

Analysis of Nutritional and Nutraceutical Properties of Wild-Grown Mushrooms of Nepal

Jitendra Upadhyaya^{1*}, Jay Kant Raut¹ and Niranjan Koirala²

¹Nepal Academy of Science and Technology, Khumaltar, Lalitpur, Nepal

²Dr. Koirala Research Institute for Biotechnology and Biodiversity, Jadibuti, Kathmandu, Nepal

*Corresponding Author: Jitendra Upadhyaya, Nepal Academy of Science and Technology, Khumaltar, Lalitpur, Nepal.

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Abstract

The present study was conducted to evaluate nutritional properties of wild grown mushrooms collected from Far western regions of Nepal, mainly from two districts Darchula and Baitadi. Herein, it was reported and compared the nutritional value and nutraceutical values of the wild mushroom species; *Laetiporus sulphureus* (chicken of the woods), *Polypore* sp., *Trametes elegans*, *Trichaptum biforme*, *Lenzites betulina*, *Stereum complicatum*, *Trametes versicolor*, *Trichaptum subchartaceum*, and *Ganoderma lucidum*. These mushrooms were found to be rich in proteins (6.8 - 60.23%), fibres ranging from 0.174 - 36.38% and also contained fat ranging from 3.642 - 14.6%. The carbohydrate contents ranged from 7.058 to 59% (on the basis of dry weight). Similarly; ash content and moisture content ranged from 10 - 19% and 10 - 16% respectively. The protein content was highest in *Ganoderma lucidum* (*G. lucidum*) and lowest in *Trametes elegans* (*T. elegans*). The fat content was highest in *L. sulphureus* and lowest in *G. lucidum*. Total phenolic content was estimated spectrophotometrically according to the Folin-Ciocalteus method. The analysis revealed that the total phenolic contents ranged from 3.95 to 10.05 mg ml⁻¹. Similarly, the total flavonoid contents ranged from 2.149 to 11.36 mg ml⁻¹. Our result indicated the high levels of antioxidants activity thus making mushrooms suitable to be used as functional foods or nutraceutical sources. Therefore, this study provides new information regarding chemical properties of wild mushrooms, which is very important for the biodiversity characterization.

Keywords: Mushrooms; *Ganoderma lucidum*; Antioxidants; Nutraceutical Value; Biodiversity

Introduction

Mushrooms are the fleshy spore-bearing fruiting bodies of fungi [1]. As the mushrooms lack chlorophyll, they don't photosynthesize like other green plants. Mushrooms are tasty, popular to eat and an important source of nutrients. The use of mushrooms as food is probably as old as civilisation and mushrooms currently have greater importance in the diet of mankind. Cultivation and production of edible mushrooms are on the increase, particularly in Europe, America and Asia. The increased nutritional importance is due to the nutritive value of high-grade mushrooms, which almost equals to that of milk [2].

Mushrooms have a great nutritional value since they are rich in protein, with an important content of essential amino acids and fibers, poor in fat and high in vitamins and minerals [3]. In general, mushroom fruiting bodies, on a dry weight basis, contain about 39.9% carbohydrate, 17.5% protein and 2.9% fats, the rest being the minerals [4]. Wild mushrooms are considered a popular delicacy in several countries all over the world and are collected and consumed seasonally. There is a well-established consumer acceptance for wild edible mushrooms, probably due to their unique flavour and texture [5]. Moreover, wild mushrooms are collected for consumption because they are a good source of digestible proteins, carbohydrates, fibres, vitamins and nutritional components [6-8]. Wild mushrooms are source of many different nutraceuticals such as unsaturated fatty acids, phenolic compounds, tocopherols, ascorbic acid, carotenoids and alkaloids and nutrients such as proteins, fats, ash, fiber, moisture and carbohydrates. Thus, they might be used directly in diet and promote health,

taking advantage of the additive and synergistic effects of all the bioactive compounds present [6,9,10]. Moreover, wild mushroom have various properties for health benefits such as antioxidative, anti-tumour and hypercholesterolic effects [11].

