56.82±0.02	1.38±0.01	17.98±0.90	24.41±3.47
L. proxima	10.32±0.26	14.06±0.18	50.75±0.03	3.40±0.03	21.52±0.91	27.87±2.03

Moisture and environmental factors have a direct impact on the nutritional value of mushrooms (Kalac, (2008). Freshly harvested mushrooms usually have 85–95% of moisture; however, samples which have been air-dried may have as little as 5–20% moisture, depending on how long they are stored (Crisan and Sands, 1978). In this study, the highest moisture content in, the moisture content was around 10% in both species (10.32% in *Laccaria proxima),* and (9.42% in *Cantharellus subcibarius*). Pushpa and Purushothoma (2010) additionally revealed that the ash content in several natural nutritious mushrooms from India, ranges from 7%-17%. The ash content reported in our study was also with in the same range. It is known that mushrooms with high ash content have a higher mineral content (Thachunglura *et al.,* 2023).

Additionally, the most prevalent component of mushrooms is carbohydrate, accounting for 35–70% of their mass in both digestive and non-digestible forms (On-nam *et al.,* 2023). The two edible mushroom species under research, *Cantharellus subcibarius* (56.82%) and *Laccaaria proxima* (50.75%), have total carbohydrates with in the reported range. The fact that multiple mushroom specimens contain carbohydrates suggests these nutrient-dense mushrooms are also important for food security in human. In general, wild mushrooms are thought to have a low-fat level and a high protein, vitamin, fiber, and mineral content (Lin *et al.,* 2023).

On average, crude protein content in nutrient- rich mushrooms range from 19 to 40% (Kurtzman, 1978). The results of the present research revealed the protein content near the lower value in the reported range (17.98% in *Cantharellus subcibarius* and 21.52% in *Laccaaria proxima*). The protein content for *Cantharellus subcibarius* in present study was lower than that reported by Devi (2017) for the same species (21.92%). The crude fat *Cantharellus subcibarius* and *Laccaria proxima* ranged from 1.38%- 3.40% included less fat than other foods, which might make it a healthy choice.

Table 2

Mineral Content of the Collected Wild Edible Mushrooms

Species	Phosphorus (mg/100g)	Calcium (mg/100g)	Iron(mg/100g)
C. subcibarius	478.82±4.45	249.97±38.04	83.56±2.29
L. proxima	410.14±17.69	171.00±11.36	88.79±0.97

It is well known that different amounts of calcium, phosphorus, and iron can be extracted from mushrooms. These minerals are crucial for human nutrition. They are necessary in healing damaged cells, strong bone and teeth, generating red blood cells and preserving osmotic balance (WHO, 1996). The result of the present study demonstrates the abundance of minerals especially the phosphorus and calcium in this species. While both of them were generally low in Iron (Table 2). Mineral concentrations are directly correlated with species, growing region, fruiting body maturity duration, substrates, and proximity to sources of pollution (Bellettini *et al.,* 2019; Gucia *et al.,* 2012). Thus, many mushrooms have high concentrations of heavy metals, which when consumed, might have a negative impact on human health (Fu *et al.,* 2020). In general, two samples are nutritional-rich, with *Cantharellus subcibarius* having the highest minerals contents like Phosphorus (478.82 ± 4.45 mg/100gm), Calcium (249.97± 38.04 mg/100g) and Iron (83.56± 2.29 mg/100gm).

CONCLUSION

The current study concluded that the studied species mushroom contained relatively small amount of fat and a high amount of protein, carbohydrates, fiber, and other minerals, it makes them an excellent source of food for human consumption. It indicates that each of these edible types of wild mushrooms can be included as component of balanced diets.

AUTHOR CONTRIBUTIONS

Merina Sapkota has done field work, lab work, data analysis and writing of the first draft of manuscript. Dr. Sanjay Kumar Jha had conceptualized and designed the research. He also supervised the research work and critically reviewed the manuscript.

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