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Mycochemical and Nutritional Analysis of Selected Wild Mushrooms from Gaurishankar Conservation Area, Nepal

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Abstract

Wild mushrooms are the major supplement of culinary in rural parts of developing world including Nepal and are often praised for their medicinal and nutritional properties. Wild mushrooms in various parts of the world have been studied for their antioxidant, nutritional and biochemical properties. However, such information is lacking in case of Nepalese wild mushrooms. In this study antioxidant activity and nutritional composition of six wild mushrooms from Gaurishankar conservation area of eastern Nepal was carried out. Radical scavenging activity, total flavonoid, total phenolic, β carotene, lycopene, Vitamin C and protein content of the samples were analyzed following established protocols. *Stereum ostrea* and *Geoglossum cookeanum* showed higher scavenging activity among all tested mushroom samples. Total flavonoid content, β carotene and lycopene were the highest in *Daldinia concentrica*. Total protein content was the highest in *Trichaptum abietinum*. Low amount of Vitamin C, β carotene and lycopene was observed in all samples.

Keywords: Wild Mushrooms; Mycochemical; Antioxidant; Nutrition; Nepal

Introduction

Mushrooms have been consumed by human beings as dietary source since time immemorial. Good numbers of mushroom are recorded as edible and most of them have medicinal importance. Ethnomycological and mycochemical studies show that mushroom is highly nutritious [1] having good quality proteins, fibers, carbohydrates and vitamins [2,3,4] which is the major reason behind its popularity in vegetarian community. Diverse color, shape and taste are other reasons for increase in market demand for mushrooms exponentially [1,2]. Mushrooms are widely distributed throughout the world in every climatic region with high humidity [5,6]. Out of 140,000 estimated mushroom species in the world around 14,000 species are only described so far [7,8]. Nepal, having diverse climatic regions within narrow geographic area, is rich in micobiota [9] and till date 1125 species of mushrooms have been identified from here [10].

With modernization, disease diversity is increasing dramatically. Complex diseases like hypertension, diabetes, cancer, cardio-vascular diseases etc. are listed at the top of the current diseases. According to WHO, cardiac

stroke is the deadliest disease followed by pulmonary dysfunction, cancer, diabetes etc. [11]. Due to high mental and environmental stress and unhealthy food habit people are being susceptible to such complex diseases contributing high mortality rate. To overcome this devastating situation some miracle compounds like antioxidants are highly required. Antioxidants are secondary metabolites such as polyphenolic compounds, terpenes, steroids etc. They act as cleansing agents by neutralizing reactive oxygen species (ROS) produced inside the body [12]. Antioxidant compounds are available in high amounts in fruits, vegetables, mushrooms etc. [13-15]. Mushrooms like *Ganoderma*, *Morchella*, *Ophiocordyceps* etc. have high medicinal value with high antioxidant activity [12,16,17]. They have traditionally been consumed as food and medicine in many Asian countries [18]. Antioxidant, nutritional and mycochemical analysis of wild mushrooms have been reported from various parts of the world. However, little information is available on Nepalese wild mushrooms [19]. The Present study was carried out to know the antioxidant activity and nutritional composition of selected wild mushrooms from Gaurishankar conservation area of eastern Nepal.

Materials and Methods

Sample Collection and Extract Preparation

Initially six wild edible mushrooms were collected from different parts of Gaurishankar Conservation Area, Central Nepal based on ethnomycological information of local people as well as literature. During collection the morphological & ecological characters of observed specimens were properly noted. Photographs of specimen from different angles were also taken for future studies. Identification of specimens were based on macroscopic and microscopic descriptions available in the standard literature [20,21]. Scientific names and authorities follow the Index Fungorum database, the online global fungal nomenclature [22]. Reference specimens were preserved at the NAST herbarium. Collected samples were air dried for a week and then oven dried at 37°C for 2 days. The dried samples were grinded to make fine powder and stored in zip locked polythene bags in dry place.

Methanolic Extract

For methanolic extract preparation, one gram of each sample was separately weighed and soaked with 20 mL of methanol. The mixture was kept in shaking incubator at 100 RPM and 37°C for 24 h. Then the mixture was filtered with Whatman's No. 1 filter paper and the residue was again soaked with 20 mL of methanol and above procedure was repeated for another 24 h. Final volume of each sample was made to 40 mL with addition of extra methanol and stored at refrigerator until further analysis.

