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Research Article

Diversity, Characterization and Nutritional Status of Wild Edible and Medicinal Mushrooms of Sikkim

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ABSTRACT

Wild edible mushroom species are used since ancient time for their unique taste and extraordinary health enhancing bioactive compounds. Mushrooms are also a class of superfoods created from the actual fruiting body as well as the mycelium biomass. With the aim to study the diversity, nutritional content and identification of some wild edible mushrooms of Sikkim, the fungal cultures were isolated from the tissue taken from their basidiocarps, using moist chamber method. On the basis of available literature the fungi were identified by matching their characteristics like external morphology and anatomical features. Eleven different species of wild edible mushrooms namely; Laetiporus sulphurous, Polyporus guianensis, Pseudohydnum gelatinosum, Grifola frondosa, Pholiota nameko, Morchella crasspes, Auriculariacornea, Lentinula edodes, Pholiota sp., Schizophyllum commune and Trametes sanguinea were selected for detailed study. The nutritional contents of the mushrooms were analysed in terms of moisture, protein and carbohydrate (sugar) contents. The present study describes the existing situation of the wild mushrooms of Sikkim and putting emphasis on ecological effects of harvest. The results could be a valuable documentation for scientific community in domestication or cultivation of wild mushroom for nutritional and medicinal purpose.

Key words: *Sikkim, Wild edible mushroom, Fungal diversity, Nutritional content, Medicinal mushroom.*

INTRODUCTION

Mushrooms the fruiting bodies macrofungi (Ascomycotas and Basidiomycota) represents short reproductive stage in their life cycle. They are important constituents of minor forest produce, there are several references which suggests wild edible mushrooms are traditionally used by many cultures as food and medicine[1,2] and are becoming popular in our diet for their nutritional properties. Edible mushrooms have long been considered to have medicinal value and to be devoid of undesirable effects[3] also they are rich in essential nutrients such as carbohydrates, proteins, vitamins, minerals, fat, fiber and various amino acids[4].

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According to Rai *et al.,* 2005 out of approximately 14,000 known species, 2,000 are safe for human consumption and about 650 of these possess medicinal properties[5]. Numerous new species have been introduced to science with some being edible and other having medicinal use. Presently 35 mushroom spices have been cultivated commercially and of these 20 are cultivated on an industrial scale. The cultivation of button and oyster is outstanding example of a biotechnological enterprise because of a solid foundation in basic scientific research in all aspects (genetics, physiology and biochemistery) and these fundamental knowledge acquired in recent years will be of considerable value for cultivations other mushrooms.

The former Himalayan kingdom Sikkim is known for its rich biodiversity owing to its altitudinal and climatic variations. Forests is are one of the rich natural resource of the Sikkim with tree cover of approximately 47.69 percent of total geographical area of the state, which is dominated by subtropical and temperate mixed broadleaved forest. Therefore abode for macrogungi or mushroom forming fungi by establishing ectomycorrhizal associations. Rich wild fungal resource have been a very significant in life of tribal people of Sikkim, they provide a source of income and significant nutritional addition to the diet. They are collected and consumed when in season from the wild by the locals. Despite the fact that ethnic people throughout Sikkim eaten them for centuries, it is only in British colonial records very little information can be found. Considering the potential economic importance of these wild mushrooms growing in the forest of Sikkim, this is possibly the first attempt that has been carried out on their diversity, identification and biochemical composition.

In the present investigation, scientific identification of wild edible mushroom of Sikkim has been made and proximate analysis of biochemical composition of 11 different wild edible mushrooms species in terms of moisture, protein, crude fat, carbohydrate were also carried out. The results could be a valuable documentation for scientific community in domestication or cultivation of wild mushroom for nutritional and medicinal purpose.

MATERIAL AND METHODS

Study area

The wild mushrooms species of Sikkim (27º 04’ 46’’ to 28º 07’48’’ North latitude and 88º00’58’’ to 88º 55’25’’ East longitudes) were collected during rainy season typically occur from May until October from the wild and some from the obtained from local farmers market in the year 2016-2018. All important morphological characters such as shape, size, texture, colour, colour change on ageing, odour, including substrata were also noted (living trees, logs, tree stumps and twigs). They were brought to laboratory where they were stored at 4°C, until further analysis.

Identification

Identification of each of the fruiting bodies of wild edible mushrooms species was done by comparing their morphological (microscopical and macroscopical) characteristics following different standard key and manuals.

