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Article

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Proximate composition and antimicrobial activity of three wild edible mushrooms consumed by ethnic inhabitants of Tripura in northeast India

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Abstract

The study was focused on edible mushrooms consumed by the ethnic inhabitants of Tripura, northeast India. Three mushrooms namely *Lentinus squarrosulus*, *Lentinus tuber-regium* and *Macrocybe gigantea* were evaluated quantitatively for proximate composition and mineral nutrients. In addition, the efficacy of the mycelial extract was tested for antimicrobial activity against the bacteria. The results of this study indicated that mushrooms were rich in protein and carbohydrate with low fat content. *Macrocybe gigantea* proved to the best source of protein and carbohydrate among the three mushrooms. There were varied amount of micronutrients recorded in all the three mushrooms. The antimicrobial activity of mycelial extract of *M. gigantea* was found against all the tested strains of bacteria. The study suggested that these mushrooms are rich in nutrients particularly *M. gigantea* which could be used as an alternative source of vegetarian food to the ethnic people of Tripura. The antimicrobial activity exhibited by these mushrooms indicated their medicinal properties.

Keywords – Antimicrobial activity – edible mushroom – ethnic tribes – macro nutrients – micro nutrients

Introduction

Mushrooms have gained attention as food, medicine and cosmetics worldwide (Halpern & Miller 2002, Sliva 2003, Boa 2004, Sliva 2006, Oboh & Shodehinde 2009, Hyde et al. 2010, Bishop et al. 2015). Mushrooms are considered as source of high protein content, fibre and minerals with low fat content (Leon-Guzman et al. 1997). The consumption of wild edible mushrooms is increasing because of good protein content and trace minerals (Ogundana & Fagade 1981, Senatore 1990, Thimmel & Kluthe 1998, Sudheep & Sridhar, 2014). The macrofungi harbours compounds with broad ranging antimicrobial activity (Barros et al. 2007). A number of compounds isolated from mushrooms possessed antifungal and antibacterial activity (Morita & Kobayashi 1967, Yasumoto et al. 1971).

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Northeast India is geographically situated in one of the most biodiversity-rich regions of the world (Chatterjee 2008). The diverse climatic conditions prevailing in northeast India harbours a wealth of mushrooms diversity. The consumption of such wild edible mushrooms used by ethnic tribes and local people of northeast from Assam, Meghalaya, Nagaland and Manipur have been reported (Sing & Sing 1993, Sarma et al. 2010, Tanti et al. 2011, Khaund & Joshi 2013). Nutritive value of seven wild edible mushrooms commonly consumed by the peoples of Khasi hills of Meghalaya was reported earlier (Agrahar-Murugkar & Subbulakshmi 2005). The nutraceutical properties of mushrooms consumed by ethnic tribes from this region were studied recently (Khaund & Joshi 2015). In addition, the nutritional analysis of *Pleurotus djamor* was also reported from Tripura, northeast India (Roy Das et al. 2014).

The rural tribal people commonly consume wild edible mushrooms growing in the forest beds of Tripura, northeast India. The studies regarding the nutritional evaluation of mushroom from northeast India are scarce. Hence, in the present study we analysed the proximate composition, mineral content and antimicrobial activity of three wild edible mushrooms of Tripura.

Materials & Methods

Sample collection

Three mushrooms were collected from Udaipur Bazar (23°32'03.40"; 91°28'44.22"; 28 masl), Lake Chowmuhani Bazar (23°50'31.52"; 91°16'55.46"; 17 masl) and homegarden of A.D. Nagar (23°47'48.51"; 91°16'20.40"; 24 masl), Tripura in northeast India during April-October, 2015. The collection sites have a humid tropical climate with large amounts of rain. The places experiences long, hot and wet summers, from March to October. Average temperatures is around 32°C, fluctuating with rainfall. The average humidity ranges between 50-80 %.

