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NUTRITIONAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF INDIGENOUS EDIBLE MUSHROOM *AGARICUS HETEROCYSTIS*

M. Manimozhi* and V. Kaviyarasan

Centre for Advanced Studies in Botany, University of Madras,
Guindy Campus, Chennai 600025, Tamil Nadu
*Corresponding author: Email: mani26_micro@yahoo.com

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ABSTRACT:
Mushrooms have been cultivated worldwide for commercial purposes. However, little research has been done to ascertain the nutritional and antibacterial properties of indigenous edible mushroom in India.The proximate nutritional composition ofindigenous edible mushroom *Agaricus heterocystis* was determined by using various laboratory analyses. The proximate nutrient contents of crude protein, crude fibre, crude fat, carbohydrate, soluble sugars, ash, mineral elements (K, Na, P, Ca, Zn, Fe, Cu, Mn, Mg) were analysed.The analysis indicated that the indigenous edible mushroom contained rich sources of crude protein (32.23 ± 0.89%), crude fibre (19.7 ± 0.09%) and carbohydrate (48.55 ± 0.21%). The mushroom studied had good amounts of minerals. Content of fat was low (2.90 ± 0.03%).The research proves that *Agaricus heterocystis* is a nutritional mushroom and has great value to health. Antibacterial activity of various solvent extracts of *Agaricus heterocystis* was tested against six species of bacteria:*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Bacillus subtilis*and *Micrococcus luteus.*The methanol extract exhibited maximum activity. It showed narrow spectrum active against gram-negative bacteria and strongly inhibited gram-positive bacteria *Micrococcus luteus,Staphylococcus aureus* and *Bacillus subtilis.*

Keywords: *Agaricus heterocystis*, proximate nutritionalcomposition,antibacterial activity

[I] INTRODUCTION

Man has been hunting for the wild mushrooms since antiquity[1]. During the early days of civilization, mushrooms were consumed mainly for their palatability and unique flavours[2,3]. Present use of mushrooms is totally different from traditional because, lot of research has been done on the chemical composition of mushrooms, which revealed that mushrooms can be used as a diet to combat diseases. Many genera of mushrooms are edible and are rich in essential nutrients such as carbohydrates with low fat and oil content, proteins, vitamins, mineral, fibres and various amino acids.One-third of the iron in the mushrooms is in available form [4].The protein content of mushrooms has been reported to be twice that of vegetables and four times that of oranges and significantly higher than that of wheat [5–7]. Mushrooms are now marketed along major highways and urban centres. They are also relatively much cheaper than beef, pork and chicken that contain similar nutrients.Due to their high content of vitamin, protein and minerals, they are considered as “poor man’s protein”.Due to the low fat and oil content, they are recommended as good source of food supplement for patients with cardiac problems or at risk with lipid-induced

disorders.They are also recommended to diabetic and anaemic persons, owing to their lowcarbohydrate and high folic acid content.Rolfe and Rolfe [8] mentioned that the mushroomslike *Agaricus campestris, Morchella esculenta, Helvellacrispa, Hydnum coralloides, Hypoxylon vernicosum* and*Polyporus mylittae* were used much earlier in India.In general, the fruiting bodies of mushrooms contain about 56.8% carbohydrate, 25.0% protein, 5.7% fat and 12.5% ash on a dry weight basis[9].Protein contents vary between 4 and 9% in *Auricularia* sp. and between 24 and 44% in *Agaricus* sp. Their energy value also varies according to species, which is about equal to that of an apple.

Mushrooms also possess bioactive natural products that are antitumor,antiviral and antibacterial. They contain many different bioactive compounds with diverse biological activity depending on the way they prepared and consumed[10]. In recent years,multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation forced scientists for searching new antimicrobial substances from various sources that are the good sources of novel antimicrobial chemotherapeutic agents[11].Benderet al.'s[12] culture extracts of *A. bisporus* and other *Agaricus* sp.showed antibacterial activity against *Staphylococcus saprophyticus*.

To the best of our knowledge, no research has been available on nutritional and antimicrobial activity of an indigenous edible mushroom *Agaricus heterocystis*.Therefore, the aim of the present work is to evaluate nutritional and antimicrobial potential of the test fungi.

