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MOLECULAR CHARACTERIZATION AND BIOCHEMICAL ANALYSIS OF SCHIZOPHYLLUM COMMUNE FROM AYODHYA, INDIA

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Abstract

Schizophyllum commune is very common in India and is found throughout the year. The study also brings to light the medicinal value of Schizophyllum commune apart from its rich nutritional value. The biochemical analysis was performed during the study which included estimation of phenolics, flavonoids and antioxidant activity. The scope of mushroom is limitless. The present study throws light on the potentiality of mushrooms in the study area. With proper infrastructural development and awareness, these mushrooms can be judiciously exploited for the greater good of the society. The ethnic people can be encouraged to work in this field by making them aware of the nutritional and medicinal benefits of mushrooms and how it can be an income generator for them. Mushrooms are nature's gift to humans.

Keywords: *Schizophyllum commune, Nutritional, Medicinal, Antioxidants, Ayodhya.*

INTRODUCTION

Fungi referred from Kingdome fungi which has broadly divided into two categories i.e., Macrofungi and Microfungi. Macrofungi refer as mushroom and has a fruiting body (Singh & Singh, 2022). Macrofungi are cosmopolitan in nature and occur seasonally in various habitats all over the world. India being a mega diverse nation and fortunate enough to have favorable agro-climatic conditions, abundance of bio wastes, cheap labor and very rich biodiversity of fungi (Borkar *et al*. 2015). Since the beginning of civilization, mushrooms have been regarded as the most prized food in comparison to other vegetables because of its unique taste and flavor (Chang and Miles, 1992). Though around 2000 species of macro-fungi are reported to be edible, but approximately 25 species are accepted as food globally and very few have attained the level of commercial item (Bonatti *et al*., 2004). *Agaricus bisporus*, *Pleurotus* species, *Auricularia* species, *Lentinula edodes* and *Volvariella volvacea* are some of the most accepted edible mushrooms globally. Mushroom research has gained prominence due to the role played by mushrooms in food and pharmaceutical industries (Wasser, 2002). Taxonomic studies of mushrooms have been mainly based on studying the morphological traits like shape, size and color of caps and gills (Lee *et al*., 2006), which sometimes fails to identify some mushrooms correctly.

Wild Edible Mushrooms (WEMs) are popularly consumed as food and used as medicines in many African and Asian countries (Boa, 2004). They content important nutrients like proteins, fibres, minerals and polysaccharides required for healthy diet and play important role in human health because of the presence of bioactive compounds (Barros *et al*., 2007; Ferreira *et al*., 2007). The secondary metabolites in mushrooms like terpenes, polyphenols, steroids etc exhibits bioactive properties which plays an important role in securing the health of consumers (Cheung *et al*., 2003; Barros *et al*., 2007). These secondary metabolites like flavonoids and anthocyanins show pharmacological activities like antimicrobial, immuno-modulatory, antioxidant, antifungal, anti-inflammatory, anticancer etc (Ramesh and Pattar, 2010; Keles *et al*., 2011). Generally, the antioxidant protection systems possessed by organisms for defense against the free radicals caused by oxidative enzymes and chemical compounds

are not sufficient (Mau *et al*., 2001). Naturally available antioxidants are studied extensively for protection from cell damage caused due to oxidative stress in organisms and these mushrooms are sources of natural antioxidants (Cazzi *et al*., 1997; Mau *et al*., 2004). In recent times, antioxidant and antimicrobial activities of mushrooms garnered attention due to the increasing awareness in human health (Singdevsachan *et al*. 2013). It is reported that bioactive compounds have already been extracted from numerous mushrooms like *Schizophyllum commune, Trametes versicolor*, *Lentinula edodes*, *Ganoderma lucidum* and more (Wasser and Weis, 1999). Mushrooms have already been extensively studied in the western countries, while in tropical countries like India especially the North-East India is less explored and it is high time, explorations need to be carried out to conserve this valuable natural resource. India is a country with rich fungal biodiversity which is a boon for the nation (Ajith and Janardhanan, 2007).

Schizophyllum commune is an edible fungus of the family Schizophyllaceae belonging to division Basidiomycota. This mushroom species is commonly referred as the split gill fungus. It causes white rot decay of wood. This mushroom is gaining importance not only because of its edibility but also for its medicinal value. This research highlights the biochemical composition of *Schizophyllum commune* collected from Ayodhya, India and also the morphological characterization of the mushroom.

MATERIALS AND METHODS

Sample collection

For the present study, the mushroom sample was collected during field explorations from Ayodhya (study area), India. The morphological characters like fruiting body color; size, shape and color of the stipe, pileus and lamellae; presence of rings or annulus; spore print color etc. were recorded. For identification of mushrooms, standard manuals and keys were consulted (Guarro *et al.*, 1999; Philips, 2006; Das, 2009; Singh *et al*., 2014; Mortimer *et al*., 2014; Monika, 2022;).

BIOCHEMICAL ANALYSIS

Sample Extraction

The mushroom sample was either sun dried or oven dried at 70-75^{o}C for 6-12 h. The samples were extracted according to the methods and procedure followed for each estimation.