Identification of mushrooms

The collected samples were placed in sterilized plastic bag and brought to the laboratory and accession numbers are MCCT 03, MCCT 04 and MCCT 05, respectively. For identification of the specimens various morphological characteristics were considered and compared (Pegler 1977, Purkayastha & Chandra 1985, Pegler et al. 1998). The mushrooms were identified by amplifying ITS region of the extracted DNA from the fruit body (Roy Das et al. 2017).

Proximate composition analysis of nutrients

Moisture, crude fat and crude fibre content were determined following the method of the Association of the Official Analytical Chemist (AOAC 1990). The total protein was analysed (Lowry et al. 1951). Carbohydrate content was estimated (Hedge & Hofreiter 1962). The ash content was determined (Raghuramulu et al. 2003). Total energy was calculated according to the following equation (Singdevsachan et al. 2015):

$$\text{Energy (kcal)} = 4 \times (\text{g protein} + \text{g carbohydrate}) + 9 \times (\text{g fat})$$

Mineral element determination

For determination of nutrient elements, 0.25g of homogenised dried mushroom samples were digested in aqua regia (15ml) and were incubated at 50ºC for 30min, and the temperature was raised to 100-120ºC for 2h. After heating, samples were cooled at room temperature, then 10ml of 0.25M HNO_3 was added, and the mixture was filtered through Whatman paper No. 42. Filtrate was brought to a volume of 25ml with 0.25M HNO_3 (Radojevic & Bashkin 1999). Then, elements were quantified by Atomic Absorption Spectroscopy (Perkin-Elmer 3110).

Determination of antimicrobial activity

Inoculation of the mushroom mycelium of seven days old cultures was done in 100ml basal synthetic medium broth (BSM) in 250 ml Erlenmeyer flasks and was incubated for 21 days at 25°C in stationary condition. The antimicrobial activity was assessed by disc diffusion method (Bauer et al. 1966). The broth of each submerged mycelium was filtered and the filtrate was used for the antimicrobial activity. The mushroom extract was tested against the bacteria procured from Institute of Microbial Technology (IMTECH) Chandigarh, India. The microorganisms used for investigation were *Bacillus subtilis* (MTCC-619), *Escherichia coli* (MTCC-40), *Pseudomonas aeruginosa* (MTCC-424) and *Staphylococcus aureus* (MTCC-96). The results obtained was compared with Streptomycin (100 µg/ml).

Data analysis

The assays were done in triplicates, and the results were expressed as mean±SD. The mean values and standard deviation was done using Statistica 9.0. The antimicrobial property was assessed after inoculation with bacteria and zone of inhibition (mm) was noted down after 24-48 h of incubation. The percentage inhibition was calculated as follows:

$$\%\text{Inhibition} = \frac{\text{Diameter of inhibition zone (mm)}}{\text{Total diameter of Petri dish (mm)}} \times 100$$

Results

Characters of wild mushrooms

The three mushrooms were collected from markets and one was also from home garden. These are highly priced edible mushrooms which are found in most of the local markets (Fig. 1 a & c) and were found to be enjoyed by the ethnic inhabitants.

Lentinus squarrosulus Mont.

Synonym: *Pleurotus squarrosulus* (Mont.) Sing.
Collection site: A.D. Nagar & Lake Chowmohani
Laboratory accession: MCCT 03 (1 b & d)

Basidiocarps solitary as well as in groups. Pileus: 3–12cm diam., depressed at the centre, often deeply infundibuliform, fleshy and pliant when fresh, surface typically white, squamose to squarrose with small concentrically arranged, innate scales which may be concolorous, margin thin, regular or lobed, curved downwards and sometimes involute. Stipe: 2–7 cm x 0.5–2cm; typically central, attenuated towards the base, cylindrical, firm, solid. Spore print: white.