Nepal possesses diverse phytogeographical zones related to altitude and other factors. Thus the vegetation varies greatly from east to west and from north to south. It is reported that in Nepal as many as 110 mushrooms are edible, 13 have medicinal importance and 45 species are found to be toxic [12]. In far western regions is rich in wild mushrooms with high medical as well as nutritional values. However, there is no any sufficient identification, processing as well as economic studies of these wild mushrooms. Therefore, the present study was focused on far western regions (Baitadi and Darchula).

Objectives of the Study

The main aim of this study is to determine nutritional and nutraceutical values of selected wild mushrooms with commercial importance.

The specific objectives of the proposed study are:

1. Estimation of the nutritional values of selected wild mushrooms.
2. To find out nutraceutical components such as flavonoids, and phenolic contents.

Materials and Methods

Selection, collection and preparation

Based on the ethnobotanical survey and literature review, the most preferred wild mushrooms were collected for the present study. The different sites of collections were: Budhi tola, Sahajpur, Ningladi, Gaira, Dharamghar, Musyachaur, Bindravan, Budha, Gajari of Baitadi and Darchula districts. The details information of sample collections is in figure 1. The collected mushrooms were dried for further studies.

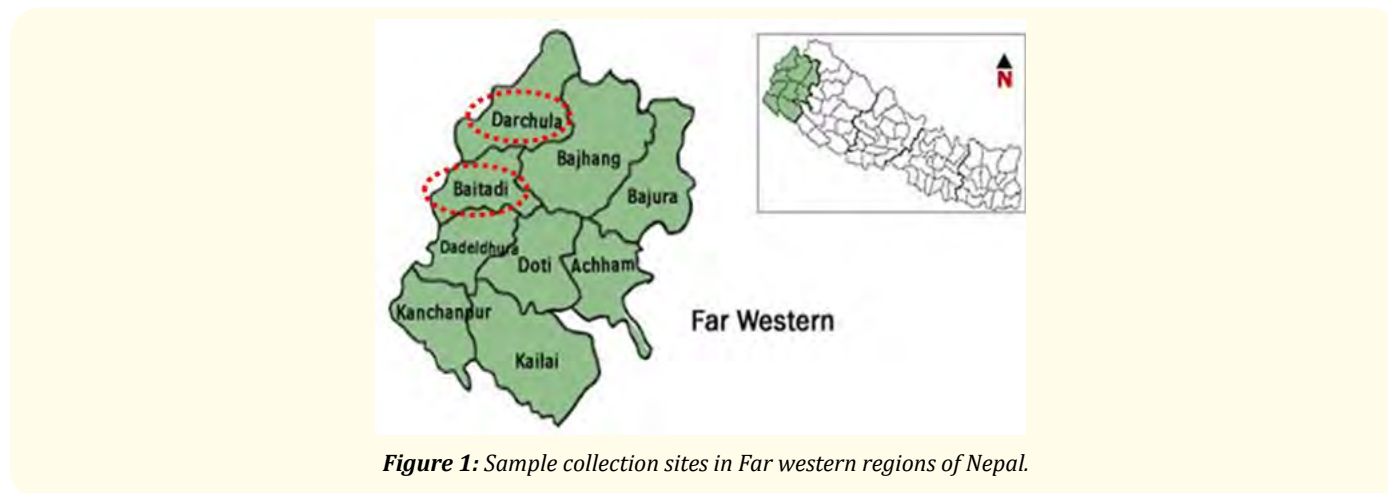


Figure 1: Sample collection sites in Far western regions of Nepal.

Processing of collected mushrooms

The collected mushrooms were dried in the shade and then ground to fine powder. The dried powdered samples were used for determination of proximate and nutraceutical analysis.

Proximate Analysis

Chemical estimation of moisture, ash, fat, fiber, carbohydrate and protein content were done by following Association of Official Analytical Chemists (AOAC),1995 guidelines [13].

