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Studies on chemical composition and proximate analysis of wild mushrooms

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ABSTRACT

Mushrooms had long been used for medicinal and food purposes since decades. It is now increasingly recognized that correct diet, controls and modulates many functions of human body and consequently participates in the maintenance of state of good health, necessary to reduce the risk of many diseases. The chemical composition and proximate analysis of 4 wild mushroom (*Microporus xanthopus, Pisolithus arrhizus, Phellinus linteus* and *Phellinus wahlbergii*) species were analyzed from young and matured sporocarps were collected from the Western Ghats of Shivamogga and Chickmagalur districts. The macro nutrient profiles in general revealed that the wild mushrooms contains the moisture in range of (26.2-90.26%), crude fat (0.35-0.67%), carbohydrate (14.2-23.76%), crude protein (5.4-33.5%), crude fiber (8.4-13.2%), ash (1.92-9.2%) and nutritive value (86.44-125.84 Cal/100 gm). Among the macronutrients, Na is dominant, which is followed by K, P, Ca and Mg; in case of micronutrients Mn was dominant followed by Cu and Zn all the mushroom samples. In the same time Fe, Pb and Cd was completely absent in all mushrooms samples. The results were subjected for statistical analysis. Hence, these nutrient contents revealed that mushrooms were low energy, healthy food and may also be used as a protein supplementary diet. This paper sums up diverse beneficial health effects of mushrooms to humans, in the form of proteins, carbohydrates, fats, vitamins, minerals, food and drugs and medicines.

Keywords: Wild macrofungi, Medicinal mushrooms, Proximate value, Micronutrient, Macronutrient and Western Ghats.

INTRODUCTION

Mushrooms are a heterogenous group of fungi with members from both Ascomycotina and Basidiomycotina. All human beings require a number of complex organic compounds as added caloric requirements to meet the need of their muscular activity. Carbohydrates, fats and proteins form the major portion of the diet, while minerals and vitamins form comparatively a similar part [1]. Mushrooms are important constituents of minor forest produce, that grow on the most abundant biomolecule of this biosphere, that is, cellulose. Presently mushrooms are regarded as a macrofungus with a distinctive fruiting body which can be either epigeous or hypogeous and large enough to be seen with the naked eyes and to be picked by hand [2]. Only fruiting body of the mushroom can be seen whereas the rest of the mushroom remains underground as mycelium. Basidiomycetes mushroom have been valued as both food and medicine for thousands of years. They have high nutritive and medicinal values and contribute to a healthy diet because of their rich source of

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vitamins, minerals and proteins [3]. Not only do mushrooms provide food, but their waste can be recycled into fertilizers and additives that improve tree plantations and soil conditions. They are low calorie food with very little fat and are highly suitable for obese persons [4]. Many genera of mushrooms are edible and are rich in essential nutrients such as carbohydrates, proteins, vitamins, mineral, fat, fibers and various amino acids [5]. A major chunk of the population consumes mushrooms because of their easy availability, flavor, meaty taste and medicinal value [6].

Mushrooms offer tremendous applications as they can be used as food and medicines besides their key ecological roles. They represent as one of the world's greatest untapped resources of nutrition and palatable food of the future. Mushrooms have been found effective against cancer, cholesterol reduction, stress, insomnia, asthma, allergies and diabetes [7]. Due to high amount of proteins, they can be used to bridge the protein malnutrition gap. Mushrooms as functional foods are used as nutrient supplements to enhance immunity in the form of tablets. Due to low starch content and low cholesterol, they suit diabetic and heart patients. One third of the iron in the mushrooms is in available form. Their polysaccharide content is used as anticancer drug. Even, they have been used to combat HIV effectively [8, 9]. Biologically active compounds from the mushrooms possess antifungal, antibacterial, antioxidant and antiviral properties, and have been used as insecticides and nematicides as well. Thus keeping in view the tremendous applications of mushrooms, the present study reviews different aspects of mushrooms towards human health benefits such as food, medicine, minerals, drugs etc.

