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Research Article

Nutritional Composition of Wild Edible Mushrooms *Scleroderma cepa* and *Laccaria laccata*

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Abstract

Wild edible mushrooms are widely recognized for their high nutritional value and used as food. This study analyzed the macronutrient and mineral composition of *Laccaria laccata* and *Scleroderma cepa*, focusing on moisture, ash, protein, fiber, fat, carbohydrate, calcium, phosphorus and iron. The quantitative estimation of these components was conducted using standard analytical techniques: oven-dry method for moisture, Soxhlet extraction for fat, Kjeldahl digestion for protein, ignition method for ash, proximate analysis for carbohydrates, acid-base digestion for crude fiber, complexometric titration for calcium, molybdenum blue method for phosphorus, and colorimetric analysis for iron. The results revealed that carbohydrates were the most abundant macronutrient (54.93–60.42%), while fat was present in the low amount (0.38–0.55%). Among the minerals, phosphorus content ranged from 424.9 to 507.72 mg/100 g, calcium from 182.83 to 243.16 mg/100 g, and iron from 43.25 to 48.14 mg/100 g. These findings highlight the nutritional significance of these wild edible mushrooms reinforcing their potential as valuable dietary food supplements.

Keywords: *Laccaria laccata*, Macronutrient, Mushroom, Nutrient analysis, *Scleroderma cepa*

Introduction

Wild edible mushrooms have been consumed as food for centuries due to their rich nutritional value and they are also utilized as traditional medicine for health benefit. They are highly valued for their unique taste and texture. They serve as a significant source of essential nutrients, including proteins, carbohydrates, dietary fibers, vitamins, and minerals, while being low in calories and cholesterol (Demirbas, 2002; Mendil et al., 2004). Compared to many conventional food sources, mushrooms contain high-quality proteins and bioactive compounds, making them an excellent dietary choice for improving human health (Chang & Miles, 2004; Kakon et al., 2012).

The nutritional composition of wild edible mushrooms varies depending on species, habitat, and environmental conditions. Many species are rich in essential amino acids, unsaturated fatty acids and antioxidants, which contribute to their role in boosting immunity, reducing inflammation and preventing various chronic diseases (Kalač, 2009; Valverde et al., 2015). Additionally, wild edible mushrooms provide a significant number of micronutrients such as potassium, phosphorus, iron, zinc and selenium, which are crucial for metabolic

functions and overall well-beings (Mattila et al., 2000; Agahar-Murugkar & Subbulakshmi, 2005).

Despite their nutritional and medicinal significance, research on the composition and potential health benefits of wild mushrooms remains limited, particularly in regions with high fungal biodiversity. According to previous studies, mushrooms with low fat content are recommended as a good source for the people with cardiac problems (Pandey and Budhathoki, 2006; Upadhyaya et al., 2017; Shrestha et al., 2023). Therefore, this study aims to analyze the nutritional composition of wild edible mushrooms.

Materials and Methods

Collection of mushrooms

Two wild edible mushrooms species; *Scleroderma cepa* Pers. and *Laccaria laccata* (Figure 1) were collected from the Chaukot community forest in Kavreplanchowk district of central Nepal (27°37′N latitude and 85°33′E longitude, and elevation range of 1,500 to 1,700 meters). The forest is characterized by montane subtropical *Schima-Castanopsis* forest, dominated by *Pinus roxburghii*, *Schima wallichii*, *Myrica esculenta*, *Rhododendron arboreum*, and *Castanopsis tribuloides*.

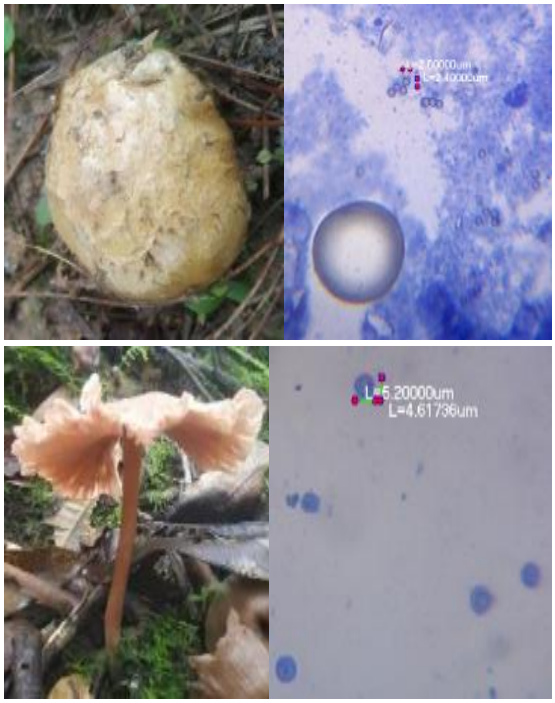

Figure 1: *Scleroderma cepa* with its spores (above) and *Laccaria laccata* with its spores (below).