Metaphosphoric Acid Extract

For the preparation of the extract, 0.2 g of each samples were weighed and soaked with 2 mL of metaphosphoric acid (1%). The mixture was kept at room temperature for 45 minutes. Then it was filtered with Whatman's No. 1 filter paper.

Antioxidant Activity

Antioxidant activity of samples were estimated using free radical scavenging activity of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) solution as described by Choi and coworkers with some modifications [23]. Different concentrations of samples (1-25 mg/mL) were mixed with 0.3 mM concentration of methanolic solution of DPPH in 1:1 ratio. The mixture was incubated in dark for 30 minutes at room temperature and absorbance was measured at wave length of 517 nm. DPPH solution and methanol were taken as positive and negative control respectively. L- ascorbic acid was taken as standard solution (10-100 µg/mL). Percentage free radical scavenging of each sample was calculated as follows:

DPPH free Radical Scavenging Activity (%) =

$$\frac{[(Ac-As) \times 100]}{AC}$$

Where Ac is absorbance of control and As is absorbance of sample respectively.

Total Flavonoid Content

Total flavonoid content (TFC) of samples was determined by using the method adopted by Chang, et al. [24] with slight modification. In brief, 100 µL of each sample was poured in 96 well ELISA plate and 100 µL of 2% Aluminium chloride ($AlCl_3$) was added to it. Above solution was incubated in dark for an hour and absorbance was measured at 415 nm in ELISA plate reader. TFC was expressed as mg Quercetin equivalent per gram of samples.

Total Phenolic Content

Total phenolic content (TPC) of samples was determined by using Folin-Ciocalteu method with slight modification [25]. Briefly, 30 µL of methanolic extract of each samples were poured in 96 well ELISA plate with 150 µL Folin Ciocalteu (10%) reagent and 120 µL of 7.5% sodium carbonate solution. The mixture was incubated for half an hour in dark and absorbance was measured at 765

nm. Total phenolic content of the samples were expressed as mg Gallic acid equivalent (GAE) per gram of samples.

Total Protein Content

Total protein content of aqueous extract of samples was determined by Boumin-serum albumin method with some modifications [26,27]. Bovine serum albumin was used as standard ranging from 10-1000 µg/mL. Firstly, 200 mg of dry mushroom sample was mixed with 20 mL double distilled water and kept into shaking incubator at 50°C in 100 RPM for 24h. Then it was filtered through Whatman's no. 1 filter paper and filtrate was used for the determination of protein content. Sample filtrate and freshly prepared Bradford reagent was taken in 1:10 ratio and vortexed for the measurement of absorbance at 595 nm against blank in ELISA plate reader.

Total Carotenoid Content

Total carotenoid content was determined by the method described by Nagata and Yamashito [28]. In brief, 0.1 g of dry powdered sample was vigorously shaken with 10 mL of acetone- hexane (4:6) for 1 minute to enhance the efficacy of extraction. Then it was filtered through Whatman's no. 1 filter paper and filtrate was used for the measurement of absorbance at 453, 505, 645 and 663 nm. Content of β- carotene and lycopene were measured using following equations:

Content of β- carotene= 0.216A663- 0.304A505 + 0.452A453

Content of lycopene= - 0.0458A663 + 0.372A505 - 0.0806A453

Total β- carotene and lycopene were expressed as mg/g of sample.

Vitamin C Content

Total vitamin C content was measured after following Klein and Perry with minor modification as suggested by Hegazy, et al. [29,30]. Briefly, 200 mg of each sample was mixed with 2 mL of 1% metaphosphoric acid solution and kept for 45 minutes at room temperature. The mixture was filtered through Whatman's no.1 filter paper and filtrate was used for vitamin C estimation. Then 2,6-dichlorophenolindophenol was mixed with above sample in 9:1 ratio and absorbance was measured at 515 nm against blank. L-ascorbic acid was taken as a standard (10-100 µg/mL) and amount of vitamin C content was expressed as mg ascorbic acid per gram of sample.

