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Research Article

Wild and Cultivated Mushrooms of Nepal as a Source of Nutrients and Nutraceuticals

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Abstract

This study was carried out on nutrient and nutraceutical analysis of wild and cultivated edible mushrooms collected from different parts of Nepal. A total number of five species of mushroom were collected including two wild (*Morchella* sp. and *Ganoderma* sp.) and three cultivated species (*Pleurotus* sp., *Agaricus* sp. and *Lentinula* sp). The moisture, protein, crude fat, total ash, dietary fibre and carbohydrates were determined as nutrients whereas phenolic content, flavonoid content, antioxidant and mineral matter were determined as nutraceuticals. In this study, the highest amount of nutrients contained was protein (34%), fat (7%), fibres (42%), ash (11%), and carbohydrate (60%). The nutraceutical in these mushrooms is phenolic (0.79 µg of GAE/gm), flavonoid (0.585 µg of QE/gm) and antioxidant (0.261±0.036 mg/ml). The concentration of iron (26.19 mg/l) was found highest in all mushrooms, whereas lead and chromium were found in negligible amounts i.e., >1. The comparative study revealed that the nutrients in cultivated mushrooms have highest than in wild mushrooms. Moreover, the nutraceutical is maximum in wild mushrooms (phenolic content of 0.79 µg of GAE/gm, flavonoid content of 0.58 µg of GAE/gm) than in cultivated mushrooms. Furthermore, wild edible mushrooms are a good source of antioxidants than cultivated ones. In conclusion, this study suggested that mushrooms are a good source of nutrients and nutraceuticals which are considered valuable good nutritional dietary supplements.

Keywords: Antioxidant, Flavonoid, Mushrooms, Nutrients, Phenol

Introduction

Mushrooms have been utilized as a portion of food from ancient times (Rahi et al., 2004) and act as a source for the development of drugs and nutraceuticals (Lakhanpal & Rana, 2005). They are responsible for their antioxidant, antitumour and antimicrobial properties (Jones & Janardhanan, 2000). The term 'mushroom nutraceuticals' has been coined by Chang & Buswell (2001) for the description of those compounds that have considerable potential as dietary supplements and use in the prevention and treatment of various human diseases without any troublesome side-effects that frequently accompany treatments involving synthetic drugs. Mushrooms boost the

immune system and also have anti-cancerous properties. It acts as an anti-hypercholesterolemia and hepato-protective agent, and showed anti-HIV and anti-viral activities, and ameliorates the toxic effect of chemo- and radiotherapy (Khatun et al., 2012). Mushrooms act as a source of folic acid, the blood-building vitamin that prevents anaemia (Kannaiyan & Ramaswamy, 1980). Some species have been properly analyzed for medicinal values such as *Ganoderma lucidum* (Reishi), *Lentinus edodes* (Shiitake), *Grifola frondosa* (Maitake), *Agaricus blazei* (Hime-matsutake), *Cordyceps militaris* (Caterpillar fungus), *Pleurotus ostreatus* (Oyster mushroom) and *Hericium erinaceous* (Lions mane).

Mushrooms contain various nutritional components like carbohydrates, adenosines, terpenoids, hormones, proteins, vitamins, amino acids, fibres, minerals, essential oils and steroids. Good quality mushrooms can even possess a similar amount of nutrients to milk (Upadhyaya, 2017). Regular consumption of mushrooms helps to boost the immune system. Mushrooms are also an important source of natural active compounds and have potential additional medicinal value (Cheung, 2010). Most of the mushrooms are wild, though considerable species are cultivated worldwide. About 38,000 species of mushrooms are known in the world, out of which around 2,000 species are edible, and more than 1,000 species are poisonous (Chang, 1995). Many countries eat mushrooms with different specifications of delicious food (Aneja, 2001). Wild mushrooms are very often collected in rural and remote areas of Nepal as a source of food. Some of the work has been done through the survey of mushroom species in Nepal which tells that there are about 1,000 species of conspicuous mushrooms in Nepal (Bhandari, 1985). Professional mushroom cultivation has started for 30 years in different parts of Nepal. It is expected the experts that there are about 200 edible varieties of mushrooms in Nepal, but less than 20 kinds only appear in local markets (Bhattarai, 2001). The research in mushroom cultivation and commercialization began in 1974 under Nepal Agriculture Research Council. Likewise, people started to cultivate white bottom mushrooms in 1977, oyster mushrooms in 1984, Shiitake and *Ganoderma* were lately introduced in 2001. About 8000 to 10000 kg of mushrooms are produced daily in Nepal, including the major cities (Poudel & Bajracharya, 2011). The farming of mushrooms has been in progress in some districts such as Kathmandu, Lalitpur, Bhaktapur, Kaski, Nuwakot, Dhading, Parbat, Baglung, Manag, Mustang, Myagdi, Palpa, Shyangja, Tanahun, Gulmi, Lamjung, Gorkha, Dhankuta, Sholukhumbu and Bhojpur (Mishra & Mishra, 2013).