Sample preparation

The mushrooms were thoroughly cleaned to remove any mud, dried on blotting paper, sliced without dividing the pileus and stipe, powdered to about 1 mm particle size, and stored at room temperature in polyethylene bottles until analysis (Mallikarjuna et al., 2013).

Determination of macronutrients

Nutrient contents in the wild edible mushroom species were determined in the National Agricultural Research Council (NARC) according to the Handbook of Analysis and Quality Control for Fruit and Vegetable Product (Ranganna, 2011).

Moisture content determination

The moisture content of the mushroom samples was determined using the hot air oven drying technique. In tarred oven dried crucible, 2 g air dried sample was placed and heated to 110 °C in hot air oven until its weight stayed constant. The dried sample allowed to cool in a desiccator until its final weight was determined. The percentage of moisture content was determined by using a formula given by (Raghuramulu *et al*., 2003).

Percentage of moisture content = (Fresh wt. – Dried wt.) / Fresh wt. x 100

Total ash content

A dried clean crucible was weighed. A dry sample of 1 g was measured. The samples were placed inside a muffle furnace at 525 ˚C and lit on a hot plate for 6 hours. After the entire process, the crucible with the ash was cooled in a desiccator and the sample's ash content and the crucible's final weight were determined. The following formula is used to get the percentage of ash content.

Percentage of total ash content = (Wt. of ash / Wt. of dried sample) x 100

Protein content determination

Kjeldal digestion technique was used to determine the total protein content. About 3 g of powdered was mixed with 10 g digesting mixture in the presence of 10 ml of Conc. H_2SO_4. It was heated till the solution turned transparent blue and white fumes started to form. Following digestion, the flask was allowed to

cool for 20 to 30 min at room temperature. Then, using a pipette, the digested sample was transferred into a volumetric flask, the volume was adjusted with distill water, and the flask was sealed.

The equipment was set up for distillation with cold water running through it constantly. A conical flask was filled with 5 ml of 2% boric acid, 4 drops of mixed indicator. Filling the burette with 0.01 N HCl. The steam trap liquid was then removed by opening the pinched clamp. Next, set the boric acid-filled conical flask underneath the condenser. After pipetting 5 ml of the digested material into the distilling flask, the funnel was washed with distilled water. When steam entered the distillation flask, 10 ml of 30% NaOH was added, stirring the sodium hydroxide and digestion mix. Thus, released ammonia escapes into the boric acid solution through the condenser along with steam, creating a solution that is bluish green.

Percentage of total Nitrogen = (14 x (V – V1) x 100 x S) / W x 1000

Percentage of total protein = Total Nitrogen (%) x 6.25

Where, 14 is molecular weight of Nitrogen, 6.25 is conversion factor, 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, W = Weight of sample taken for digestion

Fat determination

The Soxhlet extraction method was used to assess the fat content of a sample of mushrooms. An oven-dried powdered sample weighing 10 g was kept in a thimble. Next, cotton is folded and inserted into the thimble so as to cover the sample. A dried round-bottom flask weight was noted. After that, the sample and thimble were put inside the Soxhlet apparatus, where they were extracted using petrolium spirit for four to five hours. In an evaporating dish covered in tar, the solvent was evaporated and then weighed. The following calculation was used to determine fat percentage (AOAC, 2005).

Percentage of fat = (M1 – M2)/E x 100

Where, M1= wt. of round bottom flask, M2 = wt. of round bottom flask with fat, E= wt. of sample

Carbohydrate determination

Total carbohydrate was calculated by using AOAC (Association of Official Analytical Chemists), 18th edition official method (Horwitz & Latimer, 2005).

Percentage of total carbohydrate = 100 – (Ash % + Fat % + Protein %)

Crude fiber determination

Acid base digestion method was used to quantify amount of crude fiber (AOAC, 2000). Firstly, 0.5- 1 g sample was taken in crucible and assembled in (fibrotron) crude fiber instrument connected with condenser. Then, digestion is carried out with 1.25% H_2SO_4 for 30 min. It was washed with warm H_2O to remove excess H_2SO_4. For digestion, 1.25 % of NaOH was used for 30 min followed by wash with warm water to remove excess NaOH. It was then wash with alcohol. The crucible was heated at 110 °C to constant weight and cooled in desiccator and weighted. The content of the crucible was ignited in muffle furnace for 20 min. Finally, it was cooled and weighted to get crude fiber quantity.

