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NUTRITIVE PROPERTIES AND ANTIOXIDATIVE ACTIVITY OF *AMANITA CAESAREA* AND *A. LOOSII WILD* EDIBLE MUSHROOMS FROM ODISHA

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ABSTRACT

The aim of this study to determine the nutritional , mineral and antioxidant properties of two species of mushrooms *Amanita caesaria* and *Amanita loosii*, collected from dry deciduous forest of Odisha India. These edible mushrooms were analysed in terms of macro and micronutrient, mineral contents and antioxidant properties through various biochemical and spectrophotometrical analysis. Antioxidant properties were determined by evaluating the presence of phenolic content, ascorbic acid, carotenoids and flavonoids. The average protein content ranged 3.13 – 8.66 mg/gm. And carbohydrate ranged 23.61-26.00 g/100g were found. Both mushrooms have exhibited 72-73 % DPPH scavenging activity. More amounts of Fe and Zn found in *Amanita caesaria* as compared to *A. loosii.* The examined mushroom species were rich in protein and carbohydrate. The variations in both edible mushrooms are attributed to different species and/or growth stages. Results obtained through various nutritional and biochemical analysis b exhibited the exploitable potential of these species for cultivation and commercialization.

KEY WORDS: Distribution, Antioxidants, Amanita.

INTRODUCTION

Mushrooms are widely distributed all over the world and some of them have nutraceutical and pharmaceutical importance. In general the fruiting bodies of mushrooms contain about 56.8% carbohydrate, 25.05% protein, 5. 7 % fat and 12. 5 % ash on a dry weight basis [1]. Mushrooms are also gaining importance due to high protein content (19-35 %) in comparison to wheat (13.3%) and to milk (25.2 %); besides low fat content and vitamin C and B [2-4]. In addition the mushrooms are important source of food and gain income in both developing and developed countries [5,6].

Edible mushrooms attract much attention due to its medicinal value. Mushrooms are reported to protect heart health, prevent the risk of cancer and help to control blood sugar level etc [7]. Many of the diseases/disorders are mainly linked to oxidative stress due to free radical formation Barros et al [8] reported the presence of antioxidant compounds in food diets, which act as agents to reduce oxidative damage in human body. Several mushroom species collected from different parts of world have been reported for usefulness of their antioxidant activity [9]. Still there are several varieties of wild edible mushrooms whose nutritive and medicinal properties have not been studied. *Amanita caeserea* and *A. loosii* from Odisha, India are one such edible mushroom is required to be studied in detail.

The Genus *Amanita* contains about 600 species including some of most toxic as well as some edible species. In India approximately 100 species are reported [10]. It is found in North Africa and southern Europe, particularly in the hills of northern Italy. The mushroom is also distributed in Hungary, Mexico and China. *Amanita caesarea* is listed in the Red Data book of Ukraine and it is protected by law in Croatia, Slovenia. This species has also been reported in Arunachal Pradesh and Khasi hill of Meghalaya in India. The species has been found in Similipal forest of Odisha India. It has been observed that local tribals use it is as one of their food items.

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Similipal, located in the northern Odisha has a green moist deciduous forests and climate in spring and autumn provides ideal conditions for fungal growth with temperature ranging between 18-28°C. Local tribal community utilizes the forest product like mushrooms (wild) for their livelihood by collecting and selling them in local tribal market with minimal cost. Only these people know edible mushrooms and are able to identify them. Tribal forests dwellers are finding few wild edible mushrooms and use them as food. Due to scattered and minimal population of some wild mushrooms in the area, they may not include as components of their livelihood.

The two species covered in this paper i. e. *Amanita caesaria* and *A. loosii* have not been explored till date for their taxonomic characterization, identification and specialty for nutritional and medicinal properties though local people use it occasionally as food. Hence, it is necessary to have some basic information to avoid the poisoning factors associated with some the mushrooms. Hence, the samples collected from this area were evaluated for some nutritional and antioxidant properties and described here.

MATERIALS & METHODS

Collection and characterization of Mushrooms

Mushrooms samples: Healthy, fresh and succulent mushroom from two species of *Amanita caesarea* and *A. loosii* were collected from Tropical moist deciduous and semi evergreen forest of Similipal.