In the present study, we intend to evaluate the wild and cultivated edible mushrooms in their nutrients and nutraceutical values.

Materials and Methods

Materials

Three cultivated mushroom species; *Agaricus bisporus* (Gobre chyau), *Pleurotus ostratus* (Kanne chyau) and *Lentinula edodes* (Shiitake), and two wild edible mushroom species; *Morchella esculenta* (Gucchi chyau) and *Ganoderma* (Rato Chyau) were collected from the Kathmandu, Chitwan, Jumla and Tanahu districts of Nepal (Table 1, Figure 1).

Table 1: Collection of mushrooms.

Collection Area	Scientific name	Local name
Kathmandu (Ktm)	*Pleurotus ostratus*	Kanne chyau
Chitwan (Ctwn)	*Agaricus bisporus*	Gobre chyau
Jumla	*Morchella esculenta*	Gucchi
Kathmandu (Ktm)	*Lentinula edodes*	Shiitake
Chitwan (Ctwn)	*Pleurotus ostratus*	Kanne chyau
Kathmandu (NARC)	*Agaricus bisporus*	Gobre chyau
Tanahu	*Ganoderma*	Rato Chyau

Figure 1: Mushrooms collected during the study. (a) *Pleurotus ostrotus*; (b) *Lentinula edodes*; (c) *Morchella esculenta*; (d) *Agaricus bisporus*.

Preparation of the Samples

Collected samples were washed and cleaned with distilled water to remove external dirt, dust and physical impurities then dried at room temperature. The air-dried samples were crushed to powder and stored in airtight bottles to protect samples from contamination and deterioration.

Proximate Analysis

The proximate analysis contains the percentage of moisture, ash, crude protein, crude fat, crude fibre and total carbohydrate.

Estimation of moisture content: One gram of the sample was weighed in a crucible. The content was dried in an oven at 100±5℃ for 1 hour, cooled in desiccators and weighed. The process was repeated till constant weight was obtained. The loss of weight from samples represents as moisture content of the sample (AOAC, 1995).

Estimation of crude protein: Crude protein was estimated by the Kjeldhal method (AOAC, 2005). The sample was passed through three steps i.e., digestion, distillation and titration. One gram of sample along with 2.5 g of digestion mixture ($CuSO_4$ +Na_2SO_4) in the ratio of 1:5 with 10 ml of sulphuric acid at Kjeldhal flask heated till the solution turns transparent. The same process was followed for control with catalysts and conc. H_2SO_4 without a sample was taken as blank. After digestion, the solution was cooled and diluted to 100 ml with distilled water in a volumetric flask. About 10 ml of the diluted solution was mixed with 10 ml of 40% NaOH and heated until 10 ml of 4% boric acid solution with indicator methyl orange turns pink to green. The solution was titrated with standard 0.05 N H_2SO_4 till the solution turned green to pink.

Estimation of crude fat: One gram of sample was placed in a thimble of the Soxhlet apparatus. The sample was extracted for 10 hours by using hexane. The collected extract was concentrated by using a rotary evaporator to evaporate the hexane. The obtained extract was dried in a hot air oven at 105ºC and weighed (Rangana, 1986).

$$\text{Percentage crude fat} = \frac{\text{Weight of fat (W2}-\text{W1)}}{\text{Weight of sample (W)}} \times 100$$

Estimation of crude fibre: The crude fibre was estimated as per the method described in AOAC (1995) with slight modification. One gram of residue from crude fat extraction was mixed with 100ml of 1.25% H_2SO_4 and boiled for 30 minutes. The solution was filtered through the Whatman filter paper. The residue was washed with hot water till it was free from acid. Then, the residue was boiled with 1.25% NaOH for exactly 30 minutes. The solution was filtered and washed with distilled water till free from alkali. The filter paper with residue was dried at 105 ℃ for 3 to 4 hours till constant weight was obtained (AOAC, 1995).

