Nutritional values and antioxidant potential of some edible mushrooms of Kashmir valley

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Abstract: Mushrooms are considered rich in proteins, carbohydrates and other nutrients. The present study was carried out to evaluate some edible mushrooms of Kashmir valley for their protein, carbohydrate and lipid contents. The highest protein content was found in *Boletus edulis* (2.20g) followed by *Agaricus bisporus* (1.80g), *Pleurotus ostreatus* (1.68g), *Morchella esculenta* (1.62g) and *Pleurotus sajor caju* (1.6g). Carbohydrate content also showed variation in all the five tested edible mushroom species, the highest carbohydrate content observed in *Boletus edulis* (6.0g) followed by *Agaricus bisporus* (4.85g), *Pleurotus ostreatus* (4.30g), *Morchella esculenta* (4.25g) and *Pleurotus sajor caju* (3.35g) respectively. Similar results were observed for lipid content. The present study was also investigated for the antioxidant potential of aqueous extract of mushroom species by the methods of DPPH radical scavenging activity, hydroxyl radical scavenging activity and superoxide radical scavenging activity. All these *in vitro* antioxidant activities were concentration dependent, which were compared with standard antioxidant Catechin.

Keywords: Edible mushrooms, proteins, carbohydrates, lipids, antioxidant potential.

INTRODUCTION

Fungi are eukaryotic, achlorophyllous, nucleated, shade loving, gametophytic, haploid, heterotrophic, nonvascular, spore producing, cryptogamic, thallophytic plants which are surrounded by cell wall containing chitin. They do not use carbondioxide as their carbon source and hence are dependent on external sources for organic carbon. Fungi range from simple forms like thread fungi to complex forms like mushrooms. Mushrooms are good sources of energy. They are low in starch and lipids but rich in proteins. Mushrooms have been found effective against cancer, cholesterol reduction, stress, insomnia, asthma, allergies and diabetes (Bahl, 1983). Due to high amount of proteins they can be used to bridge the protein malnutrition gap. The mushrooms are used as functional foods as they are used as nutrient supplements and to enhance immunity in the form of tablets. Overall, the world production of mushrooms is dominated by those mushrooms which are both edible and have medicinal properties. The main objective of the present study was to determining the nutritional values, and to evaluate the protective effects of some edible mushrooms of Kashmir valley against free radical mediated damages under *in vitro* situations.

MATERIALS AND METHODS

Collection of mushroom species

Field trips were organized to different places of the Southern Kashmir to collect mushrooms from their natural habitats. Field trips were designed according to the method given by (Hailling, 1996). Different areas were explored at regular intervals. Different sites were selected and within a month several trips were conducted. Sites from which samples of mushroom species were taken are: Shopian, Aharbal, Achabal, Chutpora, Kulgam, Pulwam, Pampore, Kakpora, Verinag, Halsidar Kokernag, Doru Shahabad, Kapran and Nowgam.

These samples of mushrooms were brought to the laboratory and identified on the basis of morphological, reproductive and other characteristics. Final identification was done by comparing the recorded characters of mushroom species with standard field guides by Largent (1973) and (Lincoff, 1982) and after comparing with mushroom herbaria of SKUAST-K, RRL Srinagar and N.R.C.M. Solan, Himachal Pradesh, India. The mushroom specimens were also preserved in FAA (Formaldehyde acetic acid) for herbarium purposes, in mushroom collection of KASH Herbarium of Plant Taxonomy, Division of Botany, Kashmir University under reference number SH.KASH-28742 (*Morchella esculenta*), SH.KASH-29750 (*Boletus edulis*), SH-KASH-28747 (*Agaricus bisporus*), SH-KASH-28749 (Pleurotus *ostreatus*), SH-KASH-28762 (*Pleurotus sajor caju*).

Preparations of extract

The mushroom material was dried in the shade at 30 ± 2°C. The dried material was ground into a powder using mortar and pestle and passed through a sieve of 0.3 mm mesh size. The powder obtained was extracted with water by using the method of (David, Fadel, Anee & Pickering,

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2000). The extract was then concentrated with the help of rotary evaporator under reduced pressure and the solid extract was stored in refrigerator for further use.