Macroscopic examination for the pileus, stipe, veil, ring, volva, lamellae and gills etc. for the identification was done according to Largent, 1981).The mushrooms samples were carried into the laboratory and preserved in Microbiology laboratory of Regional Plant Resource Centre, Bhubaneswar, Odisha, India as specimen as well as further analytical and biochemical processing.

Estimation of protein

The protein content was determined from dried powder of mushroom (100 mesh size). 1 gram of dried powder of mushroom was treated with 10 ml of phosphate buffer (pH 7.6), and centrifuged fro 20 min at 8000rpm at 20 °C. 100 µl of supernatant was mixed with 5 ml of Bradford, incubated in dark (10 min.) and absorbance was recorded at 595nm. The values were expressed in mg protein per gram of dried sample [11].

Estimation of Total carbohydrate

The total carbohydrate was estimated by Phenol Sulphuric acid method [12]. The total carbohydrate content in dried mushroom powder was estimated by boiling of 100 mg samples in 5 ml of 2.5 N HCl.

The mixture was neutralized with sodium carbonate until the effervescence ceases. The final volume was made up to 100 ml and centrifuged. The absorbance was measured at 490 nm using UV-spectrophotometer (Analytic Jena, Germany). D-glucose at concentration of 0.5mg/ml used to make standard. The mean value expressed in mg of carbohydrate per gram of mushroom sample.

Estimation of reducing sugar

Reducing sugar was determined by treating 100 mg dried powder with 5 ml of 80% ethanol [13]. The reaction mixture was heated at 90 °C till the complete evaporation of ethanol. Residue was added with 5 ml of ethanol and mixed. 100 µl of this was added with 3 ml of DNS reagent (200mg crystalline phenol + 50 mg sodium sulphite in 100 ml of 1% NaOH).The reaction mixture was kept in boiling water bath for 5 min. followed by addition of 1ml 40% Rochelle's salt (Sod. Potassium tartarate).The absorbance was measured at 510 nm. Glucose was used as standard to calculate the concentration of reducing sugar and expressed in terms of mg per gram dry weight of the samples.

The amount of non reducing sugar was calculated as differential amount of total carbohydrate and reducing sugar and expressed in terms of g/100g of dry wt. of samples.

DPPH Free Radical scavenging activity

The DPPH activity was estimated in the methanolic extracts by a colorimetric method [14]. 1 gm of samples was added with 20 ml methanol. Methanolic extract was added with 2 ml of DPPH solution (1:2) and incubated for 30 min. in dark after vigorous mixing. Absorbance was measured at 517 .Scavenging activity of each extract on DPPH radical was calculated using the following equation:

Scavenging activity (%) = (1- Absorbance of sample/Absorbance of control) ×100.

AEAC: (Ascorbic acid Equivalent Antioxidant Capacity) was calculated by calibrating the value of above absorbance in standard ascorbic acid curve and expressed in mg per gram of dried sample.

Determination of Carotenoid content in mushroom

The carotenoid was estimated in 500 mg of dried mushroom powder treated with 10 ml of 80% acetone and centrifuged at 3000 rpm for 10 minutes at 4^0 C. This procedure was repeated until the residue became colorless. The residue was made upto 10ml with 80% acetone and measured for absorbance at 480, 645 and 663 nm separately. The quantity of carotenoids was calculated by using following formula and values were expressed in mg/gm by using formula [15]

Carotenoid = A.480+ (0.114×A.663 - 0.638 × A.645). Where A= Absorbance.

Estimation of Flavonoids

The flavonoid content of dried sample was estimated from methanolic extract. 100 µl of extract was diluted with 1.5 ml of methanol and incubated for 5 min at room temperature. 0.1ml of $AlCl_3$ was added and again incubated at room temperature for 5 min. The reaction

mixture was mixed with 0.1ml of Potassium acetate (1M) and total volume was made upto 5 ml with dist. water.

100µl of methanolic extract was taken in a clean test tube later 1.5ml of methanol was added and incubated for 5 minutes at room temperature then 0.1 ml of $AlCl_3$ was added again followed by 5 minutes of incubation at room temperature followed by addition of Potassium acetate, total volume was made upto 5ml, finally absorbance was measured at 415nm. Quercetin was taken as standard and flavonoid content was expressed in mg/gm [16].