Estimation of total ash

About 2g of the sample was weighed and taken in a vitreous basin. The basin was heated in a low flame at the beginning till no fumes were given off by the charred mass. The charred mass was broken by a glass rod carefully and burnt in a muffle furnace at 550 - 600°C for 4 - 5 hrs. The muffle was allowed to cool to 150°C. The basin was then cooled in a desiccator and the ash content was then weighed. The total ash was calculated as follows,

% of total ash = weight of the ash × 100 / weight of the sample

Estimation of moisture content

The samples were taken in a flat bottom dish and kept overnight in a hot air oven at 100 - 110°C and weighed. The loss in weight was regarded as a measure of moisture content.

Estimation of crude protein (Lowery's Method)

0.2 ml of BSA working standard in 5 test tubes and make up to 1 ml using distilled water. The test tube with 1 ml distilled water serves as blank. Add 4.5 ml of Reagent I and incubate for 10 minutes. After incubation add 0.5 ml of reagent II and incubate for 30 minutes. Measure the absorbance at 660 nm and plot the standard graph. Estimate the amount of protein present in the given sample from the standard graph.

Estimation of crude fat (Ether extract)

5 gm of dry sample was weighed on a piece of glazed paper and transferred into an extraction thimble. The thimble was introduced into soxhlet extractor over a pad of cotton wool, so that top of the thimble is well above the top of the siphon. A clean dry flask was taken, weighed and was fitted with the extractor. Ether was poured along the side of the extractor until it begins to siphon off. Then another half a siphonful of ether was added. The equipment thus assembled with the flask was placed on a water bath at 60 - 80°C and the extractor was connected with the condenser. Start cool water circulation in the condenser and allowed the extraction for 8 hours. Then thimble with the material was removed from the extractor. The apparatus was assembled again and heated on a water bath to recover all the ether from the receiver flask. The receiver flask was disconnected and dried it in a hot air oven at 100°C for 1 hour, cooled and weighed.

Determination of crude fiber

About 2 gm of moisture and fat free sample was weighed and transferred to the spout less one liter beaker. 200 ml 1.25% H₂SO₄ was added. The beaker was placed on hot plate and allowed to reflux for 30 minutes, timed from onset of boiling. The content was shaken after every 5 minutes. The beaker was removed from the hot plate and filtered through a muslin cloth using suction. The residue was washed with hot water till it was free from acid. The material was transferred to the same beaker and added 200 ml of 1.25% NaOH solution and refluxed for 30 minutes. Again filtered and the residue was washed with hot water till it was free from alkali. The total residue was transferred to a crucible and placed in hot air oven, allowed to dry to a constant weight at 80 - 110°C and weighed. The residue was ignited in muffle furnace at 550 - 600°C for 2 - 3 hrs, cooled and weighed again. The loss of weight due to ignition was the weight of crude fiber.

Determination of total carbohydrate

Carbohydrate can be calculated by following formula:

% of Carbohydrate = 100 - (Crude Protein% + Crude Fiber% + Ether Extract% + Total ash %)

Nutraceutical Analysis

- a) Phenolic content
- b) Flavonoid content

For quantification of the phenolics, flavonoids via Spectrophotometric method, the sample (2 mL) was dissolved in ethanol and mixed with 10 mL Folin-Ciocalteu's reagent diluted 1/10 with distilled water. After few minutes sodium carbonate (8 mL) was added to this solution. This solution was stored in dark place for two hours and after that, the absorbance was measured at 765 nm. A standard curve was prepared using gallic acid as standard with a concentration range from 100 to 500 µg/mL. Results are expressed in mg of gallic acid equivalents per gram (mg GAE g⁻¹) of mushrooms [14].

Result and Discussions

There is no doubt that mushroom-based products, mushroom nutraceuticals, can serve as superior dietary supplements to improve biological functions and contribute to human fitness and health. Commercialisation of these products is a rapidly expanding industry with the current market value of medicinal mushroom/dietary supplement products worldwide.

To promote the use of wild mushrooms as source of nutrients and nutraceuticals, several experiments were done in wild mushrooms. The analysis of nutrient includes the determination of protein, fats, ash, moisture, and fiber. The results revealed that the wild mushrooms to the villagers which they do not consider to be edible also contain a comparable protein, fat and ash content to the commonly known mushrooms.