Biochemical analysis of mushrooms

A study was carried out to determine protein, sugar and lipid content of some important edible mushrooms of Kashmir valley such as, *Morchella esculenta, Pleurotus ostreatus, Boletus edulis, Agaricus bisporus* and *Pleurotus sajor caju.* The methods employed to estimate the protein, carbohydrate and lipid contents of mushrooms are as under:

Estimation of protein content

Protein concentration was estimated by the method of (Lowry, Roseborough, Farr & Randall, 1951).

Estimation of total sugar

Total sugar content was estimated by the method of **(**Dubois, Gilles, Hamilton, Rebers & Smity, 1995).

Estimation of lipid content

Total lipid content of mushrooms was determined by the method of (Folch, Lees & Sloane, 1956).

Antioxidant activity of mushrooms

DPPH radical scavenging activity: The 1, 1- diphenyl-2-picryl- hydrazyl (DPPH) assay was performed by using the method of (Braca, De Tommasi Nunziatina, Di Bari Lorenzo, Pizza Cosimo, Politi Mateo & Morelli Ivano, 2001). Various concentrations of mushrooms extract (100-600µg/ml) were added to 1ml of the 0.004% methanol solution of DPPH, and the mixture was vortexed vigorously. The tubes were then incubated at room temperature for 30 minutes in dark, and the absorbance was taken at 517nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Catechin was taken as known free radical scavenger. Percentage inhibition activity was calculated by using the formula:

$$\% \text{ inhibition } = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100$$

Where A_0 was the absorbance of the control and A_1 was absorbance in the presence of *mushrooms* extract/ known antioxidant.

Assessment of Hydroxyl radical scavenging property: Hydroxyl radical, generated from the Fe^{3+}- Ascorbate-H_2O_2 (Fenton reaction), was evaluated by degradation of deoxyribose that produced thiobarbituric acid reactive species (TBARS) (Halliwell, Gutteridge & Aruoma, 1987). The reaction mixture contained 25mM deoxyribose, 10mM Ferric chloride, 100mM ascorbic acid, 2.8mM H_2O_2 in 10mM KH_2PO4 (pH 7.4) and various concentrations of mushroom aqueous extracts. The reaction mixture was incubated at 37°C for 1h. Then 1ml of 1% thiobarbituric acid and 1 ml of 3% trichloroacetic acid was added and mixture heated at 100°C for 20min. The TBARS was measured spectrophotometrically by taking absorbance at 532nm. The results were expressed as percentage inhibition of deoxyribose oxidation, as determined by the following formula:

$$\text{Percentage inhibition} = \left[\frac{(A - B)}{A}\right] \times 100$$

Where A was the malondialdehyde produced by Fenton reaction treated alone, and B was the malondialdehyde produced in the presence of mushroom extracts/ known antioxidant.

Assessment of superoxide anion radical scavenging property: Superoxide anion radical generated by the Xanthine/Xanthine oxidase system was spectrophotometrically determined by monitoring the product of nitroblue tetrazolium (NBT) using the method of (Jung, Seog, Choi, Park & Cho, 2006). A reaction mixture containing 1.0ml of 0.05M phosphate buffer (pH 7.4), 0.04ml of 0.15% BSA, 0.04ml of 15.0mM NBT and various concentrations of (mushroom extracts and known antioxidant) was incubated at 25°C for 10min, and the reaction was then started by adding 0.04ml of 1.5U/ml Xanthine oxidase and again incubated at 25°C for 20min. The absorbance of the reaction mixture was measured at 560nm. Decreased absorbance of the reaction mixture indicates increased superoxide anion radical scavenging activity. The scavenging activity of the mushroom extract on superoxide anion radical was expressed as:

$$\% \text{ inhibition } = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100$$

Where A_0 was the absorbance of the control and A_1 was absorbance in the presence of mushroom extracts/ known antioxidant.

RESULTS

Biochemical analysis of mushrooms

It was found from the results that protein content showed variation in all the tested mushrooms. However, the highest protein content was found in *Boletus edulis* (2.20g) followed by *Agaricus bisporus* (1.80g), *Pleurotus ostreatus* (1.68g), *Morchella esculenta* (1.62g) and *Pleurotus sajor caju* (1.60g) respectively (table 1).

Carbohydrate content also showed variation and the highest carbohydrate content was observed in *Boletus edulis* (6.00g), followed by *Agaricus bisporus* (4.85g), *Pleurotus ostreatus* (4.30g), *Morchella esculenta* (4.25g) and *Pleurotus sajor caju* (3.35g) (table 1). Similar results were observed with the total lipid content of tested mushrooms and the lipid content was in the following descending order (table 1).