[II] MATERIALS AND METHODS

2.1. Mushroom

The wild edible mushroom *Agaricus heterocystis*, indigenously collected and identified, was cultivated under laboratory condition (CAS in Botany, University of Madras, Chennai, India) to produce fruitbody using compost. The mushroom cultivated and harvested was shade dried at room temperature. Dried samples were crushed by using a mortar with pestle and stored in pre-cleaned polyethylene bottles until the analysis started.Dried mushroom sample (50 g) was extracted by stirring with 500 ml of boiling water for 3 h and filtered through Whatmann No. 4 filter paper. The filtered extract was then subjected in rotor evaporator to dryness and stored at 4°C for further use.

2.2. Proximate nutritional composition

The proximate analysis (carbohydrates, fats, proteins and ash) of mushroom powder were determined by using AOAC[13] methods. Crude protein (N × 6.25) was determined by micro Kjeldahl method described by Pearson [14], in which the sample was digested with a known quantity of acid. The digested material was distilled after the addition of alkali. The released ammonia was collected in 4% boric acid. The resultant boric acid, which now contained the ammonia, was then titrated against 0.1 N HCl, manually. The nitrogen content thus determined was multiplied by a factor of 6.25 to arrive at the amount of crude protein.

Crude fat content of the samples was determined by extracting a known weight of powdered plant sample with petroleum ether, using by Soxhlet. The ash content was determined by combusting the mushroom material in silica crucibles in a muffle furnace at 535 ± 150°C for 3 h. Fibre content was estimated by the method of Van [15].

The carbohydrate content was calculated by subtracting the contents of crude ash, fat, fibre and protein from dry matter. Soluble sugars were determined as follows: The powdered samples were extracted with boiling water for 30 min, absolute alcohol were added to precipitate the soluble sugars. The precipitated polysaccharides were collected by centrifugation at 5000 rpm for 10 min in a bench centrifuge and subsequently dried at 60°C to remove residual ethanol. Soluble sugars were determined by the phenol–sulphuric reaction described by Whistler and Wolfrom [16].

Minerals were determined by atomic absorption spectrophotometer (AAS-6800, Shimadzu,

Japan) after dry-ashing the samples [13]. Phosphorus was determined by using the molybdovanadate method [13].

2.3. Statistical analysis

Each experiment was carried out thrice for each parameter and we got three replications ($n = 3$) from which we derived the mean values and standard error (SE) [17].

2.4. Antimicrobial activity

The following strains of bacteria were used:*Escherichia coli* (ATCC 25992), *Pseudomonas aeruginosa*(ATCC27853), *Salmonella typhi*(clinical isolate),*Staphylococcus aureus* (ATCC 12598), *Micrococcus luteus* (clinical isolate),and *Bacillus subtilis* (ATCC 6633).

Using the polarity difference of varioussolvents, dry mushroom powder (60–100 mesh) was extracted at 60°C, in order, by hexane, chloroform, methanol, ethyl acetate and boiled water. The correspondingextraction fraction for each solvent was obtained. Each extract was dissolved in dimethyl sulphoxide (DMSO) at a concentrationof 50 mg/ml and stored at 4°C.Antimicrobial activity of the crude extracts was tested according to Ndyetabura[18] by agar well assay methods as previously described by Rojas et al.[19] and Moshi et al.[20].Bacterial cultures were grown at 37°C for 24 h in Nutrient Broth. The culture suspensions were adjusted by comparing against 3.0 McFarland. The prepared agar plates were inoculated with 100 µl bacteria culture by spreading evenly over the surface of agar plate, using an ethanol flamed glass Drigalsky spatula (spreader). Un-inoculated untreated agar plate was incubated at 37°C for 24 h before use, to ensure sterility. Wells of 5 mm in diameter and 4 mm in depth were made on the agar, using a sterile cork borer. For each test microorganism, 25 µl of each extract and of control were pipetted into different wells [19]. The wells were then labelled to correspond with the code numbers of the test crude extracts and controls. The treated plates were stored in a refrigerator at 4°C for at least 6 h to allow diffusion of theextracts into the agar while arresting the growth of the test microbes. The plates were then incubated for 24 h at 37°C. The test was carried out intriplicates. Antimicrobial activity was determined by measuring the diameters of zones of inhibition in mm using callipers.The means of the diameters of zones of growth inhibitions for the treatments were calculated and recorded.