Results

Six species of wild mushrooms from Gaurishankar Conservation Area (Central Nepal) were taken under study for mycochemical and nutritional values. Antioxidant activity, total flavonoid content, total phenolic content, protein, carotenoid (β carotene and lycopene) and vitamin C content of the samples was analyzed.

Specimen code	Name of mushrooms	% scavenging activity of DPPH free radical				
		25 mg/g	5 mg/g	2.5 mg/g	1.25 mg/g	1 mg/g
RW241	*Daldinia concentrica*	78.74±0.19	39.76±1.06	31.75±0.86	21.85±0.90	17.20±0.89
RW238	*Fomes fomentarius*	88.74±0.13	38.02±1.12	31.78±0.38	23.22±1.28	17.68±0.32
RW245	*Ganoderma applanatum*	69.32±0.78	26.91±4.50	20.67±3.96	14.58±2.17	10.48±1.12
RW250	*Geoglossum cookeanum*	39.28±0.61	35.95±10.38	22.038±3.45	13.25±1.63	2.92±0.64
RW248	*Stereum ostrea*	87.93±0.89	74.013±0.67	52.23±1.00	34.11±2.16	31.67±5.28
RW249	*Trichaptum abietinum*	29.53±1.15	16.57±0.97	14.65±1.11	12.85±0.67	10.93±0.06

Percentage DPPH radical scavenging activity of Ascorbic acid (AA) at different concentration						
AA (µg/mL)	10	20	40	60	80	100
% RSA	58.66±0.34	64.48±0.23	73.23±0.89	79.45±0.60	89.14±3.99	95.66±2.88

Table 1: DPPH radical scavenging activity of mushroom samples at different concentrations.

Antioxidants are those compounds which can neutralize the reactive oxygen and nitrogen species and maintain the optimum level of free radicals in the body of organisms [31]. Among the mushrooms, *Stereum ostrea* showed higher radical scavenging activity at all tested concentrations. At highest tested concentration, *D. concentrica* and *F. fomentarius* also reveal better antioxidant activity (Table 1). Scavenging activity of samples was concentration dependent which decreased with increase in dilution of samples. Scavenging percentage of *S. ostrea* (87.93 %) and *F. fomentarius* (88.74 %) at 25 mg/g was similar to that of L- ascorbic acid at the concentration of 80µg/mL (89.14%). However, *T. abietinum* and *G. cookeanum* showed poor activity even at the highest tested concentration.

Total amount of flavonoids, phenols, protein, carotenoids and vitamin C were calculated and presented in the Table 2. Total flavonoid, phenol, protein, β- carotene and lycopene content was found in the range of 0.34-0.26 mg/g (*D. concentrica-T. abietinum*), 5.18-1.19 mg/g (*S. Ostrea-T. abietinum*), 4.45-1.72 mg/g (*T. abietinum-G. applanatum*), 0.068-0.032 mg/g (*D. concentrica – G. cookeanum*) and 0.037-0.030 mg/g (*D. concentrica-S. ostrea*) respectively. Vitamin C content was almost similar in all the species (0.015-0.016 mg/g). Comparatively, phenolics are found to be the major component as antioxidant while vitamin C and carotenoid (β- carotene and lycopene) were in trace amount.

Sample	TFC (mg/g)	TPC (mg/g)	Protein (mg/100 mg)	β carotene (mg/g)	Lycopene (mg/g)	Vitamin C (mg/ 100 mg)
D. concentrica	0.34±0.02	3.92±0.55	2.93±0.73	0.068±0.006	0.037±0.001	0.015±0.001
F. fomentarius	0.32±0.02	2.09±0.58	3.65±0.14	0.047±0.023	0.035±0.006	0.015±0.001
G. applanatum	0.27±0.02	1.32±0.60	1.72±0.08	0.038±0.016	0.031±0.003	0.016±0.002
G. cookianum	0.29±0.02	2.29±0.58	3.13±0.03	0.032± 0.006	0.031±0.002	0.015±0.001
S. ostrea	0.31±0.02	5.18±0.54	2.04±0.05	0.046±0.014	0.030±0.004	0.015±0.001
T. abietinum	0.26±0.02	1.19±0.61	4.45±0.21	0.041±0.017	0.034±0.000	0.015±0.001

Table 2: Total flavonoid, phenolic, protein, carotenoid and vitamin C content of the samples.