Percentage of Fiber = (Wt. of dried sample – Wt. of ash) / Wt. of dried sample x 100

Determination of minerals

Preparation of ash solution

The ash solution was prepared by adding 25 ml of 10% HCl in the ash obtained from ashing. The solution was filtered through Whatman filter paper no. 1 and volume was made up to 100 ml.

Phosphorus content

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 content

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 dropwise 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 $KMnO_4$ 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 utilized to assess the mean value of nutrients among species and conduct an independent sample T-test. Significance was recognized at the 5% significance level. To ensure that the results are accurate, the analysis was done three times. The experimental result was given as the mean ± standard error.

Results and Discussion

Nutrient analysis

Two species namely, *Laccaria laccata* and *Scleroderma cepa* were edible mushrooms whose nutrients analyzed. We performed analysis of six macronutrients (fiber, protein, fat, ash, carbohydrates and moisture) and three micronutrients (iron, phosphorous and calcium). All nutrient analysis was carried out on dry weight basis.

Macronutrient profile

Mushroom contained the highest percentage of carbohydrate (54.93-60.42) % followed by fiber (37.46-48.86) % and protein (22.95-24.14) % and lowest fat percentage (0.38-0.55) (Figure 2). Null hypothesis was rejected from the independent sample t-test and shows a difference between the *Scleroderma cepa* and *Laccaria laccata* with respect to the dependent variable moisture %, carbohydrate %, and fiber %. There was sufficient evidence to say that the result is statistically significant. However, the independent sample t-test with unequal variances results acceptance of null hypothesis in case of protein %, fat% and ash % of samples. The $p>0.05$ illustrates that there was not significant difference in protein, fat and ash of two species.

Depending on the kind of mushroom, nutrient level differed because of their ability to bioaccumulate the nutrients into their cells (Mshandete & cuff, 2007). Mushrooms that have been air-dried may have as little as 5–20% moisture whereas fresh mushrooms typically have 85–95% moisture (Crisan & Sands, 1978). In our research, mushrooms sample were air dried and we found out that the moisture content of *Scleroderma cepa* was lesser (6.1%) than that of *Laccaria laccata* (11.08%) resembles with the findings of Wu et al. (2023) which states that the dense basidiome structure of *Scleroderma cepa* contributes to lower moisture content compared to other fungi because the compact morphology limits water retention. Mushrooms having more moisture spoil quickly due to susceptibility to enzymatic and microbiological degradation (Bano, 1976; Djamila et al., 2020).

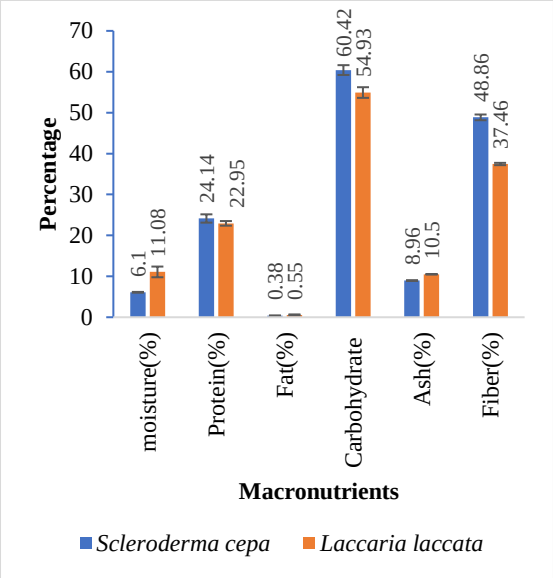

Figure 2: Macronutrient constituent in two species of mushrooms.

