

NUTRITIONAL AND NUTRACEUTICAL POTENTIAL OF WILD EDIBLE MACROLEPIOTOID MUSHROOMS OF NORTH INDIA

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ABSTRACT

Wild edible mushrooms viz. *Macrolepiota dolichaula*, *M. procera*, and *M. rhacodes* are widely consumed in India partly because of their taste, flavour and due to their rich nutritional and nutraceutical credentials.

Objective: The aim of the study was to determine the proximate composition including mineral elements, heavy metals (Ca, Mg, Cu, Fe, Mn, Zn, Cr, Se, Cd, Pb, Hg, and As), and certain nutraceutical components (flavonoids, alkaloids, carotenoids and phenolic compounds) in these mushrooms.

Methods: The samples were analyzed for proximate chemical composition (moisture, proteins, fat, carbohydrates, fiber and ash) using the AOAC procedures. The concentrations of minerals and heavy metals in the samples were determined by using atomic absorption spectrophotometer. For nutraceutical composition crude extracts were prepared from mushroom parts using methanol as the solvent following standard protocols.

Results: Proximate analysis revealed that these mushrooms contained 16.45 - 19.95% protein, 2.9 - 3.4% fat, 2.5 - 5.1% crude fibre, 56.2 - 68.19% carbohydrates, 1.93 - 7.3% ash and 7.8 - 8.8% moisture, besides 5 - 28 mg/100g Ca, 143 - 254 mg/100g Mg, 5 - 9 mg/100g Cu, 241 - 276 mg/100g Fe, 1 - 5 mg/100g Mn, 0.06 - 0.09 mg/100g Zn, 0.06 - 0.10 mg/100g Se, 0.062 - 0.087 mg/100g Hg, 0.0014 - 0.0019 mg/100g Cd and 0.074 mg/100g As. Mushrooms were found to be good sources of bioactive compounds like phenolics which are ranged from 5.9 - 16.81 mg/g phenolics, flavonoids 1.36 - 1.76 mg/g, alkaloids 0.048 - 0.103 mg/g, β -carotene 0.12 - 0.29 μ g/gm and lycopene 0.05 - 0.12 μ g/gm (dw).

Conclusion: This study shows that these mushrooms need to be domesticated for their large scale production and subsequent use as a source of natural nutrients and nutraceuticals so as to promote health, taking advantage of the additive and synergistic effects of different nutritional and bioactive compounds present in them.

Keywords: Edible mushrooms, Minerals, Nutritional and Nutraceutical components.

INTRODUCTION

Mushrooms have been a food supplement in various cultures and they are cultivated and eaten for delicacy [1]. In most countries, there is a well-established consumer acceptance for wild edible mushrooms, probably due to their unique flavour and texture but also by their chemical, nutritional components [2]. These are traditionally used in many Asian countries as food and medicine [3, 4]. Among three macrolepiotoid mushrooms represented by genus *Macrolepiota*, a basidiomycetous fungus belonging to the family *Agaricaceae* [5]. These species of this genus, namely *Macrolepiota dolichaula*, *M. procera*, and *M. rhacodes* known for their edibility are traditionally consumed in Indian subcontinent [6,7]. In the present study we intend to evaluate the proximate composition of these three mushrooms along with minerals, heavy metals and nutraceutical constituents. The evaluation of nutrient composition included the determination of proteins, fats, ash, fiber, moisture and carbohydrates. The evaluation of nutraceutical composition included the determination of phenolics, flavonoids, carotenoids, and alkaloids. Wild edible mushrooms are collected for consumption because they are a good source of digestible proteins, carbohydrates, fibres and vitamins [2,8-10]. These are reported to accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids [11]. Purified bioactive compounds derived from medicinal mushrooms are a potentially important new source of natural antioxidants that positively influence oxidative stress related diseases such as cancer [12-14]. There are several reports on mushrooms about the presence of bioactive compounds, which largely makes mushrooms important items of consumable for earning revenue [15-16]. There are number of investigations covering pharmaceutical and nutritional evaluation of mushrooms [17-21]. In view of above investigations for evaluation of nutritional and nutraceutical prospects of these species of *Macrolepiota* collected from different localities of North part of India was initiated. The results of which are presented in this manuscript.

MATERIALS AND METHODS

Samples

Fully mature samples of all three species were collected from North West India during monsoon season. The morphological identification of the wild edible macrolepiotoid mushrooms was made according to macro and microscopic characteristics, noted down on the field key to mushroom collector [22]. Identification of the samples was done by consulting Pegler [23] and representative voucher specimens were deposited at the herbarium of Botany Department, Punjabi University, Patiala (Punjab), India. These mushrooms were dried at 45°C before analysis.