(e) Determination of total Phenol Content

1 gm each of dried mushroom sample was mixed with 10 ml of methanol. Samples were grinded in mortar pestle for effective extraction and centrifuged at 2000g for 15 minutes. Supernatant collected and stored at 4^0 C for further analysis. The yield of extraction was expressed as percent on a dry weight basis. The total phenolic content in the wild edible mushroom were determined spectrophotometrically by folin phenol method described by Singleton & Rossi [17] with some modification. A sample of 100 µl was made upto 1 ml with distill water. 1 ml of folin ciocalteau reagent and 2 ml of 10% sodium carbonate solution was added. Gallic acid was used to draw the standard curve using 10-80µg of gallic acid .The total phenolic content was expressed as gallic acid equivalent (GAE) in gram per 100 gram of

(f) Determination of Ascorbic acid content

The ascorbic acid content in the wild edible mushrooms was determined by volumetric method [18]. The dye solution was prepared by dissolving 42 mg of sodium carbonate into a small volume of distill water and 52 mg of 2,6 dichlorophenol indophenols was added in it . The volume was made up to 200 ml .Sample (0.5-5g) was extracted in 4% oxalic acid and made up to a known volume (100 ml) and centrifuged at 2000g for 15 minutes . 5ml supernatant from the extract was added with 10 ml of 4 % oxalic and titrated against the dye (V2 in ml). The initial and final volume of dye consumed while the appearance of pinkish color for each sample was noted down.

Stock standard solution: Dissolve 100 mg ascorbic acid in 100 ml of 4 % oxalic acid solution in a standard flack (1mg/ml).

Working standard: Dilute 10 ml of stock solution in 100 ml of 4% oxalic acid. End point after the concentration of working standard becomes 100µg/ml. End point after titration of the working standard against dye(V1ml) results in appearance of pink color that remains for few minutes .The amount of ascorbic acid in mg/ 100 g sample is calculated by the following method.

0.5 mg/V1 ml × V2 /5 ×100/ weight of the sample ×100.

(g) Estimation of mineral content

All the samples were weighed 0.5gram and kept in 10 ml concentrated Nitric acid overnight. Then the samples were heated till the emergence of white fume and treated with diacid (Hydrochloric acid and Perchloric acid in 2:1) with the help of Microwave digestion system (Milestone stat D, Germany) .The sample volume made up to 100 ml with distilled water. Estimation of Calcium and Magnesium was done by titration method taking EDTA as titrate [19]. Estimation of Phosphorous was done by using Vandate molybdate and orthophosphoric acid giving yellow colour complex in nitric acid medium. Estimation of Sodium was done by following and estimation of Zn, Cu, Co & Cr was done by atomic absorption spectrophotometer (Analytic Jena, Germany).

RESULTS

1. Identification and characterization of mushrooms species

***Amanita caeserea (*Scop.) Pers.**

Macroscopic Features: Commonly known as Caesar's mushroom

Habitat: On soil, Association: Scattered, Present in slopes, Soil characteristics: Laterite soil

Forest type: Tropical moist deciduous, Vegetational community: Thick woody.

PILEUS:-Diameter: 10 cm (av), Colour:- When young in centre : Orange, In Margin: Yellow, Mature Specimen: Yellow. Shape: Convex. Pileus margin roll: Inflexed, Pileus surface on Touch: Moist, Hygrophanous, Scales: Fibrillose, Cover the entire surface, Cuticle:- Half peeling, Pileus consistency:- Fleshy, Flesh colour: White, No change in colour on bruishing or handling. STIPE:- Attachment: Central, Colour at top:- White, At base :- bulbous, Texture: Smooth, Size: 8 cm, Thickness at top: Equal throughout, Stipe Shape: Equal in diameter, Stipe base: Bulbous, Texture: Smooth, Consistency: Fleshy , Stipe Surface: Fibrous, Stipe context: stuffed, Trama color: White. RING: Present, Double, movable, VEIL: Absent, VOLVA: Present, lobbed, Basal Association: Mycelial. LAMELLAE:- Gill Length: 5 cm, equal, No of Sets of Lamellae: 1, Gill Colour : White ,On Maturity:- White, Consistency: fleshy, Densely crowded, Gill Attachment: Free, Gill Separation : Separable , Gill nature : Forking , Gill Shape : Normal , Edge : Smooth .