Mushroom collection and identification

As shown in table 1, mushroom samples, collected from far western of Nepal were identified by morphological observation. The results obtained from proximate analysis of the wild mushroom are presented in the table 2.

S.No.	Collection Code	Collection Date	Altitude (m)	Coordinate	Coordinate	Substrate	Identification
1	FB-72	9/18/2016	1262	N 28°56.574'	E 80°35.923'	On trunk	<i>Laetiporus sulphureus</i>
2	FB-77	9/21/2016	1881	N 29°33.319'	E 80°32.117'	On soil	<i>Polypore</i>
3	FB-85	9/22/2016	1995	N 29°30.962'	E 80°29.639'	On log	<i>Trametes elegans</i>
4	FB-87	9/23/2016	2208	N 29°28.805'	E 80°40.595'	On log (broad leaf)	<i>Trichaptum biforme</i>
5	FB-95	9/24/2016	2031	N 29°13.370'	E 80°37.516'	On trunk	<i>Lenzites betulina</i>
6	FB-97	9/24/2016	1600	N 28°59.654'	E 80°37.525'	On log	<i>Stereum complicatum</i>
7	FB-98	9/24/2016	1600	N 28°59.654'	E 80°37.525'	On log	<i>Trametes versicolor</i>
8	FB-99	9/24/2016	1600	N 28°59.654'	E 80°37.525'	On log	<i>Trichaptum subchartaceum</i>
9	FB-101	9/19/2016	1882	N 29°33.319'	E 80°32.117'	On trunk	<i>Ganoderma lucidum</i>

Table 1: Mushrooms collection and identification.

Name	% moisture	% Ash	% Fiber	% Fat	% Protein	% Carbohydrate
<i>Laetiporus sulphureus</i>	11.68	15.34	1.074	14.6	51.23	7.15
<i>Polypore1</i>	12.24	19.13	6.055	11.68	4.08	52.868
<i>T. elegans</i>	16.49	15.23	4.123	9.2	6.8	52.28
<i>Stereum complicatum</i>	11.83	16.53	28.54	12.8	12.15	46.69
<i>Lenzites betulina</i>	13.04	10.28	5.855	11.66	40.012	25.004
<i>Stereum complicatum</i>	11.19	15.9	22.09	4.368	8.7	59.842
<i>T. versicolor</i>	12.5	18.94	20.89	2.8	11.21	54.55
<i>Trichaptum subchartaceum</i>	11.44	18.74	36.38	9.376	19.02	41.424
<i>Ganoderma Lucidium</i>	10.61	18.46	14.21	36.64	60.23	7.058

Table 2: Proximate Composition (%) of nine wild growing mushrooms.

The ash content in wild mushrooms was found to be highest in *polypore* (18%) and lowest in *L. betulina* (10%) whereas *G. lucidium*, *T. Subchartaceum*, *T. Versicolor*, *S. complicatum*, *L. sulphureus*, *T. elegans* and *T. biforme* had almost same ash as shown in figure 2. The ash content observed were between 10 - 18% which indicates that the mushroom contains some nutritionally important minerals.

