Ethnomycological Studies of Mushrooms in Kamrup District of Assam, India

Dr. Karabi Devi
St. Mary's higher secondary school, Guwahati, Assam, India
Email - devikarabi@gmail.com

Abstract: Macrofungi constitute a group of the high value non-wood forest resource worldwide. Investigations on the taxonomy and diversity of macrofungi are gaining importance, as many macrofungi are facing a threat of extinction due to environment destruction. The collection of macrofungi was done in the Tropical Semi evergreen Forest (TSEF) of Kamrup district of Assam where 5 study sites belonging to 5 reserve forests were selected. The traditional knowledge of local people related to macrofungi was gathered. An extensive study was conducted covering the whole study area through semi structured questionnaire survey. The indigenous people are mostly mycophilous and are frequently used to collect wild edible fungi (WEF) from the forests. In the study area, the tribal people mostly use only 4 species of mushrooms as food and 2 species of mushrooms in medicine. The species that were used as food included *Agaricus bisporus*, *Cantharellus lateritius*, *Lentinus squarosulus* and *Termitomyces heimii.* The macrofungi which local people of Kamrup used in medicine were *Ganoderma lucidum, Lentinula edodes and Bovista plumbea.* Three most commonly available macrofungi which were consumed the most in this region and were also sold in the local markets were *Cantharellus cibarius. Cantharellus lateritius and Lentinus squarusulus* . These were hence selected for nutritional analysis. and were analysed for their macronutrients properties like carbohydrates, proteins, fibre and lipid content and micronutrients like Fe, Ca, Zn, Cu, Pb and Mg. Their physical properties such as moisture contents, dry matter and ash contents were also measured.

Key words: Kamrup district, Assam, Wild edible fungi, medicinal, macronutrient, micronutrient

1. INTRODUCTION:

Macrofungi are the higher fungi having hypogeous or epigeous distinctive fruiting bodies which can be seen with the unaided eye and be picked by hand (Chang and Miles, 1992). Macrofungi are defined as fungi that form large fructifications, such as gilled fungi, jelly fungi, coral fungi, stinkhorns, bracket fungi, puffballs and bird's nest fungi etc. (Hawksworth *et al*., 1995; Richard's and Murray, 2002; Bates, 2006). These terms reflect the morphological diversity that is encountered within the macrofungi. The term macrofungi or macromycetes has been variously defined by several authors. All the definitions however lay emphasis on the production of fruiting bodies that are visible to the naked eye (Redhead, 1997; Lodge *et al*., 2004; Da Silva, 2005; Seen *et al*., 2007). There are thousands of species of macrofungi where each and every species is unique and beautiful in its own way. The group includes mainly terrestrial species of diverse forms and habitat and is a general term used mainly for the fruiting bodies of macrofungi (Ascomycota and Basidiomycota) and represents only a short reproductive stage in their life cycle (Das, 2010). The division Basidiomycota includes mushrooms, puffballs, corals, teeth and bracket fungi while the Ascomycota includes cup fungi, morels, etc.

Wild edible fungi (WEF) have been collected and consumed by people for thousands of years. Of the 14,000 mushroom species, nearly 7000 species are well studied to possess varying degree of edibility and more than 3000 species spread over in 31 genera are regarded as prime edible. Thus far, only 200 of them are experimentally cultured, 100 economically cultivated, approximately 60 commercially grown and about 10 have reached an industrial scale (Chang and Miles, 2004), whereas, 283 species are reported to be available in India (Purkayastha and Chandra, 1985). Ethnomycology is the study of wild edible macrofungi with their identification and documentation of nutritional potential and indigenous knowledge regarding their uses as medicine or in some other cultural traditions. Edible fungi were collected from forests in ancient Greek and Roman times and highly valued, though more by high-ranking people than by peasants (Buller, 1914). Mushrooms are not only prized for their splendid tasteful flavor; they also serve as a good healthy supplement. In addition to proteins, sugars, glycogen, lipid, vitamins, amino acids and crude fibre, mushrooms possess some essential mineral nutrients which are considered as key factors for the normal functioning of the body (Gbolagade *et al*., 2006; Kalac, 2013). The awareness of wild edible fungi and their importance to people are generally poor. Subsistence uses in developing countries have often been ignored and it is only in recent years that initiatives on non-wood forest products (NWFP) have begun to explain their widespread use and roles in livelihoods.