Nutritional evaluation

The samples were analyzed for proximate chemical composition (moisture, proteins, fat, carbohydrates, fiber and ash) using the AOAC procedures [24]. The crude protein content ($N \times 4.38$) of the samples was estimated by the macro-Kjeldahl method [25] the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 525°C \pm 20°C of silica dishes containing the 5 - 10 gm sample. Total carbohydrates were calculated by difference. Energy value was estimated based on its content of crude protein, fat and carbohydrate using the at water factors of 4.0, 9.10 and 4.2 kcal/gm of each component, respectively.

Determination of minerals and heavy metals

Dried powder of each sample (1 g) was placed in a Silica crucible and ashed at 45°C for 18 - 24 hrs; then the ash was dissolved in 2 ml concentrated HNO₃, heated again at 45°C for 4 hrs and dissolved in 1 ml concentrated H₂SO₄, 1 ml HNO₃ and 1 ml H₂O₂, and then diluted with double deionised water up to a volume of 25 ml. A blank digest was carried out in the same way. The concentrations of Minerals and heavy metals in the samples were determined by using atomic absorption spectrophotometer (Perkin Elmer precisely A Analyst 400) [26].

Nutraceutical evaluation

Extraction procedure

The fruiting bodies were freeze-dried in a Lyophilizer (Ly-Christ Alpha1-2) and powdered before analysis. The dried samples (5 g) were extracted by stirring with 100 ml of methanol at $25 \pm 1^\circ\text{C}$ at 150 rpm for 24 hrs. and filtered through Whatman No. 4 paper. The residue was then extracted with two additional 100 ml portions of methanol, as described earlier. The combined methanolic extracts was evaporated at $40 \pm 2^\circ\text{C}$ to dryness and re-dissolved in methanol at a concentration of 50 mg/ml, and stored at $4 \pm 2^\circ\text{C}$ for further use.

Phenolics [27]

Phenolics were determined by a Folin-Ciocalteu assay. The extract solution (1 ml) was mixed with Folin-Ciocalteu reagent (5 ml, previously diluted with water 1:10, v/v) and sodium carbonate (75 g/l, 4 ml). The tubes were vortex mixed for 15 seconds and allowed to stand for 30 minutes at $40 \pm 2^\circ\text{C}$ for colour development. Absorbance was then measured at 765 nm. Gallic acid was used to obtain the standard curve (0.0094–0.15 mg/ml), and the results were expressed as mg of gallic acid equivalents (GAE) per g of extract.

Flavonoids [28]

For flavonoids quantification, the extract sample concentrated at 2.5 mg/ml (0.5 ml) was mixed with distilled water (2 ml) and NaNO_2 solution (5%, 0.15 ml). After 6 min, AlCl_3 solution (10%, 0.15 ml) was added and allowed to stand further for 6 minutes. NaOH solution (4%, 2 ml) was added to the mixture, followed by distilled water until a final volume of 5 ml was obtained. The mixture was properly mixed and allowed to stand for 15 minutes. The intensity of pink colour was measured at 510 nm. (+)-Catechin was used to calculate the standard curve (0.015–1.0 mM) and the results were expressed as milligrams of (+)-catechin equivalents (CE) per gram of extract.

Carotenoids [8]

For β -carotene and lycopene determination, the dried methanolic extract (100 mg) was vigorously shaken with 10 ml of acetone-hexane mixture (4:6) for 1 minute and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505 and 663 nm. Content of β -carotene was calculated according to the following equations: For β -carotene (mg/100 ml) = $0.216 \times A_{663} - 0.304 \times A_{505} + 0.452 \times A_{453}$ and lycopene (mg/100 ml) =

$-0.0458 A_{663} + 0.372 A_{505} - 0.0806 A_{453}$. The results were expressed as μg of carotenoid/g of extract.

Alkaloids estimation [29]

The total alkaloids were extracted from 5 g of each of the dried powdered mushroom samples using 100 ml of 10 % acetic acid, which was left to stand for 4hrs. The extracts were filtered to remove cellular debris and then concentrated to a quarter of the original volume. To this concentrate, 1 % Ammonia solution was added drop-wise until the formation of precipitate. The alkaloids thus obtained were dried to a constant weight at 65°C in an oven. The percentage of alkaloids was calculated by using formula.

Percentage alkaloids % = $\text{Weight of ppt} / \text{Weight of the sample} \times 100$

Statistical analysis

For each sample three extracts were obtained and all the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$. This treatment was carried out using the SPSS v. 16.0 program.