Boletus edulis > *Agaricus bisporus* > *Pleurotus ostreatus* > *Morchella esculenta* > *Pleurotus sajor caju*

Antioxidant activity of mushrooms

DPPH radical scavenging activity

The results of the DPPH scavenging activity of different edible mushrooms are shown in table 2. Results revealed that *Boletus edulis* aqueous extract showed the highest DPPH radical scavenging activity (88.54%) at the concentration of 600µg/ml followed by the *Morchella esculenta* (85.15%), *Pleurotus sajor caju* (82.50%) and *Agrocybe cylindrica* (80.02%) respectively. While as *Pleurotus ostreatus* showed moderate DPPH radical scavenging activity of 56.24% at 600µg/ml. Catechin which was used as known free radical scavenger showed significant DPPH radical scavenging activity of 96.25% at 600µg/ml.

Table 1: Quantitative estimation of proteins, lipids and carbohydrates of some mushroom species

S. No.	Name of the species	Protein (g/100g)	Carbohydrate (g/100g)	Lipids (g/100g)
1	*Pleurotus sajor caju*	1.60	3.35	1.90
2	*Morchella esculenta*	1.62	4.25	1.96
3	*Pleurotus ostreatus*	1.68	4.30	2.20
4	*Agaricus bisporus*	1.80	4.85	2.38
5	*Boletus edulis*	2.20	6.00	3.60

Table 2: Effect of aqueous extract of different mushroom species and known antioxidant on DPPH radical scavenging activity. Absorbance at 517nm. Concentration (µg/ml)

Species	100 (µg/ml)	200 (µg/ml)	300 (µg/ml)	400 (µg/ml)	500 (µg/ml)	600 (µg/ml)
Agrocybe cylindrica	47.67±0.367	61.01 ± 0.22	65.86 ± 1.18	75.36 ± 0.20	77.54 ± 0.22	80. 02± 0.85
Morchella esculenta	50.77 ± 0.11	60.27 ± 0.12	74.53 ± 0.12	81.24 ± 0.09	85.15 ± 0.10	88.39 ± 0.03
Boletus edulis	37.78 ± 0.05	50.87 ± 0.08	78.40 ± 0.19	84.80 ± 0.11	86.6 ± 1.37	88.54 ± 0.10
Pleurotus ostreatus	5.76 ± 0.54	11.34 ± 1.16	20.23 ± 0.23	33.24 ± 0.80	53.32 ±2.25	56.24 ± 0.79
Pleurotus sajor caju	20. 58 ±0.07	46.30 ± 0.37	67.60 ± 0.20	75.52 ± 0.45	80.6 ± 0.47	82.50 ± 0.28
Catechin	43.10 ± 0.06	65.36 ± 0.07	73.91 ± 0.09	80.94 ± 0.10	94.60 ± 0.11	96.25 ± 0.07

The results represent mean ± S.D of 3 separate experiments.

Table 3: Represents the effect of aqueous extract of different mushroom species and known antioxidant (Catechin) on superoxide anion radical scavenging activity (% inhibition). Absorbance at 560nm (Absorbance of control = 0.898 ± 0.01). Concentration (µg/ml)

Species	50 (µg/ml)	100 (µg/ml)	150 (µg/ml)	200 (µg/ml)	250 (µg/ml)	300 (µg/ml)
Agrocybe cylindrica	16.30 ± 1.04	21.45 ± 0.56	27.10 ± 1.74	33.55 ± 1.04	38.96 ± 1.57	44.90 ± 1.25
Morchella esculenta	19.44 ± 1.0	27.08 ± 1.36	32.34 ± 1.71	38.41 ± 1.20	43.84 ± 1.18	52.01 ± 1.67
Boletus edulis	11.03 ± 0.57	15.55 ± 0.37	18.52 ± 1.51	24.10 ± 0.88	28.70 ± 1.58	40.75 ± 1.55
Pleurotus ostreatus	6.88 ± 0.35	8.98 ± 0.50	13.90 ± 0.67	19.02 ± 0.70	26.89 ± 1.65	32.33 ± 1.61
Pleurotus sajor caju	11.22 ± 0.86	16.91 ± 0.97	24.25 ± 3.26	34.18 ± 4.33	47.17 ± 1.24	56.75 ± 1.92
Catechin	31.52 ± 1.0	38.44 ± 0.84	47.72 ± 1.72	60.30 ± 2.44	68.28 ± 2.69	91.23 ± 7.27

The results represent mean ± S.D of 3 separate experiments.