Macrofungi are the centre of attraction as food, medicine and cosmetics throughout the world (Hyde *et al*., 2010). Utilization of macrofungi e.g. mushrooms, puffballs and morels as nutritional source against plant and

animal products is one of the viable avenues to fulfil the protein–energy demand (Boa, 2004). Edible mushrooms are highly valued as good source of proteins, fibres and carbohydrates and are found to have low amount of fat (Barros *et al*., 2008, Johnsy *et al*., 2011). Wild mushroom protein also contains considerable amount of non-essential amino acids such as alanine, arginine, glycine, glutamic acid, aspartic acid, proline and serine. They can be used to solve the problem of malnutrition (Manandhar, 2003). Consequently, the high nutritional and medicinal qualities and unique flavour of these wild edible fungi (WEF) should be properly documented before they are lost due to loss of species from nature or loss of traditional knowledge descended from the elders.

2. MATERIALS AND METHODS:

2.1 STUDY AREA:

The study area is located in Kamrup district of Assam, India.

It is situated between 25.46 and 26.49 North Latitude and between 90.48 and 91.50 east Longitude.

Five reserve forests belonging to Tropical Semi evergreen forest were selected for the study The total geographical area of the district is 4, 34,500 acres and the total forest cover is approximately 1,16,694 Ha.

The climate of the district is sub-tropical with semi dry summer and cold in winter. Annual rainfall ranges from 1500 mm to 2600mm. Average humidity is 75%

Maximum temperature is 38.5˚C and minimum is 7˚ C

2.2 ETHNOMYCOLOGICAL STUDIES:

To gather and document the traditional knowledge of local people related to macrofungi, an extensive study was conducted covering the whole study area through semi structured questionnaire survey.

This study was also based personal interviews conducted with local people especially elderly and traditional knowledge holders of the forest dwellers as well as by local market survey of Kamrup district of Assam. The questionnaire included questions on their knowledge on mushrooms and their utilization patterns. The questionnaires were administered randomly to about 50 respondents selected from 5 villages (10 respondents per village) under 5 reserve forests of Kamrup district of Assam. Interviews with some group of elders were also taken to gather detail information on the mushrooms they utilize and associated tradition. The queries were made relating to the occurrence, season and time of collection, method of consumption and their dependency on macrofungi with importance in tradition and medicine. Information was also gathered on whether they sell the macrofungi in the market, if yes, how much this money helps in their livelihood sustenance, whether any case of poisoning or casualty was reported and why they consume them, etc.

2.3 NUTRITIONAL ANALYSIS OF THE SELECTED WILD EDIBLE MACROFUNGAL SPECIES:

i) Selection of WEF for nutritional studies

The macrofungi which were most frequently collected from the wild and were also sold in the local markets were selected for nutritional analysis.

ii) Sample preparation

Mushrooms were first washed thoroughly to remove dust, mud and other extraneous materials and excess moisture of samples were removed with blotting paper. The samples are then dried in a hot air oven at 60°C for 3 days and stored under air tight containers for further analysis**.**

2.4 PHYSICAL PROPERTIES

i) Moisture content

The fresh weight of each mushroom sample was taken using chemical balance. These samples were then oven dried separately at 80°Cfor 48 hours. The loss in weight obtained after drying was regarded as the moisture content (Johnsy *et al*., 2011).

ii) Dry matter Content

This was taken as the final weight obtained after the samples have been oven dried at 80°C for 48 hours (Johnsy *et al*., 2011).

iii) Ash content

The powdered mushroom sample (3.0 gram) was ashed in a muffle furnace in previously ignited and cooled crucible of known weight at 550°C for 6 hours (Manzi *et al*., 2001).