Distribution: Widely distributed in Similipal Biosphere Reserve, Banamunda, Badampahad & Gurguria forest.

Macroscopic Features: Commonly known as De loose's Caesar.

Habitat: On soil, Association: Scattered, Present in slopes & plain, Soil characterstics : Laterite soil.

Forest type: Tropical moist deciduous, Vegetational community: Thick woody, shrub. PILEUS ::-Diameter : 10 cm (av) , Colour :- When young in centre : White , In Margin : White , Mature Specimen : White . Shape:

Convex. Pileus margin roll : Inflexed, Pileus surface on Touch : Moist, Hygrophanous, Scales : Fibrillose, Cover the entire surface, Cuticle :- Half peeling, Pileus consistency :- Fleshy, Flesh color: White to yellow, No change in colour on bruishing or handling. STIPE:- Attachment: Central, Concolorus with pileus, Size: 8 cm, Thickness at top: Equal throughout, Stipe Shape: Equal in diameter, Stipe base : Bulbous , Texture : Smooth, Consistency: Fleshy, Stipe Surface: Fibrous, Stipe context: stuffed, Trama color: White. RING: Absent, VEIL**:** Absent, VOLVA: Present, lobbed, Basal Association: Mycelial. LAMELLAE:- Gill Length : 5 cm , equal , No of Sets of Lamellae: 1, Gill Colour: White, On Maturity:- White, Consistency: fleshy, Densely crowded, Gill Attachment : Free , Gill Separation : Separable, Gill nature: Forking, Gill Shape: Normal, Edge: Smooth. Distribution**:** Widely distributed in Similipal Biosphere Reserve, Banamunda, Badampahad, Kaliyani forest, and in South Kalahandi forest division.

Nutritional analysis

Two wild edible mushroom species (*A. caesarea* and *A. loosii*) from the eastern Odisha of India were evaluated for their content in total protein , carbohydrate, reducing sugar and non reducing sugar. The amount of crude protein (8.66 ± 0.90 mg/g) was higher in *A. caesarea* as compared to *A. loosii* (3. 13 ±0.20 mg/g) (Table -1) . This species showed higher amount of carbohydrate and reducing sugar comparatively the other species. However, both the mushroom frutibodies appeared more or less similar in non reducing sugar content.

2. Analysis of mineral content in *A. caesarea* and *A. loosii*

Table 2 showed a comparative data of mineral content found in both wild edible mushrooms .The higher phosphorus content i. e. 0.33%) was found in *A. loosii* whereas *A. caesarea* showed higher amount of Ca 17.65% and Mg. 12.54%. Analysis of Zn and Fe content showed difference in both the species. However, both the mushrooms species resulted similarly in analysis of Sodium i.e. 0.09 %.

3. Analysis of antioxidant parameters

Determination of DPPH scavenging % exhibited 72.43+2. 24 and 72. 31 + 5. 83 in *A. caesarea* and *A. loosii*, respectively. Table -4 shows the phenols concentration in the mushroom extracts, expressed as mg of gallic acid equivalents (GAEs) per g of extracts. The contents of total phenols in methanolic extracts of A. caesarea were (0.22+0.013 g/100g) while in *A. loosii* extracts contains only 0.10+0.016 g/100 g. The antioxidant properties in terms of total ascorbic acid (0.75+ 0.19g/100g) were higher in *A. caesarea* whereas *A. loosii* showed higher level of carotenoid (5.51+0.69 mg/g).