Sample preparation

After noting the characters the whole mushroom samples (without division into pileus and stipe) were used for the study. Fresh samples, after removal of external material such as mud, bush, soil, insects’ larvae etc. by washing with demineralised water with help of brush, approximately 10 g of each sample were taken immediately for determination of moisture content using balance. Final dry matter weight was taken immediately after drying in oven at the rate of 50-60 ºC. Dried samples were ground to a fine power and stored under vacuum protected from light for further analysis; biochemical and molecular analysis.

Biochemical characterization

Total Carbohydrates

One gram of the powered mushroom sample was extracted with 10ml of 80% ethyl alcohol by using soxhlet extractor for 6 hours. The crude extract was diluted to 50ml with 80% ethylalcohol. The quantity of ethanol soluble

sugar in the extract was determined using phenol sulphuric acid method of Dubois[6].

Protein content

Five gram of grinded mushroom was taken with 50ml of 0.1N NaOH and boiled for 30 minutes. The solution was cooled in room temperature and centrifuged at 1000rpm. The supernatant was collected and total protein was measured according to the Lowry's method[7].

Crude Fat content was analysed following the method of AOAC, 1990[8]

Crude Fibre content was analysed following the method of Maynard 1970[9]

RESULT AND DISCUSSION

Diversity of Edible Macrofungi

Species diversity in the natural environment of a region is one of the basic requirements to estimate the richness of species. The oldest record of the survey of naturally occurring mushroom of Sikkim Himalayas comes from the study of Berkley[10], who listed 131 species and provided taxonomic rank and nomenclature to 90 of them[10]. In the current study, survey was conducted to collect and identify macrofungi from different location as well as from local markets. In total 25 edible species macro fungi from the study areas were recorded which belongs to the division Basidiomycetes and Ascomycetes group, (Table 1) which have been used either in form of food or ingredient of medicine by the local community.

Table 1: List of wild edible Macofungi from Sikkim

Sl. No	Species name	Habitat	Growing period/place	Division	Traditional use
1	*Laetiporus sulphurous* (Bull.) Murrill	Dead or standing tree/stump.	April-June/Ravangla-South Sikkim	Basidiomycota	Food and medicine
2	*Polyporus tenuiculus*(P.Beauv)Fr/ guianensis Mont.	Dead tree trunk or stump.	April-July/Tadong-East Sikkim.	Basidiomycota	Food
3	*Pseudohydnum gelatinosum*(Scop.) P. Karst	Dead and decaying tree trunks	May-June/ Bhusuk East Sikkim.	Basidiomycota	Food
4	*Grifola frondosa* (Dicks.)Gray	Base of dead tree stump of hardwood tree	May-June/Bhusuk-East Sikkim.	Basidiomycota	Food/medicine
5	*Pholiota nameko (Berk.)Sacc. Pholiota sp.*	Dead, decaying wood.	April-june/ Samdong, East Sikkim	Basidiomycota	Food
6	*Morchella crassipes* (Vent.) Pers.	On floor near deciduous tree/banboo bush.	April-may/ Tadong- East Sikkim.	Ascomycota	Food and medicine
7	*Auricularia cornea (*Ehrenb.) Fr.	Dead and decaying wood	May-august, Tadong –East Sikkim.	Basidiomycota	Food and medicine
8	*Lentinula lateritia* (Berk.)Pegler	On Castanopsis dead trunk	May-July/Bhusuk-south Sikkim	Basidiomycota	Food
9	*Pleurotus ostreatus* (Jacq.) P.	Dead Castanopsis, Alnus nepalensis	Sept-Oct/Pangthang-East Sikkim.	Basidiomycota	Food
10	*Pycnoporus cinnabarinus* (Jacq.) P. Karst	On dead log of hardwood	May-Oct/Tadong-East Sikkim.	Basidiomycota	Medicine
11	*Schizophyllum commune* Fr	On dead bamboo plant, dead trunk.	June-August/ Gangtok-E-Sikkim.	Basidiomycota	Food/ medicine
12	*Tremella fuciformis* Berk.	On twig, branch of fruit tree.	June-July/Tadong East Sikkim.	Basidiomycota	Food/medicine
13	*Ganoderma applanatum* (Pers.) Pat.	On the base of stump of Alnus nepalensis	Feb-Oct/Temi, south Sikkim.	Basidiomycota	Medicine
14	*Ganoderma lucidum* (Curtis)P.Karst	Dead stump	Sept-Dec/Temi south Sikkim.	Basidiomycota	Medicine
15	*Sparassis crispa* (Wulfen) Fr.	On dead log/branch	Nov-Dec/Samdong East Sikkim	Basidiomycota	Food
16	*Dacryopinax spathularia* (Schwein.)G.W.Martin	On dead fallan hardwood log	June-August/Samdong East Sikkim.	Basidiomycota	Food
17	*Armillaria mellea* (Vahl)P.Kumm	Dead and decaying wood stump	June-August/Samdong East Sikkim.	Basidiomycota	Food
18	*Daldinia concentric* (Bolton) Cesati&de Notaris	Dead, Rotting log	August-Sept/Tadong East Sikkim.	Ascomycota	Medicine
19	*Termitomyces sp.* R.Heim	On soft ground of cardamom field	June-August/ Samdong East Sikkim.	Basidiomycota	Food
20	*Fomes fomentarius* (L.)Fr.	On dead standing hardwood	Feb-oct, Temi south Sikkim	Basidiomycota	Medicine
21	*Trametes versicolor* (L.)Lioyd	Dead tree trunk of cherry tree	Feb-June/Tadong East Sikkim.	Basidiomycota	Medicine
22	*Coprinus comatus(Mul.)Pers.*	On moist floor	May-August/Samdong	Basidiomycota	Food
23	*Xylaria polymorpha* (Pers.)Grev.	Dead , rotting wood/stump	may-August/Tadong	Ascomycota	Medicine
24	*Lycopordon sp.*	On forest walls and floor	July-october/Samdong East Sikkim	Basidiomycota	Food/medicine
25	*Ticholoma sp.*	On forest floor	July-Sept/ Samdong East Sikkim	Basidiomycota	food