2.5 DETERMINATION OF BIOCHEMICAL PROPERTIES

i) Determination of total carbohydrate

Amount of carbohydrate was determined by Anthrone method (Sadasivam and Manickam, 1996).

Principle

Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium, glucose is dehydrated to hydroxyl methyl furfural. This compound forms a green coloured product with an absorption maximum at 630 nm.

Reagents used

2.5N HCl -Anthrone reagent: Dissolve 200 mg anthrone in 100 ml of ice cold95% H_2SO_4. To be prepared fresh before use.

Standard glucose: Stock- Dissolve 100mg in 100ml water.

Working principle:

standard- 10 ml of stock diluted to 100 ml with distilled water. Stored in refrigerator after adding a few drops of toluene.

Procedure

- 100 mg of the sample was weighed into a boiling tube.
- To hydrolyze, it was kept in a boiling water bath for three hours with 5ml of
- 2.5N -HCl and cooled to room temperature. It was neutralized with solid sodium carbonate until the effervescence ceases.
- The volume was made up to 100 ml and centrifuged.
- The supernatant was then collected and 0.5 and 1 ml aliquots were taken for analysis.
- Standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard. '0' serves as blank.
- The volume was made upto 1ml in all the tubes including the sample tubes by adding distilled water.
- Then 4ml of anthrone reagent was added.
- It was heated for eight minutes in a boiling water bath.
- It was allowed to cool rapidly and then green to dark green colour was read at 630nm.
- A standard graph was drawn by plotting concentration of the standard on the X- axis versus absorbance on the Y-axis.
- The amount of carbohydrate present in the sample was calculated from the graph.

Amount of carbohydrate present in 100 mg of the sample was calculated as per following formula:

$$\text{Amount of carbohydrate} = \frac{\text{amount of glucose (mg)}}{\text{Volume of the test sample}} \text{X } 100$$

ii) Determination of Protein

Principle

Protein reacts with the Folin-Ciocalteau reagent (FCR) to give a blue- coloured complex. The colour so formed is due to the reaction of the alkaline copper with the protein as in the biuret test and the reduction of phosphomolybdic- phosphotungstic components in the FCR by the amino acids tyrosine and tryptophan present in protein. The intensity of the blue colour is measured colorimetrically at 660 nm. The intensity of the colour depends on the amount of these aromatic amino acids present and varies for different proteins.

Reagents used

2% Sodium carbonate in 0.1N Sodium Hydroxide (Reagent A)

0.5% Copper Sulphate ($CuSO_4.5H_2O$) in 1% potassium sodium tartrate (Reagent B)

Alkaline Copper solution: 50 ml was mixed of A and 1 ml of B prior to use

(Reagent C)

Folin-ciocalteau Reagent (Reagent D)- A mixture consisting of 100 g sodium tungstate ($Na_2WoO_4.2H_2O$) was refluxed gently for 10 hours; 25 g Sodium molybdate ($Na_2MoO_4.2H_2O$), 700 ml water, 50 ml of 85% phosphoric acid and

100 ml of concentrated HCl in a 1.5L flask. 150g of Lithium sulphate, 50 ml of water and a few drops of bromine water.

The mixture was boiled for 15 min without condenser to remove bromine. It was cooled, diluted to 1L and filtered.

Stock solution of Protein solution

Accurately 50 g of bovine serum albumin was weighed (Fraction V) and dissolved in distilled water and made up to 50ml in a standard flask.

Working Standard

10 ml of the stock solution was diluted to 50 ml with distilled water in a standard flask. 1 ml of this solution contains 200ug protein.

Procedure

Extraction of protein from sample

Extraction was carried out with buffers used for the enzyme assay. 500 gm of the sample was weighed and ground well with a mortar and pestle in 5-10 ml of the buffer. It was centrifuged and the supernatant was used for protein estimation.

Estimation of protein

- 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard was pipetted out in to a series of test tubes.
- 0.2 ml of the sample extract was pipette out in another test tube.
- The volume was made 1 ml in all the test tubes. A tube with 1ml of water served as the blank.
- 5 ml of the reagent C was added to each test tube including the blank. They were mixed well and allowed to stand.
- Then 0.5ml of reagent D was added and then incubated at room temperature in the dark for 30 min. till blue color developed.
- Readings were taken at 660 nm.
- A standard graph was drawn and amount of protein and amount of protein present in sample was determined using standard graph.