Crude protein content in edible mushrooms usually ranges from 19 to 40% (Kurtzman, 1978). According to our finding, *Laccaria* lacc*ata* had lower protein content (22.95%) than *Scleroderma cepa* (24.14%), as protein content fluctuates depending on the growing environment supported by the research of EC et al. (2011) stating that denser fungi like *Scleroderma cepa* tend to accumulate more protein due to their compact structures. Ash contents in two wild mushrooms range from 8.96% to 10.5% of the total weight of the mushroom. These results were similar to those published by (Singha et al., 2017; Shrestha et al., 2021) and lesser than as reported by those of (Panday & budhathoki, 2007; Egwim et al., 2011). Ash content varies in mushrooms may be due to substrate composition (Boadu et al., 2023). Mushrooms appear to be an excellent source of energy in the diet based on the measured content of carbohydrate. The results showed that *Laccaria laccata* had 54.93% carbohydrates in its total weight which is almost identical to the data from Shrestha et al. (2021). A substantial amount of carbohydrate is dietary fiber (Hamano, 1997). Mushrooms contain both soluble and insoluble dietary fiber (Park & Nile, 2014). Dietary fiber content in *Scleroderma cepa* was 48.86%. Fiber in both mushrooms differ in their content due to the fiber composition of edible mushrooms changes substantially depending on their morphological phases, such as the fruit body, mycelium, and sclerotium (Cheung, 2013). *Scleroderma cepa* and *Laccaria laccata* had fat contents of only 0.38% and 0.55%, respectively which indicate they may be an appropriate healthy diet for local people of Chaukot (Jequier & Bray, 2002).

Micronutrient profile

Phosphorus (424.9–507.72 mg/100 g) was the most prevalent micro element in all samples. It was followed by calcium (182.83-243.16 mg/100 g) and iron (43.25–48.14 mg/100 g) (Figure 3). Both of the micronutrients (phosphorus and calcium) were significantly higher ($p<0.05$) in *Laccaria laccata* compared to *Scleroderma cepa*. The iron content showed no significant differences ($p>0.05$) between two wild edible mushrooms.

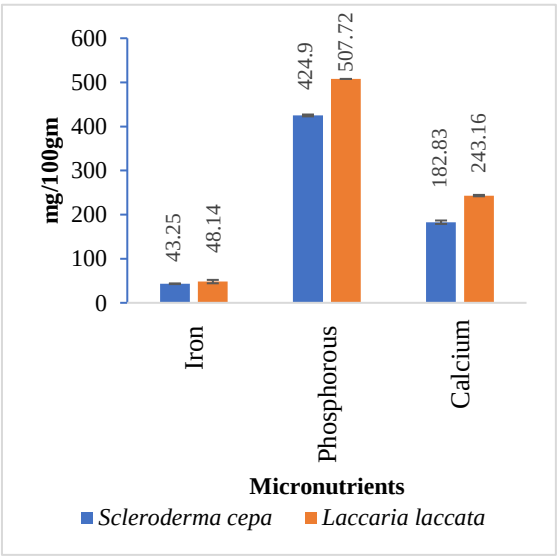

Figure 3: Micronutrient constituent in two species of mushrooms.

According to Duarte et al. (2006), concentration of micronutrients is influenced by the physiology of the species and by its ecological pattern. The study revealed high content of phosphorus as compared to iron and calcium which is in line with the result from Colak et al. (2009). It may be due to higher accumulation capacity of phosphorus by these mushroom as it was recorded in Zuo (2022) that ectomycorrhizal mushroom *Scleroderma* species improve plant growth and can replace the use of phosphate fertilizer in nursery. In comparison to *Scleroderma cepa*, *Laccaria laccata* had a greater calcium concentration. *Laccaria laccata* have a 395.5 mg/100g concentration (Egwim et al., 2011) which is higher in comparison to calcium content analysed in current investigation. The higher calcium in *Laccaria Laccata* in comparison with *Scleroderma cepa* is attributed by their effective nutrient uptake capability which is higher in comparison to calcium content analysed in current investigation. The higher calcium in *Laccaria laccata* in comparison with *Scleroderma cepa* is attributed by their effective nutrient uptake capability (Gu et al., 2019). In *Scleroderma cepa*, the iron concentration was approximately 43 mg/100g, while in *Laccaria laccata*, it was 48 mg/100g. Egwim et al. (2011) reported that the iron nutritional content was 177.69 mg/100g. Different varieties of mushrooms caused varying differences in iron concentration. These mushrooms have a mineral content range comparable to that of farmed species as noted by Crisan & Sands (1978).

Conclusion

The findings highlight that two wild edible mushrooms (*Scleroderma cepa* and *Laccaria laccata*) are an excellent source of proteins, fiber, carbohydrates, and essential minerals while being naturally low in fat, making them a highly nutritious and easily digestible food option. They contribute to improved dietary diversity and food security. Furthermore, the presence of both macro- and micronutrients in these mushrooms underscores their potential as a functional food with significant health benefits.

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