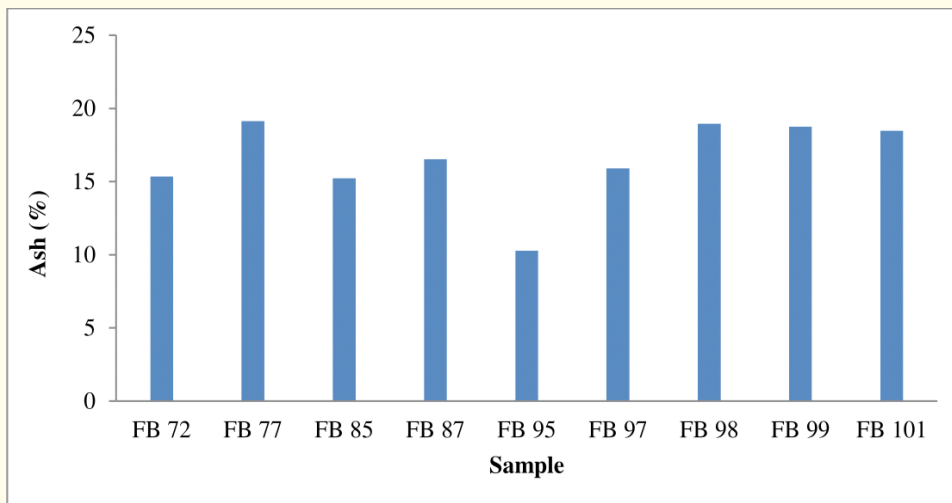


Figure 2: Ash (%) present in different wild mushrooms.

Previous studies conducted in Nigeria showed that wild mushroom species *Plerotus ostreatus*, *Agraricus bisporus*, *Auricularia polytrichia* and *Lennus sajor* also contained ash range from 4 - 16% which is nearly constancy with our result [1].

Moisture (%)

Among the nine samples, we found that *T. elegans* contained the highest moisture (16.46%), and *G. Lucidium* contained least moisture contain (10.61%). The *L. sulphureus*, *S. complicatum*, *T. subchartaceum*, *S. complicatum* contained same percentage of moisture. Similarly, *Polypore1*, *L. betulina* and *T. versicolor* contained slightly lower than *T. elegans* shown in figure 3.

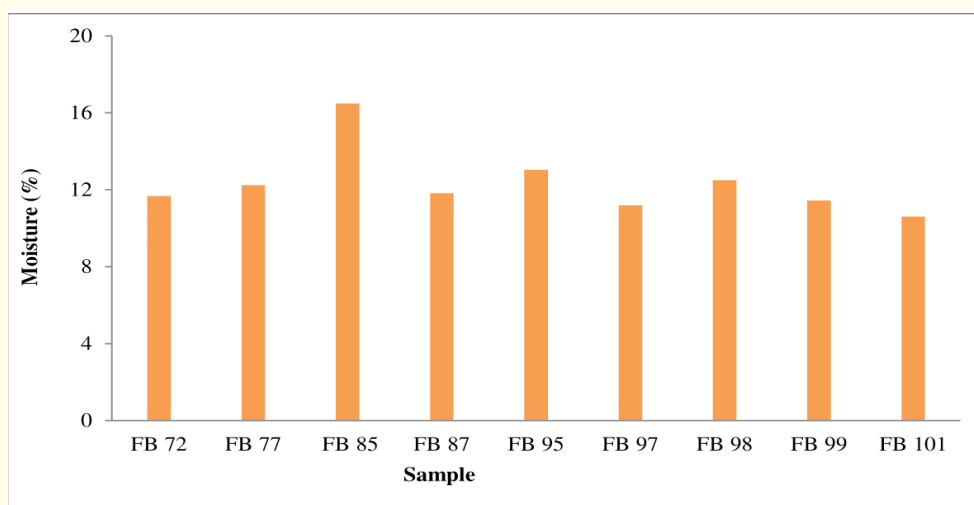


Figure 3: Moisture (%) contents present in different wild mushrooms.

The moisture contents of the mushrooms indicate that most of them are low perishable. The moisture content of wild mushroom studied in Nigeria namely coral mushroom, *Plerotus ostreatus*, *Agraricus bisporus*, *Auricularia polytrichia* and *Lennus sajor* contained high amount of moisture range 30.43- 82.17 % which is contradictory to our studies [1].

Fiber (%)

The fibre contents of the mushrooms were appreciably high, suggesting that the mushrooms would be valuable in improving human health by quickening the excretion of wastes and toxins from the body. In the present study (Figure 4), we found that *T. subchartaceum* contained high amount of fiber (36.38%), the *L. sulphureus* (10.61%), *S. complicatum*, *T. subchartaceum*, *S. complicatum* contained same percentage of moisture contain. *Polypore1*, *L. betulina* and *T. versicolor* contained slightly lower than *T. elegans*.