Mushrooms have been used as food and medicine in many parts of the world since time immemorial. Although mushrooms are often grouped with vegetables and fruits, they are actually fungi. They are macro-fungi which belong either to Basidiomycetes or Ascomycetes and they are very distinct from plants, animals and bacteria [10]. It is evidently clear that the growing interest in the cultivation of mushrooms can help in solving many problems of global importance such as protein shortage as well as improving the health and well being of people, considering that mushrooms are valuable health foods which are low in calories and provide essential minerals.

Mushrooms are fruit bodies of macroscopic, filamentous and epigeal fungi and they are made up of hyphea which forms interwoven web of tissues known as mycelium in the substrate upon which the fungus feeds [11]. Most often, their mycelia are buried in the soil around the root of trees beneath leaf litters in the tissue of a tree trunk, on a fallen log of wood or in other nourishing substrates [12]. Mushrooms are of great economic importance to man; their occurrence is dated back to the time of the early man as Mushrooms appear in traditional Yoruba art works known as "tie and die" which are materials of traditional costumes [13]. Many genera of mushrooms are edible and are rich in essential nutrients such as carbohydrates, proteins, vitamins, mineral, fat, fibres and various amino acids [5]. Most people eat mushrooms, mostly because of its flavour, meaty taste and medicinal value [6]. Mushrooms generally possess most of the attributes of nutritious food as they contain many essential nutrients in good quantity [14]. It must however be emphasized that some mushrooms are poisonous and may claim lives within few hours after consumption [15].

The present study describes the existing situation putting emphasis on the importance of the mushrooms hunted in the wild and more particularly the nutritional and ecological approach of harvest. This study was therefore aimed at chemical composition and nutritive evaluation of wild mushrooms.

MATERIALS AND METHODS

Collection of Mushrooms: The mushrooms were collected from forests and hills of Shivamogga (13° 30' N 75° 05' E, 2154 Ft) and Chickmagalur (13° 17' N 75° 08' E, 2421 Ft) districts. The mushrooms were carefully uprooted by gently lifting them up and holding the stipe gently but firmly close to the rhizomorph, thus carrying some soil along with it. This is to avoid damaging the tissue of the mushroom. Each specimen was carefully labeled before transporting to the laboratory. The mushrooms were air-dried and stored in transparent polythene bags that were loosely kept to allow for proper aeration of the mushrooms [1].

Characterization and Identification of Mushrooms: Each of the wild species of the mushrooms were characterized and identified was comparing their morphological, done by anatomical and physiological characteristics as described by Fasidi [16], monographs with descriptions given in the manual [17] and also through the electronic data on identification keys of mushrooms [18]. All the specimens were deposited at the herbarium of Department of Applied Botany, Jnana Sahyadri, Kuvempu University, Shivamogga, Karnataka, India. The analysis was made at the same Department and the Central Coffee Research Institute (CCRI) Balehonnur, Chikamagalur district of Karnataka, India.

ANALYSIS OF MATERIALS FOR PROXIMATE VALUE

For determination of proximate value, the following parameters were studied by using the mushroom material.

Moisture content: The fresh weight of each mushroom sample was taken using chemical balance. These samples were then oven dried separately at 80°C for 48 h. The loss in weight obtained after drying was regarded as the moisture content [19].

Determination of crude fat: Crude fat was determined by extracting 2gm moisture free samples with petroleum ether in a soxhlet extractor, heating the flask on sand bath for about 6 h till a drop taken from the drippings left no greasy stain on the filter paper. After boiling with petroleum ether, the residual petroleum ether was filtered using Whatman No. 40 filter paper and the filtrate was evaporated in a pre-weighed beaker. Increase in weight of beaker gave the crude fat.

Carbohydrates: 1g of the powdered mushroom sample was extracted with 30 cm³ of 80% ethyl alcohol by using Soxhlet extractor for 6 h. The crude extract was diluted to 100 cm³ with 80% ethyl alcohol. The quantity of ethanol soluble sugar in the extract was determined using phenol sulphuric acid method [20].