Estimation of total ash: About 5 g of sample in a dried silica crucible was ignited in a hot plate till the fuming ceased and cooled in desiccators to room temperature. It was weighed and the dish was transferred to a muffle furnace at 550ºC till light grey ash was obtained, and the ash was weighed **(**Rangana, 1986).

Estimation of the energy value: The energy value is calculated according to Atwater W.O. 1900 general factor system (Merrill & Watt, 1973). Energy value in food is calculated by multiplying per gram of carbohydrate, protein and fat by 4, 4 and 9 respectively.

Estimation of carbohydrate value: Carbohydrate content was estimated by subtracting the summation shown below.

% Carbohydrate = 100 – (Crude protein% + Crude fiber% + Hexane extract% + Total ash)

Determination of Nutraceuticals

Phenolic content: About 20 µl of mushroom extract (0.5 mg/ml) was mixed with 100 µl 1:10 Folin-Ciocalteu reagent along with 0.8 ml deionized water. Gallic acid was used as standard with different concentrations varying from 0.005-0.05 mg/ml for a standard curve. The sample was incubated for about 3 minutes. After incubation, 80 µl of 1M Na_2CO_3 was added and allowed to stand for 30 minutes at room temperature. The absorbance was observed at 765 nm in 96 well plates ELISA reader (BIOTEK, USA). Methanol was used as a blank. The total phenolic content was calculated as the mg GAE/ gram of dry extract with the help of the equation obtained from the standard curve. All the tests were done in triplicate (Ainsworth et al., 2007).

Flavonoid content: An equal volume (20 µl) of methanolic extracts (0.5 mg/ml) and standard quercetin were mixed with 5 µl 10% $ALCL_3$ and incubated for six minutes. After incubation, 5 µl of 1M potassium acetate was added and the volume was made up of 110 µl with deionized water. Then, 60 µl pure ethanol was added. The reaction mixture was shaken, and the absorbance was measured at 415 nm in 96 wells plate ELISA reader. The total flavonoid content was expressed as the mg equivalents to quercetin per gram of dry extracts with the help of the equation obtained from the standard. The entire test was performed in triplicate (Chang et al., 2002).

Antioxidant assay: The DPPH assay was performed by a previously described procedure (Mensor et al., 2001) with some modifications. About 2.5 ml of 7 different concentrations (3-100 µg/ml) of all mushroom extracts were mixed with 1 ml of 0.3 mM freshly prepared DPPH in methanol. The solution was incubated in dark for 30 minutes. After the incubation, the absorbance was measured at 518 nm in a 96-well plate by an ELISA reader. The ascorbic acid was used as standard and methanol as blank. All the tests were carried out in triplicate. The percentage of radical scavenging activity (RSA) was calculated by using the following formula. Inhibition concentration 50 (IC_{50}) was calculated based on average RSA values.

$$\%RSA = \frac{\text{absorbance (control)} - \text{absorbance (sample)}}{\text{absorbance (control)}} \times 100$$

Mineral matter: About 5 grams of dry samples were ashed in a porcelain crucible in a muffle furnace at 550ºC for 4 hours. Then, 5 ml of 6M HCl was added. The solution was kept on a hot plate for digestion to obtain a clean solution. Then, 0.1 M HNO_3 solution was added to a digested sample and made up the volume to 50 ml. Minerals were determined by using atomic absorption spectrophotometer equipped with a flame furnace (AOAC, 1995).

Results and Discussion

The nutritional and medicinal benefits of mushrooms have been mentioned in various religions and in Ayurveda. Various research has already shown the nutritious values and health benefits of mushrooms. The current study also explored the nutritive and nutraceutical perspective of five wild and cultivated species of mushrooms. Mushrooms provide a high value of protein in the human diet and can be a good source of protein. Mushrooms' advantages are not only limited to their nutritious but are equally beneficial for chronic diseases like cancer, CVDs, obesity, diabetes and many more due to their antioxidative properties and bioactive compounds.