Calculations

The amount of protein was expressed in percentage.

Determination of Crude fiber

For determination of crude fibre method followed by Sadasivam and Manickam, 1996 was followed.

Principle

During the acid and subsequent alkali treatment, oxidative hydrolytic degradation of the native cellulose and considerable degradation of lignin occurs. The residue obtained after final filtration was weighed, incinerated, cooled and weighed again. The loss in weight gives the crude fiber content.

Reagents

Sulphuric acid solution (0.255±0.005N):1.25G concentrated sulphuric acid diluted to 100 ml
Sodium hydroxide solution (0.313± 0.005N):1.25 g sodium hydroxide in 100 ml distilled water.

Procedure

- 2 g of ground material with ether or petroleum was extracted ether to remove fat (Initial boiling temperature was 35°C-38°C and final temperature was 52°C)
- After extraction with ether 2 g of dried material was boiled with 200 ml of sulphuric acid for 30 min with bumping chips
- It was filtered with muslin cloth and washed with boiling water until washings were no longer acidic.
- It was boiled with 200 ml of sodium hydroxide solution for 30 min.
- It was filtered through muslin cloth and then washed with 25 ml of boiling
- 1.25% H_2SO_4, three 50 ml portions of water and 25 ml alcohol.
- The residue was removed and transferred to ashing dish (preweighed dish W1).
- The residue was dried for 2 hr at 130±2ºC. The dish was cooled in a desiccator and weighed (W2).
- The residue was ignited for 30 min at 600 ±15ºC
- The residue was then cooled in a desiccator and reweighed (W3)

Calculation

% Crude fiber in ground sample

$$= \frac{\text{Loss in weight on ignition}(W2-W1)-(W3-W1)}{\text{Weight of the sample}} X100$$

Lipid Content

Two grams (2.00 gm) of powdered sample was extracted with 30 ml of petroleum ether by using Soxhlet extractor for 4 hr. The extract was evaporated to dryness in a weighed flask using a vacuum evaporator. The weighed flask was dried in the oven at 80º C for 2 hr, allowed to cool and reweighed. The difference between the initial and final weights was regarded as the lipid content of the sample.

3. ELEMENTAL ANALYSIS:

The elements iron, calcium, magnesium, zinc, copper and lead were analyzed by Atomic Absorption Spectrophotometer.

Determination of Calcium/Magnesium/ Iron/ Lead/ Copper and Zinc

Test portions of fruiting body of WEF were dried and then ashed at 450˚C under a gradual increase (about 50˚C/hr) in temperature, 6N HCl was added and the solution was evaporated to dryness. The residue was then dissolved in 0.1N HNO_3 and the analytes were determined by atomic absorption spectrophotometer (AAS Model: Perkin Elmer 3110; at a range of 193.7 nm-780 nm)

Reagents

Hydrochloric acid (6N), Nitric acid, Concentrated nitric acid, Water, Standard solutions of Cu, Zn, Pb, Fe, Mg and C.

Working of the spectrophotometer The spectrophotometer was set. Sample was converted into liquidform. An out I ml of the sample was sufficient for detection of a single element. Microwave digestor (Perkin Elmer: Multiwave 3000) working in the range of upto 400 C and
1250 psi was set.Absorbance of sample solution and blank were determined. Metal content was found detered from standard curve.

4. STATISTICAL ANALYSIS:

Proximate and elemental analysis was carried out three times for each parameter of a macrofungal sample. Hence, three replicates (n=3) were obtained from which the mean and standard deviation (SD) was calculated.