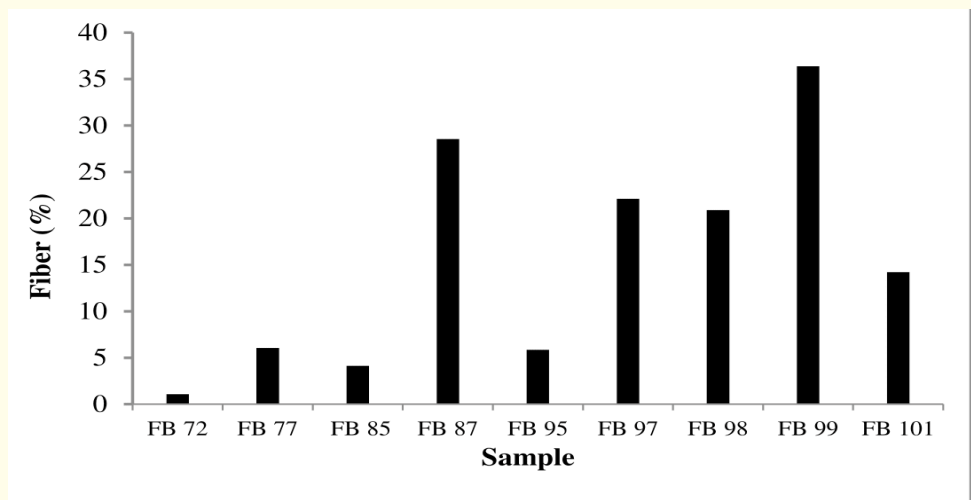


Figure 4: Fiber (%) contents of different wild mushrooms.

Fat (%)

The results showed that *L. sulphureus* contained high amount of fat whereas *S. complicatum* contained low amount (Figure 5). The fat content of these is low when compared to carbohydrates and proteins. This agrees with earlier reports by Wani., et al [15].

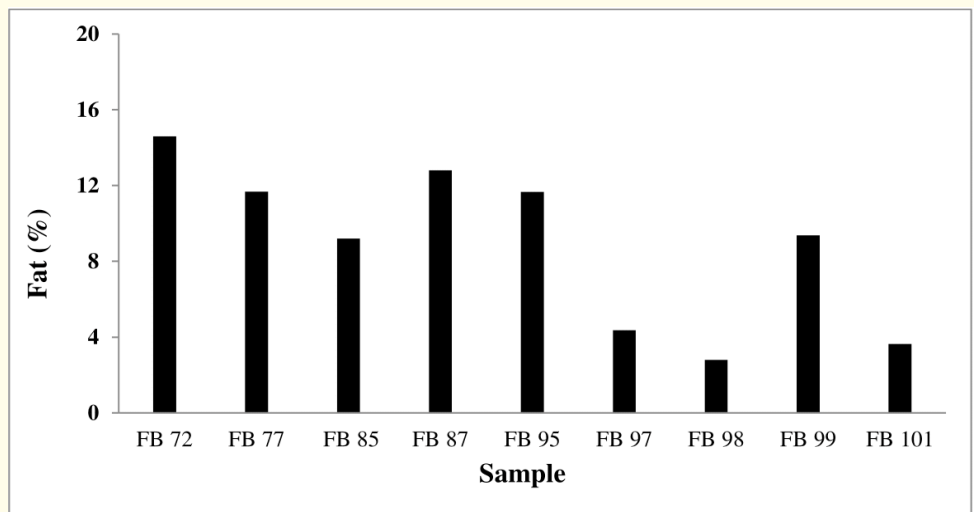


Figure 5: Fat (%) contents in different wild mushrooms.

Protein (%)

We analysed the protein contents of the nine mushroom samples collected. We found that *G. Lucidium* contained highest amount of protein and *Polypore* contain least amount of protein. *L. sulphureus*, *L. betulina*, *S. complicatum*, *T. subchartaceum*, *T. versicolor*, *T. elegans* contained 81.23%, 60.012%, 24.7%, 35.02%, 34.21%, 16.8% respectively as shown in figure 6.