Proximate Analysis

The moisture content of different species of mushrooms was found in different ranges 92% to 78%. *Agaricus bisporus*, having a high percentage of mushrooms and *Lentinula edodes* the lowest. The moisture content of different samples is posted in Table 2 below. Mushrooms contain a high moisture proportion depending on the mushroom species and other factors related to harvest, growth, culinary and storage conditions (Guillamon et al., 2010). The higher moisture content of mushrooms obtained in this work also suggested that fresh mushrooms cannot be stored for a longer time, as water activity enriches microbial growth (Aletor, 1995). Protein content was found highest in *Agaricus bisporus* 34% and lowest in *Pleurotus ostrotus* 18% which was collected from Chitwan. The protein contents of mushrooms have been reported to contrast according to the genetic structure of species, and the physical and chemical differences of the growing medium (Sanme et al., 2003). The fat percentage ranges from 2% to 7% in most of the edible mushrooms found in Nepal. It was also found that the fat percentage in mushrooms depends on climate, topography and seasons. Among the edible sample tested in this study, *Ganoderma* contained the highest percentage of fat i.e., 7% and *Morchella esculenta* contained the second highest amount of fat i.e., 4%.

Crude fibre is the measure of the quantity of indigestible cellulose, lignin, and other compounds. It is the residue of plant material remaining after the solvent extraction followed by digestion of dilute acid and alkali. These crude fibres are responsible for reducing the risk of chronic diseases like obesity, CVDs, DM. Crude fibre content was found lowest in *Agaricus bisporus* i.e., 9% and highest in *Ganoderma* i.e., 42% which was collected from Tanahu. Ash content is the measure of the total amount of mineral present in food. Ashing of mushroom samples was done in a muffle furnace at

a temperature of 550°C for about 5 hours. About 5 gm sample was taken. Among the sample tested in this study, *Agaricus bisporus* contained the highest percentage of ash i.e., 11% and Ganoderma contained the lowest amount of ash i.e., 2%. Carbohydrates are rich in sugar, starch and cellulose materials. In our study, carbohydrate content ranges from (31% to 60%). *P. ostratus* contain the highest percentage followed by *A. bisporus* and *Lentinula edodes*.

Generally, high protein and carbohydrate contents, and low-fat levels were also found by previous authors (Diéz & Alvarez, 2001). Nevertheless, the differences between the nutrient concentrations may be characterized to a number of factors, such as mushroom strain/type, composition of growth media, time of harvest, management techniques, handling conditions, and the preparation of the substrates (Manzi et al., 2001). Mushrooms contain a high moisture proportion depending on the mushroom species and other factors related to harvest, growth, culinary and storage conditions (Guillamon et al., 2010). The higher moisture content of mushrooms obtained in this work also suggested that fresh mushrooms cannot be stored for a longer time, as water activity enriches microbial growth (Aletor, 1995). A wide-ranging was observed in the protein content depending on the type of mushroom species. Among these species, the maximum protein content was found in *Morchella esculenta* (wild species) which was almost double that of other species.

The protein contents of mushrooms have been reported to contrast according to the genetic structure of species, and the physical and chemical differences of the growing medium (Sanme et al., 2003). Among these mushroom samples, *Ganoderma* (wild species) ranked highest in fat content (6.82%) and fibre content (42%) while *Agaricus bisporus* was lowest (1.5%). These mushrooms contained an appreciably high fibre content (9%) which suggested that the mushrooms are valuable diet for the digestion system of humans. The result showed that *Ganoderma* contained the highest fibre content of 42% whereas *Agaricus bisporus* contained the lowest fibre content of 9%.

Table 2: Proximate constituents in percentage of various mushroom species. * Collected in dry form

Samples	Moisture	Crude Protein	Crude Fat	Crude Fiber	Total Ash	Carbohydrates	Energy Value Kcal/g
Pleurotus ostratus (Ktm)	91	18	2	13	8	60	3.28
Agaricus bisporus (Ctwn)	92	22	2	12	11	54	3.17
Lentinula edodes	78	20	2	21	7	52	3.06
Pleurotus ostratus (Ctwn)	79	19	3	26	7	46	2.82
Agaricus bisporus (Ktm)	86	34	3	9	9	45	3.43
Ganoderma	*	18	7	42	2	31	2.58
Morchella esculenta	*	28	4.0	14	8	45	3.28