Table 4: Represents the effect of aqueous extract of mushroom species and known antioxidant on hydroxyl radical scavenging activity (% inhibition). Results are reported as the percentage of the maximum formation of $OH^{\bullet}$ radical (100% deoxyribose oxidized): in absorbency, 100% is 0.61 ± 0.043 (control). Absorbance at 532nm. Concentration (µg/ml)

Species	50 (µg/ml)	100 (µg/ml)	150 (µg/ml)	200 (µg/ml)	250 (µg/ml)	300 (µg/ml)
Agrocybe cylindrica	16.30 ± 1.04	21.45 ± 0.56	27.10 ± 1.74	33.55 ± 1.04	38.96± 1.57	44.90 ± 1.25
Morchella esculenta	19.44 ± 1.0	27.08 ± 1.36	32.34 ± 1.71	38.41 ± 1.20	43.84 ± 1.18	52.01 ± 1.67
Boletus edulis	11.03 ± 0.57	15.55 ± 0.37	18.52 ± 1.51	24.10 ± 0.88	28.70 ± 1.58	40.75 ± 1.55
Pleurotus ostreatus	6.88 ± 0.35	8.98 ± 0.50	13.90 ± 0.67	19.02 ± 0.70	26.89 ± 1.65	32.33 ± 1.61
Pleurotus sajor caju	11.22 ± 0.86	16.91 ± 0.97	24.25 ± 3.26	34.18 ± 4.33	47.17 ± 1.24	56.75 ± 1.92
Catechin	31.52 ± 1.0	38.44 ± 0.84	47.72 ± 1.72	60.30 ± 2.44	68.28 ± 2.69	91.23 ± 7.27

The results represent mean ± S.D of 3 separate experiments.

Superoxide free radical scavenging activity

The Superoxide radical scavenging activity of different mushroom species was studied in compared with Catechin. *Agrocybe cylindrica* aqueous extract showed strong superoxide radical scavenging activity of 70.56% at 300µg/ml concentration. The *Pleurotus ostreatus* and *Pleurotus sajor caju* extract showed weak superoxide radical scavenging potential of 46.41 and 51.86% at 300µg/ml, respectively. The *Boletus edulis* and *Morchella esculenta* extract showed superoxide radical scavenging activity of 56.08 and 69.80%, respectively, at 300µg/ml concentration. Overall the aqueous extract of mushrooms showed a dose dependent superoxide radical scavenging activity. Catechin, a known free radical scavenger inhibits the superoxide radical formation of 89.05% (table 3).

Hydroxyl radical scavenging activity

The results of hydroxyl radical scavenging activities of different mushroom species are presented in table 4. The results revealed that all the mushroom species showed moderate hydroxyl radical scavenging activity of 44.9% (*Agrocybe cylindrica*), 52.01% (*Morchella esculenta*), 40.75% (*Boletus edulis*), 32.33 (*Pleurotus ostreatus*) and 56.75% (*Pleurotus sajor caju*) respectively at 300µg/ml. Catechin which was used as known free radical scavenger showed significant hydroxyl radical scavenging activity of 91.23% at 300µg/ml. Overall the scavenging activity was found in the following decreasing order.

Catechin > *Pleurotus sajor caju* > *Morchella esculenta* > *Agrocybe cylindrica* > *Boletus edulis* > *Pleurotus ostreatus*