[III] RESULTS

3.1. Proximate nutritional composition

The results of the proximate and mineral composition are shown in Table-1. It shows that the dominant compounds are protein, fibre and carbohydrates and the contents are32.23 ± 0.89, 19.7 ± 0.09and 48.55%, respectively. Crude fat and ash contents are 2.90 and11.42%,respectively. Of the mineral analysed, the potassium, sodium, phosphorus, calcium content are 422 ± 0.49, 39 ± 0.23,327 ± 0.29, 81 mg/100 g and the iron, copper, manganese, zinc, magnesium content are 39 ± 0.32, 3.72 ± 0.02, 0.8 ± 0.01, 1.9 ± 0.09, 39 ± 0.31 mg/100g, respectively.

Parameters	Results obtained
Ash (%)	11.42 ± 0.12
Fat (%)	2.90 ± 0.03
Protein % (*N* × 6.25)	32.23 ± 0.89
Carbohydrate (%)	48.55 ± 0.21
Crude fibre (%)	19.7 ± 0.09
Sodium (mg/100 g)	39 ± 0.34
Potassium (mg/100 g)	422 ± 0.93
Calcium (mg/100 g)	81 ± 0.87
Iron (mg/100 g)	39.0 ± 0.32
Copper (mg/100 g)	3.72 ± 0.17
Zinc (mg/100 g)	1.9 ± 0.09
Magnesium (mg/100 g)	39 ± 0.31
Manganese (mg/100 g)	0.8 ± 0.05

[Table-1]: Proximate nutritional composition of *A.heterocystis*

3.2. Antimicrobial activity

The antimicrobial effect of different solvent extracts of *A. heterocystis*was tested against three species of gram-positive bacteria,three species of gram-negative bacteria. Among the different solvent extract, methanolic extract showed more effective inhibitory activity against bacteria. As summarized in Table-2, *A. hetercystis*methanolic extracthad a narrow antibacterial spectrum against gram-negativebacteria and strongly inhibited the

growth of thegram-positive bacteria tested, including *B. subtilis*. Themaximal zones of inhibition ranged from 6 to 18 mm.The most susceptible bacterium was *M.luteus*for both boiled and raw extract with a diameter of 14 ± 1.2 and 18 ± 1.03 mm, respectively. Against *P.aeruginosa*, both the extractsof *A. heterocystis*showed antibacterial activity but theactivity range was less in boiled extract (2 ± 0.25 mm) whencompared with raw mushroom extract (7 ± 0.13 mm). Thismight be due to the influence of temperature that disturbedthe compound, which is responsible for the activity.

S no.	Test organisms	Hexane	Chloroform	Methanol	Ethyl acetate	Boiling water
1	*E. coli*	2 ± 0.01	4 ± 0.12	8 ± 0.01	6 ± 0.03	7 ± 0.16
2	*P. aeruginosa*	–	–	7 ± 0.13	4 ± 0.04	2 ± 0.25
3	*S. typhi*	–	2 ± 0.14	13 ± 0.19	8 ± 0.1	3 ± 0.13
4	*S. aureus*	3 ± 0.23	5 ± 0.16	16 ± 0.12	12 ± 0.32	11 ± 0.17
5	*B. subtilis*	–	–	12 ± 0.18	7 ± 013	8 ± 0.29
6	*M. luteus*	4 ± 0.12	7 ± 0.09	18 ± 1.03	6 ± 0.02	14 ± 1.2

[Table-2]: Antibacterial activity of different organic solvent extract of *A. heterocystis* (zone of inhibition in mm)

Activity expressed in millimeter—mm concentration, (–) no activity.