The morphological description of selected mushroom species is represented in Figure 1

Table 2: They were further analysed in terms of their biochemical composition

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Fig. 1: *Laetiporus sulphurous(1), Polyporus tenuiculus/ guianensis (2,2b), Pseudohydnum gelatinosum(3), Grifola frondosa(4), Pholiota sp.(5,5b), Morchella crassipes* (Vent.) Pers. *6), Auricularia cornea (*Ehrenb.) Fr. *(7), Lentinula edodes lateritia(8), Pleurotus ostreatus (9), Pycnoporus cinnabarinus* (10), *Schizophyllum commune(11), Tremella fuciformis(12,12b), Ganoderma applanatum(13,13b), Ganoderma lucidum(14), Sparassis crispa(15), Dacryopinax spathularia(16), Daldinia concentric(18), Termitomyces sp.(19), Fomes fomentarius(20,20b), Trametes versicolor(21), Coprinus comatus(22), Xylaria polymorpha(23),*

Table 2: Morphological description selected of mushrooms

Sl. no	Species name	Local name	Description of mushroom
1	*Laetiporus sulphurous* (Bull.) Murrill	Mirge chyau	Fruitbodies 19-25 cm width/ 5-14 cm length. Overlapping fruitbodies. Woody pleasant smell, Cap wavy edged thicker base than margin. Orange yellow, smooth surface with margin, pore very minute, underside creamy white to yellowish. Young bracket are soft and spongy. Spore print white, ecology is saprotrophic/weak parasitic.
2	*Polyporus tenuiculus*(P.Beauv)Fr /guianensis Mont.	Chambre chyau	Fruitbodies 4-10cm wide/2-4cm length. Annual basidiocarp grows on dead and decaying wood. The pileus is white when fresh and becomes paler/light beige on maturity. Short stipe present. Wet woody smell, pileus surface smooth with a poroid himenium. Pores honycomb shaped. In bunch/overlapping sometimes. Spore print white/off-white.
3	*Pseudohydnum gelatinosum*(Scop.) P.	Zibro chyau	Fruitbodies 4-10cm wide/ 6-7 cm length, tongue shaped, Cap light brown, fresh smell, smooth wrinkled surface. Broadly convex or flat, base thicker than margin, inner tissue white, soft and delicate. Short stipe, ventral pore deep, spine like, whitish. Spore print white. Found in patches or alone on dead trunks, logs and stumps.
4	*Grifola frondosa* (Dicks.)Gray	Fool/ Thakre chyau,	Fruiting body, 10-25cm occurring in a cluster consisting of multiple grayish-brown caps which are often curled/overlapped or spoon-shaped/fan shaped, with wavy margins and 3-4 cm thickness. Smooth surface, pleasant smell, undersurface minute pore tubes. The short tough stipe has a branchy structure, wavy caps which are organised in large clusters of rosettes arising from a single branched stem. Spore print white. Weak parasitic to saprobic on decaying wood.
5	*Pholiota nameko(Berk.)Sacc.*	Chipley chyau,	Fruitbodies 2-5cm length, cap 2cm wide /1.5cm long. Cluster mushroom with small cap and large quantity. Smells fruity good. Stem not delicate solid, gills present, orangy- yellowish in colour, the cap has a smooth surface with mucilage or sticky, annulus cobwebby in young. Spore print brown; Spore is thick walled, ellipsoid-oval, smooth, thick walled.
6	*Morchella crassipes* (Vent.) Pers.	Guchhi chyau	Fruitbodies 10-15cm length, colour light brown, smooth, delicate, honeycomb like wrinkly. Caps are hollow, and attached to the stem at the lower edge, Stipe also hollow leathery (2–9 cm). Pits and ridges raised on a large whitish stem, somewhat oval in outline. Found in solitary, but more often in groups, on the ground. The spores are formed in asci lining the pits, eight-spored, and paraphyses present.