Determination of crude protein: Protein content was determined using folin phenol reagent of 0.5 g of the powdered mushroom sample was extracted with 50 cm of 2% NaCl in a water-bath at 60°C for 1 h. The extract was filtered out and 50.0 cm of 3% copper acetate monohydrate was added to the filtrate to precipitate protein. The precipitated protein was then centrifuged out and dissolves in 50 cm of 0.1 m NaOH. The quantity out and dissolves in 50 cm of 0.1 m NaOH. The quantity of protein in the alkaline solution was then determined using the folin-phenol method [21].

Crude fibre: Crude fibres of the mushroom samples were determined according to the standard method Association of Official Agricultural Chemists [22].

Ash content: The powdered mushroom sample (3g) was ashes in a Gallenkamp furnace in previously ignited and cooled crucible of known weight at 55°C for 6 h. fairly cooled crucibles were put in desiccators and weighed [23].

Nutritive value: Nutritive value was finally determined by using the formula:

Nutritive value = $4 \times Percentage$ of protein + $9 \times Percentage$ of fat + $4 \times Percentage$ of Carbohydrate

ELEMENTAL ANALYSIS

The microelements sodium and potassium were analyzed by Flame Photometer- Jenway-PFP-7 FPM Compressor Unit-122. The phosphorus was analyzed by Jenway 6300 Spectrophotometer. The microelements calcium, magnesium, zinc, copper, manganese, lead and cadmium were analyzed by Atomic Absorption Spectra GBC 932 AA/AAS.

DETERMINATION OF MACRONUTRIENTS

Determination of Sodium / Potassium: The concentration of potassium was determined with the help of flame photometer using separate standards of potassium. The yellow colored solution was aspirated at the wavelength of flame photometer to detect the concentration of potassium. Finally the percentage of potassium was calculated with the help of following formula.

% of Na / K = Graph ppm / 10^6 x Dilution factor x Volume of sample digestion made / Weight of the mushroom sample x 100

Determination of Phosphorous: Orthophosphate (phosphorous) present in the mushrooms was determined by Vanado molybdate yellow colour method. The 5 ml of aliquot of mushroom digested was taken in 50 ml volumetric flask and mixed with 10 ml vanado molybdate reagent. Having thoroughly mixed the final volume was adjusted to 50 ml by distilled water. After 30 min the developed yellow colour was measured on a spectrophotometer at 470 nm. The concentration of phosphorous was calculated with the help of standard graph. The percentage of phosphorous is calculated with the help of following formula.

% of P = Graph ppm / 10^6 x Volume of digestion made / Aliquot x Volume of sample digestion made / Weight of the mushroom sample x 100

Determination of Calcium and Magnesium: One ml of aliquot mushrooms digested material was taken in 50 ml by volumetric flask, final volume was adjusted to 50 ml by adding distils water. The presence of calcium and magnesium were determined at the wavelength 422.7 and 228.2 nm of AAS respectively. The percentage of calcium and magnesium were calculated with the help of following formula.

% of Ca / Mg = Graph ppm / 10^6 x Dilution factor x Volume of sample digestion made / Weight of the mushroom sample x 100

DETERMINATION OF MICRONUTRIENTS

Analysis of Zinc, Copper and Manganese: The 2 ml of digested samples were taken and diluted to 50 ml and the sample was aspirated of at the wavelength of 213.9, 324.75 and 279.5 of AAS to detect concentration of Zn, Cu and Mn respectively. Finally, the values of micronutrients are expressed in ppm by the help of following formula [24].

ppm of Zn / Cu / Mn = ppm / 1000 x Dilution factor x Volume of sample digestion made / Weight of the mushroom sample x 100

Analysis of Iron, Lead and Cadmium: The 2 ml of digested samples were taken and diluted to 100 ml. The presence of lead and cadmium were detected with the help of AAS by aspirating the sample at the wavelength of 217 nm and 228 nm with appropriate lamps. The ppm of lead and cadmium were calculated by the help of following formula [24].

ppm of Fe / Pb / Cd = ppm / 1000 x Dilution factor x Volume of sample digestion made / Weight of the mushroom sample x 100

STATISTICAL ANALYSIS

Experimental values are given as means \pm standard deviation (SD). Statistical significance was determined by one-way variance analysis (ANOVA). Differences at P < 0.05 were considered to be significant.