RESULTS

A. Nutritional studies

The results of the studies carried out on the three species of *Macrolepiota* namely, *Macrolepiota dolichaula*, *M. procera*, and *M. rhacodes* on dry weight basis is depicted in Table 1. In these mushrooms protein content varied between 16.45% in *M. rhacodes* to 19.95 % in *M. procera* and *M. dolichaula*, the value of the crude fat was evaluated between 2.9-3.4 % in *M. rhacodes* and *M. procera* respectively. Crude fibres were found to be maximum in *M. procera* (5.1%) while lowest in *M. rhacodes* (2.5%) and ash content varied from 1.93% in *M. procera* to 7.3 % in *M. dolichaula*. The average moisture content in dried samples was highest in *M. dolichaula* (8.8%) followed by *M. procera* (8.5%) and minimum in *M. rhacodes* (7.8%). Carbohydrates, calculated by difference, ranged from 56.2g/100 g in *M. dolichaula* and 68.19 g/100 g in *M. rhacodes*. On the account of proximate analysis, it was observed that 100 g of *M. rhacodes* assure, on an average, 364.7 kcal, which was found higher as compared to other wild edible mushrooms (Table 1).

Table 1: Proximate chemical composition (g/100 g) and energetic value (kJ/100 g) of wild *Macrolepiota* species (On dry weight basis) (mean \pm SD; n = 3)

S. No.	Species	Proteins (%)	Moisture (%)	Crude fat (%)	Fibers (%)	Ash (%)	Carbohydrates (%)	Energy (kJ/100 g)
1	<i>M. procera</i>	19.95 \pm 1.06	8.5 \pm 0.62	3.4 \pm 0.08	5.1 \pm 0.22	1.93 \pm 0.06	60.82 \pm 0.11	353.7 \pm 0.06
2	<i>M. rhacodes</i>	16.45 \pm 0.54	7.8 \pm 0.62	2.9 \pm 0.11	2.5 \pm 0.01	2.16 \pm 0.14	68.19 \pm 0.17	364.7 \pm 0.60
3	<i>M. dolichaula</i>	19.95 \pm 1.35	8.8 \pm 1.47	3.2 \pm 0.20	4.85 \pm 0.18	7.3 \pm 0.15	56.2 \pm 0.10	333.4 \pm 0.11
	CD ($p \leq 0.05\%$)	0.30	0.54	0.12	0.11	0.06	0.82	0.26

* Significant at 0.05 level

Table 2: Proximate Mineral elements (mg/100 g) of wild *Macrolepiota* species (On dry weight basis) (mean \pm SD; n = 3)

S. No.	Name of Species	Mineral elements (mg/100gm) of dry samples						
		Fe	Mg	Cu	Mn	Ca	Se	Zn
1	<i>M. procera</i>	276 \pm 0.87	254 \pm 2.00	9 \pm 0.32	5 \pm 0.30	14 \pm 0.61	0.08 \pm 0.03	0.06 \pm 0.03
2	<i>M. rhacodes</i>	248 \pm 1.74	217 \pm 0.50	8 \pm 0.71	3 \pm 0.53	28 \pm 1.40	0.06 \pm 0.03	0.09 \pm 0.02
3	<i>M. dolichaula</i>	241 \pm 1.73	143 \pm 1.00	5 \pm 0.92	1 \pm 0.23	5 \pm 0.72	0.10 \pm 0.05	0.08 \pm 0.01
4	CD ($p \leq 0.05\%$)	1.23	0.82	0.54	0.18	0.65	0.08	0.06

* Significant at 0.05 level

B. Evaluation for mineral elements and heavy metals

During the present investigation minerals elements and heavy metals conc. evaluation was estimated on dry weight basis and results obtained are documented in Table-2 and 3 respectively. Out of 3 wild samples of *Macrolepiota* examined for mineral estimation, Fe content varied from 241 mg/100 g in *M. dolichaula* to 276 mg/100 g in *M. procera*. Cu content ranged from 5 mg/100 g in *M. dolichaula* to 9 mg/100 g in *M. procera*. Mn content in *M. procera*, *M.*

dolichaula, *M. rhacodes* were 5, 1 and 3 mg/ 100 g, respectively. Mg content varied from 143 mg/100 g in *M. dolichaula* to 254 mg/100 g in *M. procera*. Ca content ranged from 5 mg/100 g in *M. dolichaula* to 14 mg/100 g in *M. procera*, Se content varied from 0.06 mg/ 100 g in *M. rhacodes* to 0.10 mg/100 g in *M. dolichaula*, Zn content ranged from 0.06 mg/100 g in *M. procera* to 0.09 mg/100 g *M. rhacodes*. The results of mineral values of the three edible species of mushrooms clearly indicate the potential for their use as sources of good quality food.