Lentinus tuber-regium (Fr.) Fr.
Syn. *Pleurotus tuberregium* (Fr.) Sing.
Collection site: Lake Chowmohani
Laboratory accession: MCCT 04 (1 e)

Basidiocarps solitary. Pileus: 10–17cm diam., infundibuliform then expanding although remaining depressed at the centre, flesh becoming coriaceous, surface glabrous, often with small, scattered, isolated appressed squamules particularly towards the centre, creamish white. Stipe 9–12 x 0.8–4 cm, central occasionally excentric, cylindrical, solid surface concolorous with the pileus, generally with isolated appressed squamules similar to the pileal surface. Spore print: white.

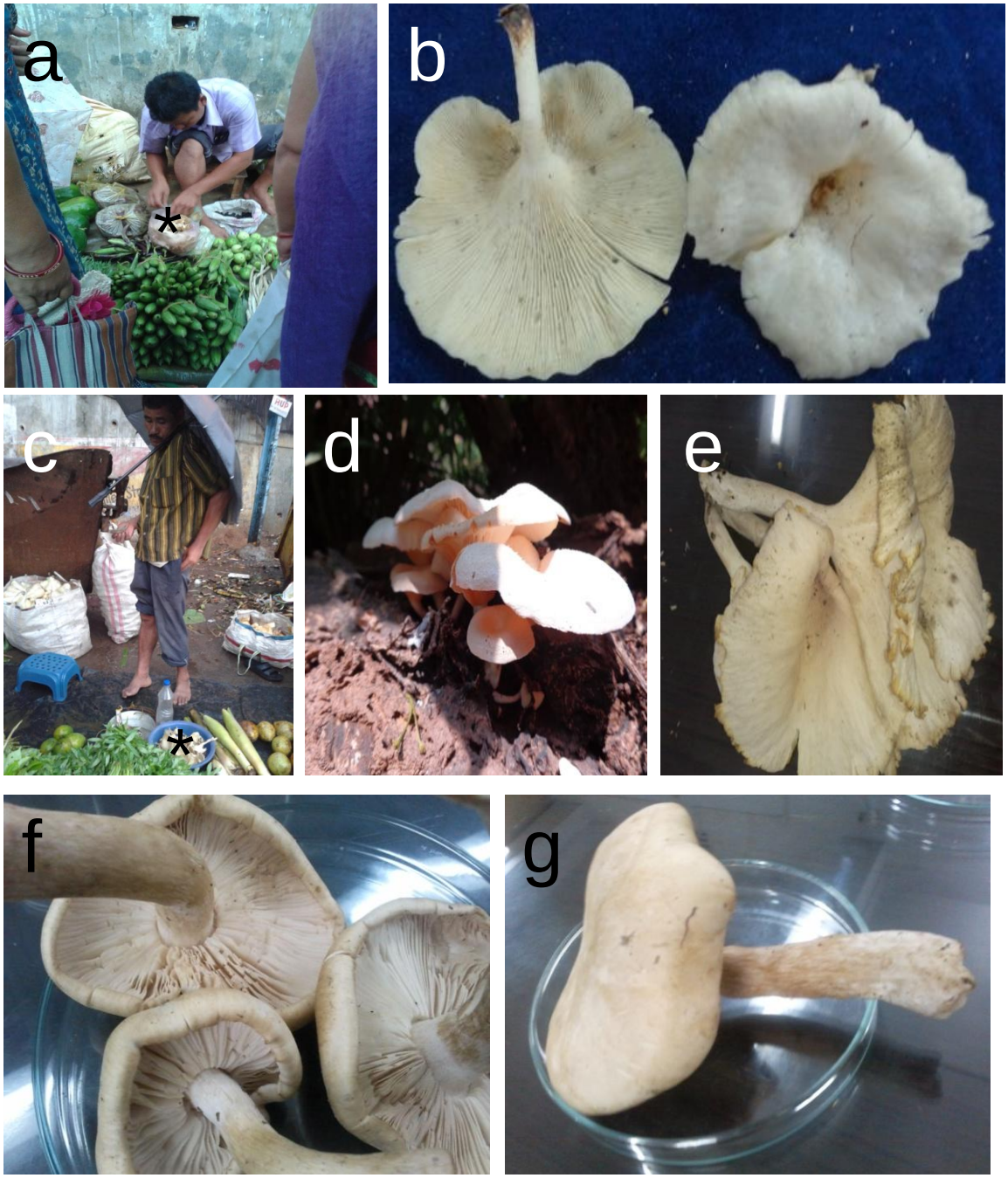

Fig. 1 – The fruit body of mushrooms. a-c The local people selling mushrooms (*Lentinus squarrosulus* and *Lentinus tuber-regium*). b-d Fruit body of *Lentinus squarrosulus* MCCT03. e *Lentinus tuber-regium* MCCT04. f, g *Macrocybe gigantea* MCCT05.

Macrocybe gigantea (Massee) Pegler & Lodge
Syn: *Tricholoma giganteum* Massee
Collection site: Udaipur
Laboratory accession: MCCT 05 (1 f & g)
Pileus 15–25 cm diam, conicoconvex then expanding; surface greyish green with a glaucous tint, glabrous and silky smooth. Stipe 15–17 x 3–6 cm, cylindrical, often elongate, solid finally fistulose; surface concolorous with pileus, fibrillose-striate, same colour as pileus. Gills white. Spore print white.

Proximate content of nutrients

The carbohydrate, protein, fibre and fat content in the mushroom are presented in Table 1. The moisture content of three mushrooms was in the range of 71.13 % and 83.33 %. The carbohydrate content obtained in different mushrooms ranged from 48.84 g/100g to 52.01g/100g. The