RESULTS AND DISCUSSION

Data presented: The several naturally growing wild mushrooms could be found in different places of Shivamogga and Chickmagalur districts. This result is not a surprise because the vegetation of these areas is typical of wild fungi. Specific mushroom tropical rainforest, which support the luxuriant growth of species were collected from different forest study areas. The results only provide indication of the areas where the sporocarps could be collected in large quantities [25].

Proximate analysis: The results of the nutritional analysis of the mushroom samples showed that all the specimens have high moisture content (Table-1). The moisture content of the mushrooms analyzed is high, indicating that mushrooms are highly perishable. High moisture content promotes susceptibility to microbial growth and enzyme activity. In the present study it was observed that the moisture content of the collected mushroom

samples ranges from 26.2% to 90.26%. However, the bodies of young mushrooms are soft and brittle and therefore contain higher moisture than fully matured ones which are often tough, almost leathery and must have probably lost some of their water content [26]. In those studies most fresh mushrooms contained about 90% moisture and 10% dry matter and dry mushrooms contained about 90% dry matter and 10% moisture [27, 28] Edible mushrooms are highly valued as a good source of carbohydrates and their contents usually range from 38.27% to 48.4% of dry weight [29, 30]. In the present study the highest carbohydrates content usually ranges from 14.2% to 23.73%. The relatively high carbohydrates content recorded in the samples is a proof of their being highly nutritious and good for human consumption. Fat content ranged from 0.35% to 0.67% in the present study (Table-1). This means that they contained less fat in comparison with other common mushrooms [28-31]. From the results are shown in Table-1, the macronutrient profile, in general, revealed that mushrooms had rich sources of protein, fiber (8.4%-13.2%) and had low amounts of fat. This high protein and low fat characteristics of the edible wild mushrooms has been previously reported [32-34]. Edible mushrooms are highly valued as a good source of protein and their protein contents usually range from 24.89% to 36.61% of dry weight [27, 31]. In the present study, the highest protein contents (33.5%) were obtained from P. arrhizus, while the lowest (5.4%) was obtained from M. xanthopus. Protein contents of mushrooms were reported to vary according to genetic structure of species and physical and chemical differences in growing medium [27, 31, 35].

Generally, fresh mushrooms contain a relatively high amount of fiber which may be responsible for its relatively high amount of ash [36]. The highest ash content was (9.2%) obtained in the *P. arrhizus*. The results also revealed that the specimens have highest calories of nutritive value (125.88 Cal/100gm) found in *P. arrhizus followed* by *Ph.linteus* (100.74 Cal/100gm) *M. xanthoporus* (86.88 Cal/100gm) and *Ph. Wahlbergii* (92.43 Cal/100gm) which normally has a tiny size and soft fruity body which could account for its highest calories of nutritive values. The ash content varied between 6.3% in *Calvulina cinerea*.

Elemental composition analysis: The average values of macro, micro, toxic elements are given in the Table-2 and 3. Na was moderately dominant macroelement which is followed by K, P, Ca and Mg in all the 4 mushroom samples. Mn was dominant microelement which was followed by Cu and Zn. Fe, Pb and Cd was absent in mushrooms.

The increasing populations of the world food demands have over whelmed the available land resources. It has been reported that protein-calories malnutrition deficiencies is a major factor responsible in nutritional pathology [37]. The dietary fibre plays an important role in decreasing the risks of many disorders such a constipation, diabetes, cardiovascular diseases, obesity etc. [38]. The carbohydrates are main source and store of energy. They are the starting substances for biological synthesis of many compounds. The trace elements, together with other essential nutrients, are necessary for growth, normal physiological functioning, and maintenance of life. They must be supplied in the food, since the body cannot synthesize them. Some of them are vitally important for health. Recommended intakes have been set for some trace elements and their deficiency can lead to disease [39]. Mn and Cu play important role in enzymatic catalysis and crucial to virtually all biochemical and physiological process [40]. Cu is a component of many enzyme systems such as cytochrome oxidase, lysyl oxidase and cerulo plasmin, an iron-oxidizing enzyme in blood [41]. The observation of anaemia in Cu deficiency may probably be related to its role in facilitating iron absorption and in the incorporation of iron into haemoglobin [42]. Zn is a component of many metallo enzymes, including some enzymes which play a central role in nucleic acid metabolism [43]. In addition, Zn is a membrane stabilizer and a stimulator of the immune response [44]. Its deficiency leads to impaired growth and malnutrition [45]. Manganese is essential for haemoglobin formation but excess is harmful [46]. K is of importance as a diuretic [47]. The present study has been carried out in order to determine the proximate and mineral contents of the mushrooms by various methods [48]. The macro, micro and toxic elemental content also varied not only with respect to the regions of the mushrooms where they grow, but also with their ages of the mushrooms. It also depends upon the genetic structure of mushroom species, physical and biochemical constituents [25].