DISCUSSION

From nutritional point of view, mushrooms are highly valued due to high proteins and other nutrients than most of the plants (Chang, 1980). So in the present study some species of mushrooms such as: *Pleurotus sajor caju, Morchella esculenta, Pleurotus ostreatus, Agaricus bisporus* and *Boletus edulis,* were investigated for protein, lipid and carbohydrate content. It was revealed from the present study that carbohydrate content showed variation in all the investigated mushrooms, however the highest carbohydrate content was found in *Boletus edulis* and lowest concentration of carbohydrate was found in *Pleurotus sajor caju.* Carbohydrate content of these mushroom species has been determined for the first time in Kashmir valley of India. However, several reports confirmed the variation in the carbohydrate content in different species of mushrooms. Florczak *et al.,* (2004) puts the value of carbohydrate for *Coprinus atramentarious* as high as 24g/100g. In another study carbohydrate content of *Agaricus bisporus* and *Pleurotus ostreatus* was found to be 2.6-5.2 g/100g and 3.8-6.7g per 100g on dry weight basis respectively by Kunachowicz *et al*. (1999) and Manzi *et al.* (2001). In the present study the protein contents of 5 species of mushrooms also showed a great variation (table 1). Similar results were reported by Souci *et al.* (1989) in *M. esculenta* and *C. cibarius*. Lipid content of all the five investigated mushroom species showed significant variation. The highest lipid content was found in *Boletus edulis* (3.60g) and lowest lipid content was found in *Pleurotus sajor caju* (1.90g) respectively. Similar studies were carried out by Shah *et al.* (1997) who reported lipid content in *Agaricus bisporus* and *Pleurotus ostreatus* as 2.6g and 1.8g respectively. It was therefore observed from the study that out of three nutritional constituents of the mushrooms viz. proteins, carbohydrates and lipids, the carbohydrate was found at highest concentration in all the investigated mushrooms. This is similar to the findings of Blumenthal (1976). The chemical composition of mushrooms determines their nutritive value and this nutritive value differs from one species of mushrooms to another species and that depends upon the nature of substrate, atmospheric conditions, stage of development of the mushrooms and part of fruiting body used besides the conditions of storage after harvest (Manzi. *et. al.,* 2001; Adejumo & Awesanya, 2005). Under *in vitro* conditions, the aqueous extract of five mushroom species has been evaluated in a series of tests like DPPH free radical scavenging, hydroxyl radical scavenging and superoxide anion scavenging activities.

In our study a dose dependent decrease in the concentration of DPPH radical scavenging was observed, and the scavenging effect of mushroom extract decreased in the order of *Boletus edulis* > *Morchella esculenta* > *Pleurotus sajor caju* > *Agrocybe cylindrica* > *Pleurotus ostreatus* at the concentration of 600µg/ml respectively.

Superoxide anions are precursors to active free radicals that have potential for reacting with biological macromolecules and thereby inducing tissue damages (Halliwell & Gutteridge, 1984). In our study we have shown that all the five mushroom species possessed excellent superoxide radical scavenging activity, suggesting that these mushrooms could be effective in reducing oxidative stress related diseases.

The hydroxyl radical is the most reactive of the reactive oxygen species, and it induces severe damage in adjacent biomolecules (Gutteridge, 1984). The hydroxyl radical can cause oxidative damage to DNA, lipids and proteins (Spencer, Jenner & Aruoma, 1994). The Fenton reaction generates hydroxyl radicals which degrade deoxyribose of DNA using Fe^{2+} salts as an important catalytic component. Oxygen radical may attack DNA either at the sugar or the base, giving rise to a large number of products. The potential of an aqueous extract of five mushroom species to inhibit hydroxyl radical-mediated deoxyribose damage was assessed by means of the iron (II)-dependent DNA damage assay. In the present study,

the hydroxyl radical-scavenging effect of the different mushroom species at a concentration of 300µg/ml, was found to be 44.90% (*Agrocybe cylindrica*), 52.01% (*Morchella esculenta*), 40.75% (*Boletus edulis*), 32.33% (*Pleurotus ostreatus*), 56.75% (*Pleurotus sajor caju*) and Catechin, which was used as a standard since it is reported to be significantly effective in inhibition of hydroxyl radicals, showed 91.23% scavenging effect at a concentration of 300µg/ml (table 4).

CONCLUSION

Of the five species screened for different nutrients such as carbohydrates, proteins and lipids, *Boletus edulis* showed highest values for all the three nutrients followed by *Agaricus bisporus, Pleurotus ostreatus, Morchella esculenta* and *Pleurotus sajor caju*. Biochemical analysis of these mushrooms have been carried out for the first time in Kashmir valley but further investigation is needed for complete analysis of these mushrooms for proteins, carbohydrates, lipids, vitamins and minerals.

All the species of mushrooms screened showed positive results for antioxidant activity. This indicates the medicinal importance of these mushrooms. Our study may help in a long way for exploration of mushrooms for medicinal properties. However, a through screening is needed to delimit their different medicinal properties which will not only help in solving the food crisis which is prevalent in the rural poor population but will also add medicinal touch to their food.

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