protein content ranged from 20.97 g/100g to 31.04 g/100g. The fibre content is highest in *M. gigantea* (10.69 g/100g) and lowest in *L. squarrosulus* (8.32 g/100g) where as fat content is maximum in *L. tuber-regium* (1.77 g/100g) and minimum in *L. squarrosulus* (0.85 g/100g). The ash content obtained is higher in *L. tuber-regium* (7.73 g/100g) and lower in *M. gigantea* (4.53 g/100g). *M. gigantea* showed the highest energy contribution (336.81 kcal/100 g dw) and lowest in *L. tuber-regium* (291.04 kcal/100 g dw). The moisture content is higher in *L. squarrosulus.* The carbohydrate content, fibre content and protein content is higher in *M. gigantea* and *L. tuber-regium* shows high fat content compared to other two mushrooms (Table 1).

Table 1 Proximate analysis and energy value of three wild edible mushrooms

Proximate content	***Lentinus squarrosulus***	***Lentinus tuber-regium***	***Macrocybe gigantea***
Moisture content (%)	83.33±0.83	76.20±0.60	71.13±1.14
Fibre content (g/100g)	8.32±0.00	9.45±0.28	10.69±1.55
Fat (g/100g)	0.85±0.11	1.77±0.13	1.27±0.04
Carbohydrate content (g/100g)	48.84±0.39	50.03±0.13	52.01±0.04
Protein content (g/100g)	27.07±0.31	20.97±0.67	31.04±0.76
Ash (g/100g)	6.32±0.22	7.73±0.23	4.53±0.32
Energy (kcal/100 g)	307.19±2.24	291.04±6.97	336.81±3.55

Mean values (n=3) with ± Standard Deviation

Mineral element content

The mineral nutrients vary in the wild edible mushrooms. *L. squarrosulus* possesses highest amount of Ca, Fe, Cu and Mn, *L. tuber-regium* harbours higher amount of Zn whereas *M. gigantea* was higher in Mg, Cd and Cr (Table 2).

Table 2 Mineral element analysis (mg/kg) of three wild edible mushrooms.