Table 3: Heavy elements (mg/100 g) of wild *Macrolepiota* species (On dry weight basis) (n = 3, ND = Not detected).

S. No.	Name of Species	Heavy metals (mg/100 g) of dry samples				
		As	Cr	Hg	Pb	Cd
1	<i>M. procera</i>	ND	ND	0.087	ND	0.0019
2	<i>M. dolichaula</i>	ND	ND	0.062	ND	0.0019
3	<i>M. rhacodes</i>	0.0074	ND	0.069	ND	0.0014
4	Permissible limit in individual food article	1 mg/kg	5.3 mg/kg	1 mg/kg	2.5 mg/kg	1.5 mg/kg

Traces of some heavy metals viz. As, Pb, Hg, Cd and Cr were found to be present in some of these samples. Out of three wild samples examined, the amount of Hg ranged from 0.069 mg/100 g in *M. dolichaula* to 0.087 mg/100 g in *M. procera*. As was only found in *M. rhacodes* (0.06 mg/100 g). Cd content ranged from 0.014 mg/100 g in *M. rhacodes* to 0.019 mg/100 g in *M. procera*. Cr and Pb were found to be absent in all these samples. Maximum permissible concentrations and approximately permissible levels of harmful effects of heavy metals in food stuff have been depicted in table-3. Results showed that these mushrooms possessed below the range of permissible limits of toxicity caused by heavy metals and the

concentrations obtained for heavy metals in these species seems to be within acceptable limits for human consumption [30].

C. Evaluation for nutraceutical components

The present study indicates the presence of phenolics, flavonoids, carotenoids and alkaloids in the extracts of the wild edible macrolepiotoid mushrooms. Phenolics were the major component detected in the extracts (5.90 – 16.81 mg/g), followed by flavonoids (1.36 – 1.76 mg/g). Alkaloids was found in small amounts (0.053 – 0.103 mg/g), and β - carotene and lycopene ranged from 0.12 - 0.29 μ g/gm and 0.05 – 0.12 μ g/gm respectively (Table 4)

Table 4: Phenolic compounds (mg/g), Flavonoids (mg/g), β -carotene (μ g/g), Lycopene (μ g/g) and Alkaloids (mg/gm) in wild *Macrolepiota* species (On dry weight basis) (mean \pm SD; n = 3)

S. No.	Species	Phenolic compounds (mg/gm)	Flavonoids (mg/gm)	β -carotene (μ g/gm)	Lycopene (μ g/gm)	Alkaloids (mg/gm)
1	<i>M. dolichaula</i>	5.90 \pm 0.58	1.76 \pm 0.20	0.12 \pm 0.02	0.05 \pm 0.01	0.103 \pm 0.01
2	<i>M. procera</i>	11.0 \pm 0.87	1.46 \pm 0.04	0.29 \pm 0.07	0.07 \pm 0.00	0.048 \pm 0.03
3	<i>M. rhacodes</i>	16.81 \pm 0.05	1.36 \pm 0.03	0.26 \pm 0.01	0.12 \pm 0.02	0.053 \pm 0.02
	CD at P \leq (0.05%)	0.30	0.91	0.05	0.10	0.052