CONCLUSION

In conclusion, the tested mushrooms possess nutritive value, followed by moisture. carbohydrates, crude protein and crude fibre content in rich quantity, where as ash and crude fats are low content. The ash and fat content were less than other foods of plant and animal origin. Overall, the rich nutritional composition makes wild mushrooms. So, mushrooms are a promising food that may overcome protein energy malnutrition problem to human beings. The carbohydrates, protein, fiber, ash and fat content in mushrooms make them a much sought after ideal vegetable by diabetic, cancer and cardiac patients. The current environmental issues of global warming and climate change would adversely affect the regeneration and growth pattern of the delicate fungi which requires a specific microclimate. Consequently, the high nutritional quality and unique flavor of these mushrooms are likely to be lost if these wild edibles are not properly documented. From the results of the analyses it can be shown that mushrooms as a source of nutrient supplements other major sources. Chemical analysis alone however, should not be the sole criteria for judging the nutritional importance of mcrofungal fruiting bodies. Thus, it becomes imperative to consider other aspects such presence antinutritional, toxicological factors and biological evaluation of nutrients content.

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Mushroom species	Moisture (%)	Crude fat (%)	Carbohydrate (%)	Crude protein (%)	Crude fibre (%)	Ash (%)	Nutritive value Cal/100gm
M. xanthopus	26.2±0.36	0.35±0.10	15.8±0.38	5.4±0.37	8.4±0.33	1.92±0.12	86.44±7.27
P. arrhizus	90.26±0.73	0.67±0.03	20.38±1.71	33.5±0.33	12.66±0.11	9.2±0.46	125.84±4.44
Ph. Linteus	48.4±0.32	0.54±0.01	23.73±0.28	9.55±0.45	13.2±0.43	6.3±0.41	100.74±1.18
Ph. wahlbergii	36.6±0.34	0.48±0.10	14.2±0.46	7.5±0.30	10.50±0.44	5.56±0.70	92.43±0.76

X 1 ·	Na	K	Р	Ca	Mg			
Mushroom species	Macronutrients in ppm							
M. xanthopus	1.01±0.39	0.57±0.01	0.33±0.00	0.18±0.01	0.10 ± 0.00			
P arrhizus	1 29+1 20	0 51+0 03	0.37 ± 0.04	0.20 ± 0.07	0.11 ± 0.02			

 0.41 ± 0.02

0.51±0.02

0.38±0.0.02

 0.29 ± 0.08

 0.22 ± 0.00

 0.18 ± 0.01

Meghalatha *et al.*, World J Pharm Sci 2014; 2(4): 357-363 **`Table 2: Percent concentration of various elements from Wild mushrooms**

0.81±0.03

 0.65 ± 0.04

Table 3: Micronutrients (in percentage) of various elements from Wild mushrooms

 1.68 ± 0.14

 1.91 ± 0.24

Muchanom anosioa	Zn	Cu	Mn	Fe	Pb	Cd
Mushroom species	Micronutrients in ppm				PD	Ca
M. xanthopus	11.45±0.17	26.25±1.93	32.89±0.093	-	-	-
P. arrhizus	32.50±0.12	41.13±0.58	54.07±0.58	-	-	-
Ph. linteus	35.00 ± 0.45	60.38±0.20	83.5±0.70	-	-	-
Ph. wahlbergii	20.56±0.002	32.38±0.22	47.5±0.91	-	-	-

Note: '- '= Absent

Ph. linteus

Ph. wahlbergii

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