Mineral Elements	***Lentinus squarrosulus***	***Lentinus tuber-regium***	***Macrocybe gigantea***
Fe	3.6±0.07	0.49±0.01	0.27±0.03
Cr	0.08±0.005	0.07±0.001	0.10±0.008
Ni	0.12±0.006	0.12±0.02	0.09±0.006
Zn	0.23±0.01	0.24±0.03	0.15±0.02
Cd	0.19±0.02	0.27±0.008	0.30±0.01
Mn	0.54±0.003	0.25±0.001	0.35±0.001
Cu	0.89±0.002	0.44±0.01	0.55±0.03
Ca	6.46±0.12	5.90±0.04	3.74±0.16
Mg	7.12±0.002	9.25±0.01	21.63±0.14

Mean values with ± Standard Deviation

Antimicrobial activity

The antimicrobial activity of three wild edible mushroom mycelial extract against Gram-negative bacteria and Gram positive bacteria is depicted in Table 3. Among the three mushrooms, *M. gigantea* showed activity against all the tested bacteria. The zone of inhibition of mycelial extracts of mushrooms against bacteria was comparatively less than the standard antibiotic used.

Table 3 Inhibition zone of mycelial extract of three wild edible mushrooms

Bacteria	*Lentinus squarrosulus*	% Inhibition	*Lentinus tuber-regium*	% Inhibition	*Macrocybe gigantea*	% Inhibition	Streptomycin	% Inhibition
Bacillus subtilis	10.23±0.11	12.79	10.35±0.35	12.94	10.55±0.39	13.19	28.26±0.40	35.33
E. coli	-	-	-	-	10.21±0.38	12.76	24.85±0.53	31.07
Pseudomonas aeruginosa	7.59±0.23	9.49	7.43±0.46	9.29	8.18±0.57	10.23	18.33±0.58	22.92
Staphylococcus aureus	8.10±0.07	10.13	-	-	8.88±0.30	11.10	33.67±1.53	42.08

Mean values (n=3) with ± Standard Deviation
-No activity observed

Discussion

The variability in moisture content observed in the present study was dependent on the mushroom species and other parameters such as environment, temperature and relative humidity during growth and relative metabolic water that may be produced or utilized during storage (Crisan & Sands 1978). The protein and carbohydrate content in our study is in accord with the earlier studies (Hung & Nhi 2012, Chang & Miles 1989, Khan et al. 2008, Manjunathan & Kaviyarasan 2011, Rizal et al. 2015).

Out of nine mineral elements detected by AAS analysis, highest amount of four elements were found in *L. squarrosulus*, three were high in *M. gigantea* and one was high in *L. tuber-regium* and Ni was equally found higher in *L. squarrosulus* and *L. tuber-regium*. The mineral content of mushrooms was highly variable in the studied mushrooms which is in accord with an earlier report (Khaund & Joshi 2015).

With an increasing number of bacteria developing resistance to commercial antibiotics, extracts and derivatives from mushrooms hold great promise for novel medicine in modern times (Chikara 1992, Mizuno et al. 1995). In recent decades, various extracts of mushrooms have been of great interest as sources of natural products (Alves et al. 2012a). In the present study, the extracts of three mushrooms inhibited both Gram positive and negative bacteria suggesting broad–spectrum antimicrobial potential, *M. gigantea* exhibiting the most satisfactory results by inhibiting all the four tested bacterial strains. The mushroom species are potentially a rich source of antimicrobial agents (Kalyoncu 2009, Vamanu 2012, Alves et al. 2012b) which is in accord with our study.

Studied species are good sources of nutrients with high carbohydrate and protein content having varying amount of mineral nutrients. The cultivation of these mushrooms in Tripura particularly *M. gigantea* would possibly supply a constant alternative vegetarian food to the locals and ethnic people of Tripura. The antimicrobial activity exhibited by these mushrooms indicated their medicinal properties. The commercial cultivation of these mushrooms could capture the market as popular dietary items of the locals and ethnic people of this region.

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