Table 1. Analysis of nutritional properties of *A. caeseria* and *A. loosii*

Nutritional parameters	*A. caeserea*	*A. loosii*
Protein (mg/gm)	8.66 ± 0.90	3.13±0.20
Carbohydrate gm/100gm	26.00 ±1.43	23.61±4.68
Red. Sugar mg/g	39.44 ± 7.12	16.25 ± 5.79
Non red. Sugar gm/100gm	22.03 ± 2.10	22.05 ± 4.72

Table 2. Analysis of mineral contents of *A. caesarea* and *A. loosii*

Mineral content	*A. caesarea*	*A. loosii*
P %	0.25	0.33
Na %	0.09	0.09
Ca %	17.65	11.4
Mg %	12.54	9.02
Fe ppm	2137.5	1530
Zn ppm	11	4.6

Table 3. Analysis of Antioxidant contents of *A. caesarea* and *A. loosii*

Antioxidant parameters	*A. caeserea*	*A. loosii*
DPPH Scavenging%	73.43 ± 2.24	72.31 ± 5.83
AEAC mg/gm	0.56 ± 0.02	0.54 ± 0.08
Ascorbic Acid (g/100g)	0.75 ± 0.19	0.59 ± 0.14
Total Phenolics (g/100g)	0.22 ± 0.013	0.10 ± 0.016
Carotenoids (mg/g)	3.12 ± 0.23	5.51 ± 0.69
Flavonoids (mg/gm)	0.96 ± 0.15	0.53 ± 0.13

± Standard deviation, n = 3 (replications)

Fig. 1. Sporocarp of *A. loosii* grow in leaf litter substratum found in Similipal forests *Amanita loosii* (Beeli)

Fig. 2. Sporocarp of *A. loosi* grow in leaf litter substratum found in Similipal forests Nutritional analysis

DISCUSSION

Occurrence of *Amanita caesarea* and *A. loosii* found in Similipal forests of Eastern India is good record on biodiversity of mushrooms in the world as *A. caesarea* has been reported in few habitat of India. No reports are available on *A. loosii* as edible mushrooms from India.

Wild mushrooms are becoming more and more important in our diet for their nutritional and pharmacological characteristics [20]. Simultaneously, their consumption is also increasing due to a good content of proteins and trace minerals

The protein content analyzed in these two edible mushrooms were seems to be very less as compared to other wild edible mushrooms like *Agarius arvensis*, *lactarius deliciosus*, *Leucopaxillus giganteus*). The edibility seems not be related to the nutritional factors only. The analysed mushroom especially *A. caesarea* contains good amount of iron and calcium besides useful phytochemicals such as phenolics , ascorbic acid, caroenoids and revealed the antioxidant properties.

Fee radical scavenging is one of the mechanisms in inhibition of lipid oxidation commonly used to estimate antioxidant activity. The methanol extract of *A. caesarea* and *A. loosii* showed the good scavenging activity. It is apparently higher with reference to *Agaricus bisporus* (67.86%). The scavenging activities of these two edible mushrooms are also well comparable with *Russula nigricans* that showed 78.16 %. Polyphenolic compounds, Ascorbic acids and flavonoids are considered as antioxidants in many fruits, vegetables and mushrooms. Ascorbic acid content in *A. caesarea* showed higher amount as compared to *A. rubescens* (29.33 mg /kg). Flavonoid content was found to be very low in both the species as it has been reported quite higher in other wild edible mushrooms like *Ramaria botrytis* (16.56 mg/g) and non edible *Hypholoma fasciculare* (5.09mg/g) [21].

It had been reported that the antioxidant activity of plant materials is well correlated with the content of their phenolic compounds [22] especially; BHT (butylated hydroxytoluene) and Gallate are known to be effective antioxidants. The results obtained on total phenol of *Amanita caesarea* (2.2 mg per gram) is also corroborated with the reports of Anguiano et al [23] who reported 1.95mg /g total phenols in same species found in Europe.

Both the two Amanita species in the present study were found to contain significant amount of nutrients like protein, carbohydrates and reducing sugar along with good amount of phenol, ascorbic acid and flavonoids. Hence, in growing demand of food and pharmaceutical industries, such wild edible mushrooms can be important candidates. This is for the first time; wild edible *Amanita caesarea* and *A. loosii* were observed, collected and subjected to preliminary nutritional and biochemical analysis. A detailed biochemical and analytical profiling on amino acid content, fatty acids and phenol content may give more positivity to its edibility.

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