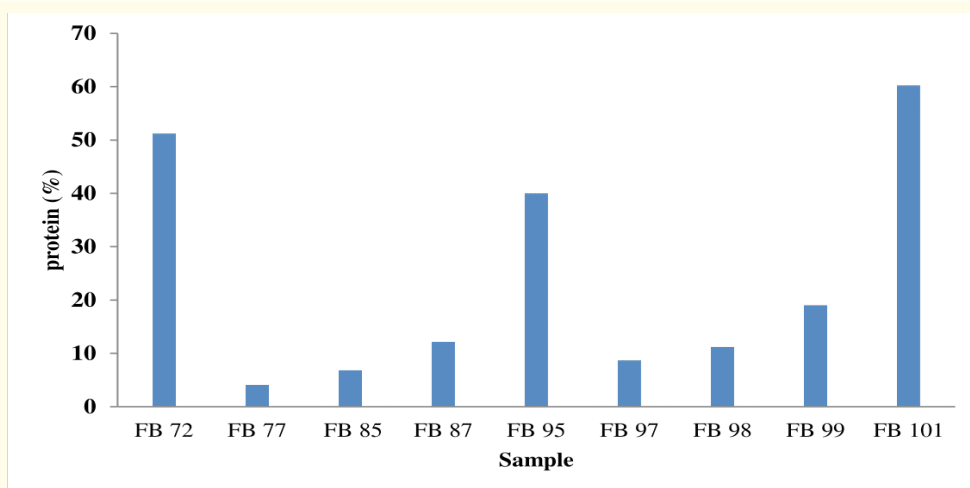


Figure 6: Protein (%) contents of the different wild mushrooms.

These are appreciable amounts of protein from nutritional perspective, suggesting that some of the mushrooms are good sources of protein.

Determine carbohydrate (%)

The obtained values of carbohydrate indicate that the mushrooms are good energy food resources. The carbohydrate concentration of the mushrooms varied from 7.0 to 59%. *S. complicatum* (which had the low value of protein) had the highest amount of carbohydrate (59.842%) followed by polypore (52.86%) and lowest in *G. lucidium* (7.058%) (Table 2).

Mineral contents

The iron (Fe), zinc (Zn), copper (Cu), lead (Pb), and manganese (Mn) contents of wild mushrooms, collected from far western regions of Nepal were analyzed. Our results indicated that the Iron content was found to be the highest in mushrooms and lead was found in negligible amount.

SN		Fe	Cu	Zn	Mn	Pb
FB87	<i>T. biforme</i>	21.71	0.238	1.107	0.322	0.1
FB98	<i>T. Versicolor</i>	35.17	0.074	0.607	0.311	0.1
FB99	<i>T. subchartaceum</i>	26.19	0.196	0.731	0.395	0.08
FB101	<i>G. lucidium</i>	23.62	0.244	0.556	0.439	0.12

Nutraceutical Analysis

Phenolic and flavonoids are considered one of the major groups of nonessential dietary components which have been suggested to be beneficial for human health [1,16-18]. The phenolic and flavonoids content of collected mushrooms are presented in figures 7 and 8.