7	*Auricularia cornea (*Ehrenb.) Fr.	Kane chyau/Namcho shamo	The fruiting body 3-8cm wide 2-4cm length is distinguished by its noticeably ear-like shape or cup-shaped and brown colouration, it grows upon dead wood with by a very short stalk. The species has a tough, gelatinous, elastic texture when fresh, but it dries hard and brittle. The colour becomes darker with age. The inner surface is a lighter grey-brown in colour and smooth. The spores can sometimes be seen in a whitish mass on the underside of the fruit body, spores are white, cream or yellowish smooth, hyaline.
8	*Lentinula edodes lateritia* (Berk.)	Katuse chyau	Fruitbodies 4-8cm wide 2-4cm long, sweet smell, cap light brown, smooth, convex soft, adnate, margin thin, incurved, Veil absen. Spore print white, spore ovoid to ellipsoid, hyaline. . Stipe 2-4cm central, often curved, solid. Flat topped with slightly sunken central region. Tough fibrous stem cottony. Cap texture is rubbery. Gills off white. Found to grow on dead Castanopsis, Quercus tree
9	*Pholiota sp.*	Chipley chyau/Phechung kangru shamo	Fruitbodies 2-6cm length, 2-4cm wide /1.5cm long. Cluster mushroom with small cap and large quantity. Wetly smells. Stem solid, gills present, brownish in colour, the cap has a smooth surface with mucilage. Annulus cobwebby in young. Spore print brown; Spore is thick walled, ellipsoid-oval, smooth, thick walled.
10	*Schizophyllum commune Fr.*	Mujurey chyau/Phiche kangru	Fruitbodies 1-4cm wide/2-3cm long. Whitish grey when dry, brownish when wet. Stem absent, margin lobed,split gills distinct, spore white, found to grow in bamboo, wood log, dead branches alone or in groups. Spore cylindrical, smooth

Table 3: Biochemical content per gram of dry weight results of three replicates

Sl.No	Mushroom sp.	Moisture (%)	Protein µg/g dry wt	Sugar/carbohydrates µg/g dry wt
1.	*Laetiporus sulphurous* (Bull.) Murrill	87%	157.3	932.6
2.	*Polyporus tenuiculus* (P.Beauv)Fr	86%	265.6	827.7
3.	*Pseudohydnum gelatinosum* (Scop.) P.	96%	365.0	1256.4
4.	*Grifola frondosa* (Dicks.) Gray	90%	1724.6	1710.7
5.	*Pholiota nameko(Berk.)Sacc.*	92%	479	2207.5
6.	*Morchella esculenta Fr.*	92%	380	847.7
7.	*Auricularia auricula judae* (Bull.) Quel	96%	537	1572.9
8.	*Lentinula lateritia* (Berk.) Pegler	92%	402	1090.5
9	*Pholiota sp.*	92%	420	1713.1
10	*Schizophyllum commune Fr.*	80%	280	1734.2

The results of the biochemical analysis of the wild mushroom samples from Sikkim showed that all the specimens have high moisture content (Table-3), indicating that mushrooms are highly perishable. In the present study it was observed that the moisture content of the collected mushroom samples ranges from 80% to 96%. Edible mushrooms are highly valued as a good source of carbohydrates and proteins their contents in the analysed mushroom samples were found to be significant. The carbohydrates percentage is more than protein with very negligible fats constituents and fibre content in most of the species is good.

The relatively high carbohydrates and protein content with low amounts of fats recorded in the samples is a proof of their being highly nutritious and good for human consumption.

CONCLUSION

The wild mushroom were found to be rich in carbohydrates, protein, fiber and with negligible fat content, that made them healthy nutritional alternative food with promising medicinal properties. With increasing demand for edible and medicinal mushroom, it becomes a necessity to unravel the rich biodiversity of wild macro fungi. Nevertheless, these mushrooms are likely to be lost if these wild edibles are not documented. Therefore, it is now imperative that a nutritional database of these mushrooms is set up to retain the information for a better management and conservation of this natural resource in their habitats. Besides, its potential in commercial utilization can be explored through biotechnological intervention by culturing or domestication for long term sustainability.

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