* Significant at 0.05 level

DISCUSSION

Mushrooms are widely appreciated for their unique taste and flavour, but also for their chemical and nutritional properties [2]. The nutritional analysis carried out on these mushroom samples showed that they are all rich in proteins, fibers, moisture and carbohydrate contents, in contrast to low fat levels, which make them suitable to incorporate into low caloric diets. These results are in agreement with different studies conducted by various workers [2, 8, 10, 31, 32]. The relatively high protein and carbohydrates, content recorded in these samples (Table 1) is a proof of their being highly nutritious and good for human consumption. The samples showed appreciable quantities of phenolics, flavonoids and carotenoids. The data obtained in the present study are in most within these ranges with some slight variations as reported by Ayaz et al [33] (ash 6.8%, moisture 9.8%, carbohydrate 54.7%, fat 2.4% and protein contents 26.35%), while minerals content (Ca 4100 mg/l, Mg 3300 mg/l, Fe 300 mg/l, Mn 432 mg/l, Zn 74.5 mg/l, Cu 91.5 mg/l, Pb 2.58 mg/l and Cd 0.37 mg/l) of *Macrolepiota procera* were very high then present findings. Fernandes et al [34] evaluated Ash (9.86%), Moisture (90.01%), Fat (1.45%), protein (7.6%), Carbohydrates (80.2%), energy value (365.01%) in *Macrolepiota procera* which is low, high and similar ranges of present study. Shirmila and Radhamany [35] observed methanolic extract of the *Macrolepiota mastoidea* revealed the presence of total phenolics (5.5 mg/g) and flavonoids (3.6 mg/g) which is more than present investigation. There are several reports which showed that mushrooms are rich source of alkaloids and other essential nutritional components [36-40]. Alkaloids were also found in very small concentrations ranging from 0.046-0.077 mg/g which is higher than reported earlier in *Schizophyllum commune* (0.015%) and *Polyporus* spp. (0.013%) [41]. Further these mushrooms compared with other mushrooms which are ranged 0.17-0.78 % [42]. Ayodele and Jokhuoya Asuquo and Etim screened phytochemical properties of the mushroom *Oxyporus populinus* and *Psathyrella atroumbonata* indicated presence of moderate amount of alkaloids [43-44]. The presence of alkaloids in the extract is an indication that these mushrooms are of pharmacological importance

[45]. *B. edulis* was clearly the species presenting significantly higher contents of total alkaloid 96.5 mg/kg which is much higher than present findings [46]. Kumari and Atri [47] evaluated alkaloids in termitophilous mushrooms (0.046 – 0.077 mg/g) which is less as compared to present findings (0.048- 0.103 mg/g). Present findings are in line with those of Puttaraju et al. [48], Barros et al. [30], Ramesh et al. [49] on their research on bioactive compounds. Olfeti et al. [50] documented the presence of P (8.8 mg), K (23.9 mg), Na (1.1 mg), Ca (1.4 mg) and Mg (1.8 mg) in *Macrolepiota procera* which is low from present study.

Vetter [51] analyzed highest arsenic levels in fruit bodies of *Laccaria amethystea* (146.9 mg/kg DM), *Collybia butyracea* (58.3 mg/kg DM) and *Macrolepiota rhacodes* (42.6 mg/kg DM), respectively which is much more than present investigation. Falandysz and Gucia [52] determined the total mercury content in individual caps and stipes of *Macrolepiota procera* 0.05-22 mg/Kg which is much more than present investigation.

Pelkonen [53] extracted mineral elements in fresh *Macrolepiota procera* 0.059 (Cd), 0.215(Pb) 0.113(As) 0.121 (Ni) mg/Kg which is higher than present findings. Slávik et al. [54] observed the highest amount of total mercury content is 176.40982 mg/Kg dry matter in two fruit bodies of *Macrolepiota procera* which is much more than our present investigation. Falandysz [55] determined Selenium in fruitbody of *Macrolepiota* spp., with an average range of \sim 5 to < 10 μ g/g dw and *Macrolepiota rhacodes* with < 10 μ g/g dw which is less than present findings. Mushrooms have been reported to possess very effective mechanisms that enable them to readily absorb heavy metals from their substrates [56-59]. High concentrations of heavy metals have been observed in the fruiting bodies of mushrooms collected adjacent to heavy metal smelters and oil polluted areas [58, 60-62]. The results of this investigation revealed that these mushrooms are quite rich in comparison to results obtained by Sarla et al [63]. Stihl et al. [64] studied *Lycoperdon perlatum* and *Pleurotus ostreatus* containing minerals such as Fe, Zn, Mn, Cu, Cr and Se and also toxic elements, such as Cd, Ni and Pb and found these to possess permissible levels of toxic elements as has been found in the present

findings. The heavy metals in these samples may be due to the occurrence of these metals at the sites possessing these metals. The results of the present study indicate that mushrooms are rich in nutrients, nutraceutical components and minerals and low in fat.

CONCLUSION

In view of the results achieved during the present investigation it is apparent that these mushrooms are nutritionally and nutraceutically important which makes these suitable for the menu of modern calorie conscious society. Its commercial production not only promises a strong food alternative for mushroom lovers with a potential to provide equally potent culinary option as is provided by edible species of *Agaricus*, *Pleurotus*, *Volvariella*, *Lentinula*, etc. There is an urgent need to understand the diversity and potential of these mushrooms by undertaking further investigations for more effective utilization, their conservation. All the studied mushrooms have high nutritive value, specific aroma and can serve as good supplement to a healthy diet. The significant levels of proteins, fats, carbohydrates, fibers and some nutraceuticals in their fruit bodies are of considerable value. Studies undertaken points towards their role as precise source of nutritional and nutraceutical component.

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