Nutraceutical Contents

The total phenol content, flavonoid contents and antioxidant activities of various mushroom samples were calculated. A high amount of phenolic content was 0.79 µg of GAE/gm in *Morchella esculenta*. Both wild edible mushrooms showed a good amount of phenolic content as compared to commercially cultivated mushrooms. While flavonoid content in all samples ranged from (0.17-0.585 µg of QE/gm), in which *Ganoderma* possess the highest. Both cultivated and wild mushrooms showed considerable amounts of polyphenols and flavonoids (Table 3). Inhibitory concentration (IC_{50}) of antioxidant activity was calculated by graph pad prism 5. The highest IC_{50} value found in *Ganoderma* was 0.261±0.036 followed by *Morchella* 0.307±0.01 which indicates the strongest antioxidant activity (Table 4). Those samples which have a value greater than one do not exhibit good antioxidant activity. The highest DPPH free radical scavenging activity is related to their phenolic content. *Pleurotus*, *Agaricus* and *Lentinula* showed the lowest scavenging activity. Those samples which revealed a value greater than one do not exhibit good antioxidant activity. The iron (Fe),

copper (Cu), zinc (Zn), manganese (Mn), Lead (Pb) and Chromium (Cr) content of wild and cultivated mushrooms were determined (Table 5). The concentration of iron was found highest in all samples ranged 26.19-13.25 mg/L, whereas lead and chromium were found in negligible amounts. Mushrooms are known to accumulate heavy metals, the levels of which are determined by species, substrate composition and environmental factors (Svoboda et al. 2006; Garcia et al. 2009).

The comparative study revealed that the nutrients contained in cultivated mushrooms have the highest amount i.e., proteins (34%), fats (7%), fibres (42%), carbohydrates (60%) and ash (11%) than in the wild mushrooms which are proteins (18%), fats (4%), fibres (14%), carbohydrates (31%) and ash (2%). Similarly, the nutraceutical content is maximum in wild mushrooms i.e., phenol content 0.79 µg of GAE/gm, flavonoid content 0.58 µg of GAE/gm and antioxidant activity 0.307±0.01 mg/ml than in the cultivated mushroom i.e., phenol content 0.26, flavonoid content 0.03 and antioxidant activity >1.

Table 3: Total phenolic (TPC) and flavonoid content (TFC) of mushroom species.

Samples	TPC (µg of GAE/gm)	TFC (µg of QE/gm)
P. ostratus (Ktm)	0.39	0.17
A. bisporus (Ctwn)	0.54	0.18
L. edodes	0.35	0.10
P. ostratus (Ctwn)	0.26	0.07
A. bisporus (Ktm)	0.35	0.13
Ganoderma	0.47	0.58
M. esculenta	0.79	0.17

Table 4: Inhibitory Concentration (IC50) of samples.

Sample	IC_{50} (mg/ml)
Pleurotus ostratus (Ktm)	>1
Agaricus bisporus (Ctwn)	>1
Lentinula edodes	>1
Pleurotus ostratus (Ctwn)	>1
Agaricus bisporus (Ktm)	>1
Ganoderma	0.261±0.036
Morchella esculenta	0.307±0.01
Ascorbic Acid	0.0092±0.007

Table 5: Mineral content in different samples.

Sample	Conc.(mg/L)					
	Fe	Cu	Zn	Mn	Pb	Cr
Pleurotus ostratus (Ktm)	20.79	0.23	1.20	0.20	<0.01	<0.01
Agaricus bisporus (Ctwn)	22.16	0.09	0.80	0.30	<0.01	<0.01
Morchella esculenta	26.19	0.19	0.53	0.48	<0.01	<0.01
Lentinula edodes	17.69	0.24	0.95	0.79	<0.01	<0.01
Pleurotus ostratus (Ctwn)	18.22	0.25	0.12	0.21	<0.01	<0.01
Agaricus bisporus (Ktm)	14.56	0.09	0.98	0.36	<0.01	<0.01
Ganoderma	13.25	0.21	0.08	0.12	<0.01	<0.01

Conclusion

In this current study, the highest amount of nutrients contained was protein (34%), fat (7%), fibres (42%), ash (11%) and carbohydrate (60%). The nutraceutical in these mushrooms is phenolic (0.79 µg of GAE/gm), flavonoid (0.585 µg of QE/gm) and antioxidant (0.261±0.036 mg/ml). The concentration of iron (26.19 mg/l) was found highest in all samples, whereas lead and chromium were found in negligible amounts i.e., >1. Moreover, the cultivated mushroom contained the highest amount of proteins than the wild mushroom. Wild mushroom has a maximum amount of polyphenols and flavonoids than cultivated mushroom. From this study, it can be concluded that wild edible mushrooms are a good source of antioxidant activity than commercial species. This suggested that they are a good source of nutrients and nutraceuticals which are considered valuable good nutritional dietary supplements.

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