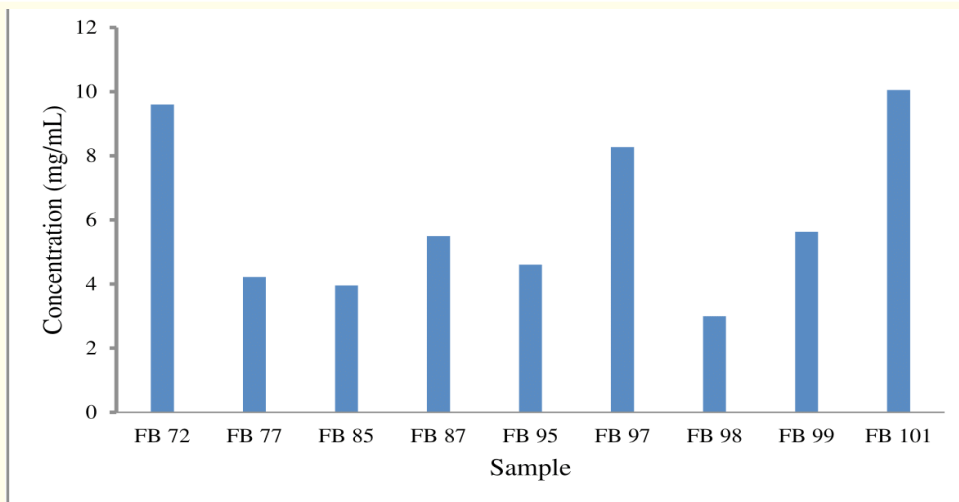


Figure 7: Concentrations of phenolic contents of different mushrooms.

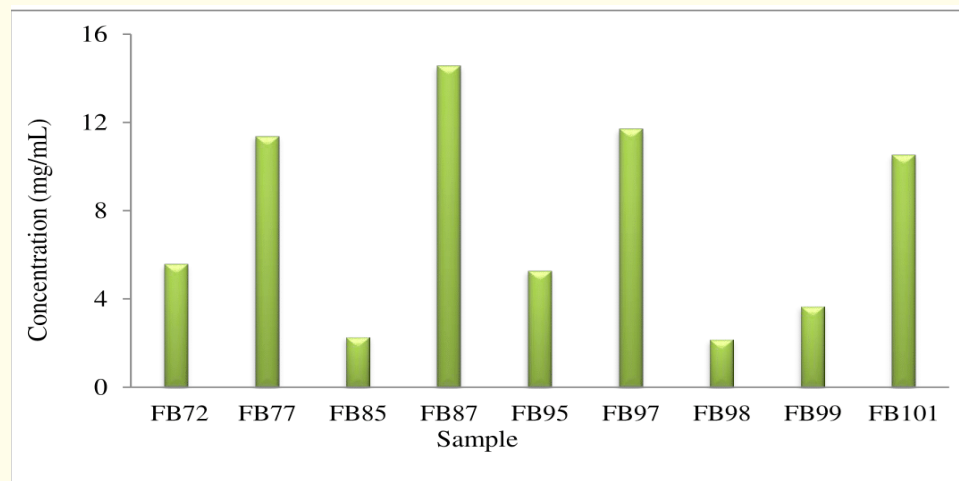


Figure 8: Concentrations of flavonoid contents of different mushrooms.

Our analysis revealed that the total phenolic contents ranged from 3.95 to 10.05 mg/ml. Moreover, the total flavonoid contents ranged from 2.14 to 11.36 mg/ml. The result indicated the high levels of antioxidants thus making mushrooms suitable to be used as functional foods or nutraceutical sources.

Conclusion and Recommendation

Here we report an evaluation of the wild mushrooms in context to their nutritional and nutraceutical composition. This work presents knowledge about the utilization of the various species of wild mushrooms. Our research shows that the wild mushrooms contain very useful nutrients and nutraceuticals such as phenolics and flavonoids which could be extracted for the purpose of being used as functional ingredients. The results of this work might be the basis that wild mushrooms can be used directly in the diet and promote health, taking advantage of the additive and synergistic effects of all the bioactive compounds present. From the results we obtained from far western regions of Nepal, it can be recommended that the highly nutritious and nutraceutical-valued mushrooms can be cultivated.

Moreover, these important mushrooms can be produced in large scale in different aspects such as medicine, foods for commercially purpose. Commercialisation of these products can be an expanding industry with the current market value of medicinal mushroom/ dietary supplements increasing worldwide. The most important thing is that, the government should review the taxation of wild mushrooms (for collection from forest) so that government revenue will increase.

Future Perspectives

Wild mushrooms are diverse and play vital roles in many local communities in Nepal but surprisingly very few published research can be found on the analysis of nutritional and nutraceutical composition of wild mushrooms. From this result, the future perspectives are as follows;

1. Cultivating the wild mushrooms that possess a high nutritional and nutraceutical values.
2. Commercially production of wild mushrooms that are highly nutritional and possess nutraceutical values.
3. Increase the revenue of government.

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