[IV] DISCUSSION

4.1. Proximate compositions

In the samples, the dominant compounds were protein, fibre and carbohydrates. Some edible fungi were highly valued as a good source of protein and their protein contents usually range from 19 to 35% of dry weight [21], from 15.4 to 26.7% [22], from 16.5 to 59.4% [23]. Here, protein content in *A. heterocystis*(32.23%) seemed to have a normal protein content compared with other edible mushrooms. These relatively high protein values obtained can enrich human diet especially in villages where meat is rare and expensive. It would appear then that gram for gram of mushroom contain more protein than either potato or cabbages (can supplement diet). The protein content of mushroom is known to be highly variable due to strain of some species, tissue type and stage development, substrate and method analysis. However, inspite ofall these high values, mushrooms are still inferior in protein to such standard protein sources as meat, fish and eggs. It is also unlikely that in spite of the relatively high protein values that mushroom can serve as a sole source of protein. Crude fibre content in this mushroom (19.1%) was quite high in comparison with many other edible mushrooms (3–32%) [21, 24].The fairly high level of fibre in the mushroom was a desirable characteristic since fibre plays an important role in human diet. Obviously, the fruit bodies of *A.heterocystis*were a good source of fibre. Carbohydratecontent of *A.heterocystis* (48.55%) was similar to those reported by Crisan and Sands [21] and Kalač [23] in the range 44.0–74.3% and 16.4–75%,respectively. Soluble sugars in edible mushrooms were highly regarded as biologically or medically active compounds and were used as functional food ingredients ornutraceuticals [25]. The content was 7.07% for this mushroom. Crude fat and ash contents here were 2.90 and 11.42%, respectively. These results were similar to those obtained by Crisan and Sands [21], Yang et al.[22]and Kalač [23]in several edible mushrooms. From the results shown in Table-1, the macronutrient profile, in general, revealed that *A.heterocystis* had rich sources of protein and fibre and had low amount of fat. This high protein and low fat characteristic of the edible wild mushrooms has been previously reported by many workers[26, 27].

4.2. Mineral elements

The metal content in the mushrooms are mainly affected by acidic and organic matter content of their ecosystem and soil[28]. The uptake of metal ions in mushrooms is in many respects different from other plants. For this reason, the concentration variations of metals depend on mushroom species and their ecosystems[29]. In general, most of the mushrooms studied had good amount of minerals including trace minerals.K, P, Na and Mg constitute about 56–70% of the total ash content of the mushrooms [30],whereas potassium alone forms 45% of the total ash. Abou-Heilah et al. [31]found that content of potassium and sodium in *A. bisporus*

was 300 and 28.2 ppm, respectively.The concentration of sodium is relatively low and is of very great nutritional benefit to the consumer, a finding that has been corroborated by Vetter [32]. It is known that adequate iron in a diet is very important for decreasing the incidence of anaemia. The iron values of *A. heterocystis*are in agreement with those reported in the literature[33, 34]. Copper concentrations in the accumulating mushroom species are usually 100–300 mg/kg of dry matter, which is not considered as a health risk [34,35]. Copper contents in mushrooms, higher than those in vegetables, should be considered as a nutritional source of the element. Nevertheless, for people, bioavailability from mushrooms was reported to be low, due to limited absorption from the small intestine. Copper contents found in this study are in agreement with results reported in the literature [33]. Mushrooms were known as zinc accumulator and sporophore, substrate ratio for Zn ranges from 1 to 10 mg kg^{-1}[36]. The zinc values of *A.heterocystis*are in agreement with the reported values of the literature. Minerals generally in the diet are required for metabolic reactions, transmission of nerve impulses, rigid bone formation and regulation of water and salt balance among others.

4.3. Antibacterial activity

The increasing trend of resistance to the antibiotics in current use has drawn the attention of researchers to natural alternative treatments of bacterial infections as potential sources of novel antimicrobial agents. Gram-negative bacteria are reported to be resistant against most antibacterial agents as a result of the more complicated nature of their cell wall compared to gram-positive bacteria[37–39]. The antimicrobial activity of the extract *A. heterocystis*showed prominent result against gram-positive organismswhen compared with gram-negative organisms. The antimicrobial properties of the *A. heterocystis*confirm previous studies that mushroomspossess antimicrobial effects[40,41]. Similar antimicrobial potentials have been observed in the culture extracts *Agrocybe* sp. [42, 43] and juice of *L. edodes*[44].

[V] CONCLUSION

On the whole, the mushrooms studied were found to be a good source of protein, fibre and minerals.The results from the present analysis allow a direct comparison of the chemical composition of *A. heterocystis* with those of other mushrooms species.In conclusion, the edible mushroom *A. heterocystis* consumed has essential medicinal properties. Boiling orcooking did not dilute or reduce the medicinal properties.Hence, it is necessary to identify the biological andpharmacological potential of mushrooms especiallyedible mushrooms that are collected indigenously and cultivated locally or sold in local and international market.The production and marketing of mushrooms and their products is vital for an economic importance. Therefore, it is also necessary to intensify research in identifying and isolating different varieties of mushrooms having nutraceutical and medicinal properties and to commercialize their production and marketing, which will boost the food industry and createemployment especially in villages.

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