Discussion

Antioxidant activity of the samples was found to be concentration dependent. All the samples except *T. abietinum* and *G. cookeanum* showed good radical scavenging activity (RSA). *S. ostrea* and *F. fomentarius* had the highest RSA among the tested species. Similar to our findings, *F. fomentarius* is reported to exhibit stronger DPPH scavenging activity, especially at higher concentrations [19,32,33]. Moreover, it showed superior antioxidant activity in all tested antioxidant model systems viz: DPPH scavenging, reducing power, total antioxidant, superoxide anion radical and metal chelating and the activity was increased with increasing sample concentration [34]. Higher RSA of mushrooms is reported previously [3,35]. This may be because of presence of antioxidant compounds such as phenolics, flavonoids, vitamin C, carotenoids etc. (Table 2). *G. applanatum* in present study showed concentration dependent antioxidant activity (10.48- 69.32%). Ganoderma species are reported to exhibit quite variation in antiradical activity from one species to another [19]. *G. applanatum* in the present study exhibit moderate RSA. Among the tested samples, extract of *T. abietinum* showed the poorest antioxidant activity as described previously [36]. Similarly, poor antiradical activity of *Trichaptum* species from Poland is reported [33]. However, Tamrakar, et al. [19] reported higher DPPH radical scavenging activity of *T. abietinum*.

Total phenolic content of the mushroom samples was determined using Folin–Ciocalteu's assay. TPC in *S. ostrea* was much higher than other species. TPC in *Ganoderma spp.* ranged between 39.5 - 124.2 mg GAE/g [19]. While *F. fomentarius* possess 32.4 mg GAE/g and *T. abietinum* have 9.5 mg GAE/g [19]. according to Karaman and coworkers *F. fomentarius* contained 43.06 mg GAE/g of ethanolic extract [37]. While analyzing polyphenolic composition of Polish wild mushrooms, Nowacka et al [33] found higher amounts of polyphenols in *F. fomentarius* (53.13 mg GAE/g). According to them *S. hirsutum* showed moderate amount (8.70 mg GAE/g), while low amount in *Trichaptum* species (2.79 mg GAE/g). However, phenolic content depends upon biological (species variation, maturity of collected sample etc.) and environmental (light intensity, insect/ pathogen infection status etc.) factors [38]. Among all antioxidants, phenolic compound was found to be the major component while carotenoid was the least. The results are supported by previously studies [39,40,41]. Similarly, protein content was the highest in *T. abietinum* (4.45%) and the least in *G. applanatum* (1.7%). The previous study also showed the content of protein in mushroom samples in the range of 2-7% [42,43]. Likewise, Barros and coworkers found it in the range of 2-3% in wild edible mushrooms of Northeast Portugal [4]. Protein determination of some mushrooms of Nepal has been done by Pandey and Budhathoki which found low level of protein content in which *Ganoderma tsugae* has 0.316% of protein; very lower than *G. applanatum* (1.7%) [44]. However, protein content is affected by type of mushroom sample, collection stage, parts collected, nitrogen availability etc. [45].

Very low amount of carotenoids and vitamin C was observed in all tested mushroom samples as reported previously [12,46,47]. Similarly, meager amount of

flavonoids was observed in mushroom samples which range from 0.26-0.34(mg/g). Low amount of flavonoids from *D. concentrica* (2.97 mg/kg) and *Cantharellus* species (0.81- 1.94mg/g) was reported previously [47,48]. Similarly, content of the flavonoid that ranges from 0.67-3.40 mg/g was obtained from various wild and commercial mushrooms [49].

The wild mushrooms under present investigation contain various useful constituents such as β carotene, Lycopene and Vitamin C, phenolics and flavonoids. Interestingly, some of these (*F. fomentarius, S. ostrea, D. concentrica, G. applanatum)* demonstrated stronger antioxidant ability and could have beneficial therapeutic and health-promoting effects in inflammations and related health problems [50].

Conclusion

The samples contain various useful nutraceuticals and *F. fomentarius* and *S. ostrea,* in particular demonstrated stronger antioxidant ability. Further study should be carried out on their micronutrient profile and antioxidant activity using various model systems.

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