5. RESULTS:

The macrofungi which local people of Kamrup use in medicine were *Ganoderma lucidum, Lentinula edodes* and *Bovista plumbea. Auricularia judae* is used for the treatment of healing wounds. *Ganoderma lucidum* was used as soups and teas to boost immune system and to cure weakness, having antibacterial, antiviral effects, *Lentinula edodes* to increase general strength and immunity while *Bovista plumbea* they use to treat sores and wounds. The indigenous people of the forests have long been using some selected mushrooms traditionally as medicine for cure of some ailments and to increase immunity. Three most commonly available macrofungi which were consumed the most in this region and were also sold in the local markets viz. *Cantharellus cibarius Cantharellus lateritius and Lentinus squarusulus.* Different tribal population thriving in the forest villages generally consume mushrooms as a very delicious food item the taste of which they compare with that of meat. People of all ages are interested in foraging for mushrooms growing in the wild. They generally collect the mushrooms early in the morning or sometimes in evening without the knowledge of the nutritional benefits of these WEF. These three WEF were most preferred species for consumption by local people of Kamrup district hence were selected for their nutritional analysis They were analysed for their physical properties such as moisture contents, dry matter and ash contents. The moisture content of the mushrooms ranged from 86.9%-89.5% where *Lentinus squarusulus* was found with highest amount of moisture (89.5 %) followed by *Cantharellus lateritius* (87.93%) and least value was obtained in *Cantharellus cibarius* (86.9%). Relatively, dry matter was observed maximum in *Cantharellus lateritius* (11.09%) followed by *Lentinus squarusulus* (11.07%) and *Cantharellus cibarius* (6.9%) (Table 1).The three selected macrofungi were analysed for their macronutrients properties like carbohydrates, proteins, fibre and lipid content were determined (Table 2) and micronutrients like Fe, Ca, Zn, Cu, Pb and Mg (Table 3). Carbohydrate content was found maximum compared to other nutritional components. *Lentinus squarusulus* exhibited the maximum amount of carbohydrates, proteins and fibre (47.83%, 35.13% and 11.33% respectively) followed by *Cantharellus cibarius* having 45.83% of carbohydrates and *C. lateritius* (32.25%). As for protein, *Cantharellus lateritius* was having more amounts (21.92%) than *Cantharellus cibarius* (21.057%). *Cantharellus cibarius* was having more amount of fibre (11.5%) than *Cantharellus lateritius* (10.3%). Fat content was the highest in *Cantharellus cibarius* (0.62) followed by *Lentinus squarusulus* (0.58) and *Cantharelus lateritius* (0.52). Minerals like Fe, Ca, Zn, Cu, Pb and Mg were also analysed for three selected WEF. *Cantharellus cibarius* was analysed with the highest amount of Fe (1.92ppm) and Ca (0.50ppm). *Cantharellus lateritius* and *Lentinus squarusulus* was with maximum Zn content (0.15ppm). Cu content was highest in *Lentinus squarusulus* (0.09ppm) while Mg and Pb was the highest in *Cantharellus lateritius* (0.71ppm and 0.07ppm respectively). Among minerals, highest Fe (1.92ppm) and Ca (0.50ppm) was reported in *Cantharellus cibarius* while *Cantharellus lateritius* had maximum Zn (0.15ppm), Mg(0.71ppm) and Pb (0.07ppm). *Lentinus squarusulus* was analysed with highest Cu (0.09ppm) and Zn content (0.15ppm.

Table 1: Physical properties of the three edible mushrooms of Kamrup district of Assam (Mean value and SD given)

Sl. No.	Wild edible mushroom	Moisture content (%)	Dry matter (%)	Ash content (%)

1.	*Cantharellus lateritius*	90.9±0.46	10.09±0.57	6.05±0.30
2.	*Cantharellus cibarius*	87.93±0.45	11.09±0.55	8.87±0.56
3.	*Lentinus squarusulus*	89.5±0.41	11.07±0.43	8.38±0.7

(Values are means of 3 replicates ± Standard error)

Table 2: Macronutrient composition of three wild edible mushroom species of Kamrup district of Assam, India

Sl. No.	Name of WEM	Carbohydrate (%)	Protein (%)	Fat (%)	Crude fibre (%)
1.	*Cantharellus lateritius*	35.27±5.34	21.927±0.57	0.52	10.3±0.56
2.	*Cantharellus cibarius*	45.87±0.97	21.057±0.59	0.62	11.33±0.72
3.	*Lentinus squarosulus*	46.63±0.65	35.13±1.09	0.58	11.53±0.37

(Values are means of 3 replicates ± Standard error

Table 3: Concentration (ppm) of different elements in three wild edible mushroom speciesof Kamrup district of Assam, India

(Values are means of 3 replicates ± Standard error)

Sl. No.	Name of wild edible mushroom	Fe	Ca	Zn	Cu	Pb	Mg
1.	*Cantharellus lateritius*	1.92±0.003	0.45±0.03	0.15±0.008	0.05±0.002	0.07±0.001	0.71±0.07
2.	*Cantharellus cibarius*	4.56±0.008	0.50±0.01	0.14±0.001	0.06±0.002	0.05±0.007	0.13±0.08
3.	*Lentinus squarosulus*	3.21±0.001	0.42±0.09	0.15±0.004	0.09±0.002	0.04±0.006	0.65±0.03

6. DISCUSSION:

Documentation of information on edible, medicinal and poisonous mushrooms as well as the different social and cultural practices associated with ethnomedicinal practices in different parts of India is very important to sensitize the communities about the value of these mushrooms Macrofungi are used both as a food and as a medicine in different parts of the world. The knowledge and the composition of nutrients of WEF have been limited as compared to other NWFPs. This might be because the mushrooms are perceived only as a delicacy (Kalac, 2013) however their real benefit is not known to rural people. Due to high protein content they can be used to bridge the protein malnutrition gap. Edible mushrooms are sources of food and are cogitated as one of the delicious food all over the world. They have a high nutritional value almost twice that of any vegetable or fruit (Sivrikaya *et al*., 2002). In the present investigation, the three-way nutritional analysis of three selected WEF i.e. *Cantharellus cibarius, Cantharellus lateritius and Lentinus squarusulus* were conducted. Neutraceutical composition of wild edible fungal species of genus *Lentinus* viz. *L. sajor-caju*, *L. connatus*, *L. torulosus*, *L. cladopus* and *L. squarrosulus* was determined (Sharma and Atri, 2014).Three edible wild mushrooms *Cantharellus cibarius* (yellow mushroom), *Lactarius piperatus*, and *Boletus edulis* were studied for chemical composition and nutritional value (Neclaet *al*., 2002).

First, the physical properties like moisture, dry matter and ash content **(Table 1)** were determined; second, the nutritional properties like carbohydrates, proteins, lipids and fibres were analysed **(Table 2)** and third, the mineral contents like Ca, Zn, Mg, Cu, Pb and Cu were determined **(Table 3)**. The results showed that *Lentinus squarusulus* contained the highest amounts of carbohydrates (47.83%), proteins (35.13%), fibres (11.33%) and ash (15.8%) Biochemical and statistical analyses conducted earlier also showed that mushrooms have the crude protein, crude fibre, crude fat, carbohydrate, soluble sugars, ash and mineral elements etc. (Moore and Chiu, 2001; Konuk *et al*., 2006; Liu *et al*., 2010).

CONCLUSION:

- The present study illustrates the unexplored biodiversity of macrofungi in Kamrup district of Assam, India and provides documentation of this important mycological group.
- The indigenous people of Assam are mostly mycophilous and are frequently used to collect wild edible fungi (WEF) from the forests.
- Three wild edible fungi viz. *Cantharellus cibarius, C. lateritius* and *Lentinus squarusulus* were found more nutritional than the cultivated mushroom species hence make wild mushrooms a promising future food.
- Older people of the society had more traditional knowledge about macrofungi than the younger ones and itwas limited only in certain pockets of this region.

- The present study provides a database on macrofungal diversity of Kamrup district, Assam, India which was not documented earlier in the North eastern region of India
- Thus, on the basis of nutrient status, *Lentinus squarusulus* is recommended for large scale cultivation whereas other two species can also be cultivated on a local scale for use and consumption throughout the year.

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