Moisture Content

The moisture content was estimated as given below:

$$\text{Moisture Content (\%)} = \frac{\text{Loss in Weight}}{\text{Weight of Sample}} \times 100$$

Dry Matter Content

The dry matter content was estimated as given below:

$$\text{Dry Matter Content (\%)} = \frac{\text{Final Weight}}{\text{Weight of Sample}} \times 100$$

Total Protein

Lowry's protocol (Lowry *et al*., 1951) was employed for estimation. Bovin Serum Albumin (BSA) used as the standard for calculating the protein in the samples. About 0.5 g of mushroom sample was used to get extract using 10 ml of 0.1 M phosphate buffer (*p*H 7.4). Then 5 ml of the reagent mixture which is prepared by mixing 2%, w/v sodium carbonate (prepared in 0.1 N sodium hydroxide solution) and 1%, w/v copper sulphate (prepared in 1%, w/v potassium sodium tartarate solution) was added to 0.1 ml of

the sample. The solution was then incubated for 10 min at RT then 0.5 ml of 1 N Folin- Ciocalteau reagent was added and incubation in the dark for 20-30 min. The absorbance of the solution was measured at 660 nm using UV-Vis spectrophotometer (ELICO Double Beam SL 210).

Total Carbohydrate

Phenol Sulphuric Acid method (Dubois *et al*., 1956) was followed for quantification of total carbohydrate. To 0.1 ml of the sample, 1 ml phenol solution (5%, v/v) and 5 ml of H_2SO_4 (96%, v/v) were added. The volume of the test sample made 10 ml with pure water and mixed well followed by incubation for 20 min at 25-30°C in water bath. The absorbance was measured at 490 nm against glucose as standard.

Reducing Sugar

Reducing sugar was determined following DNS method (Miller, 1972). To 0.1 ml of sample extract, 3 ml DNS reagent (Take 1 g NaOH, 19.2 g Sodium Potassium Tartarate, 1 g DNS powder, 0.05 g Sodium Sulphite and 0.2 g Phenol crystals for preparing 100 ml of DNS reagent) was added and the mixture was kept for 5 min in a boiling water bath. Rochelle salt solution (1 ml, 40%) was added and the volume of the test sample made 10 ml with pure water. After cooling, the absorbance was calculated at 510 nm against glucose as standard. The non-reducing sugar content was determined as the difference of total carbohydrate and reducing sugar and expressed as g/100g of dry weight.

Crude Fiber

The amount of crude fiber was estimated by the procedure of AOAC (2000). The samples were treated with petroleum ether to remove fat and boiled with 0.255 N sulphuric acid (200 ml) for 30 min. The solution was filtered and boiled with 0.313 N sodium hydroxide solution (200 ml) for 30 min followed by filtration and the residue washed with boiling 1.25% sulphuric acid (25 ml), 50 ml of H_2O and 25 ml of alcohol. The residue was transferred to ashing dish (pre-weighed, W1) and dried for 2 h at 130±2°C, then cooled the dish in a dessicator and weighed (W2). The dish was heated at 660±15°C for 30 min and cooled and weighed again. The difference in weights represents the amount of crude fiber. The amount of crude fiber calculated by using the formula:

$$\text{Crude Fiber Content (\%)} = \frac{\text{Loss in Weight}}{\text{Weight of Sample}} \times 100$$

Ash Content

The powdered mushroom sample (about 1.0 g) was ashed in muffle furnace in previously ignited and cooled crucible of known weight at 550±5°C for 1 hr. The crucible and its contents were then cooled in desiccators and reweighed. The rate of the incombustible residue accounts for ash content (AOAC, 2000).

$$\text{Ash Content (\%)} = \frac{\text{Weight of Ash}}{\text{Weight of Sample}} \times 100$$

Determination of Antioxidant Activity, Total Phenolic Content, Total Flavonoid Content

Preparation of Methanol Extract

Methanolic extraction procedure was used for sample extraction. About 10 g of dried mushroom was grinded using liquid nitrogen. The powder extract was mixed with methanol (100 ml) and incubated under continuous stirring for 24 h at 150 rpm. The process was repeated till the solvent became

colorless. The extract was filtered and the filtrate was used directly for antioxidant analysis.

2, 2-Diphenyl-1-picrylhydazyl (DPPH) radical scavenging assay

The DPPH radical scavenging assay was used for analysis of antioxidant activity (Aoshima *et al*., 2004). In 0.1 ml of mushroom methanol extract, 2.9 ml DPPH (0.1 mM) was added and then vortexed. The reaction mixture was incubated at 30°C for 30 min and the absorbance measured at 517 nm. Trolox was the standard for the evaluation of antioxidant activity. Inhibition of free radical by DPPH was calculated as:

DPPH scavenging activity (%) = $[(A_o-A_1/A_o) \times 100]$

Where- A_o is the control reaction absorbance and A_1 is the sample absorbance.

TOTAL PHENOLIC CONTENT

The presence of polyphenols was calculated as per Folin-Ciocalteu method (Singleton and Rossi, 1965) with slight modifications. To 0.1 ml filtered extract, 1 ml of the Folin-Ciocalteu reagent were added. Then incubation was done for 5 min followed by addition of 2 ml saturated sodium carbonate (75 g L^{-1}). Incubation of reaction mixture for 90 min was done in the dark. The resulting absorbance was checked at 765 nm. Quantification was performed based on gallic acid standard in methanol (80%, v/v) and the total phenolic content was expressed in mg of gallic acid equivalents (GAE)/g of the sample extract.

TOTAL FLAVONOID CONTENT

The presence of flavonoids was estimated by colorimetric method of Sahreen *et al*. (2010). To 0.3 ml extract solution, 3.4 ml methanol (30%), 0.15 ml sodium nitrite (0.5 M) and 0.15 ml aluminium chloride hexahydrate (0.3 M) were added. The solution was incubated for 5 min at RT and 1M NaOH solution was added. The absorbance was checked at 510 nm. Quercetin was the standard used for this experiment and the results expressed in mg of quercetin equivalents (QE)/g of the sample extract.

STATISTICAL ANALYSIS

All the experiments were repeated thrice and the results represent the mean of three replicates. Data expressed as Mean ± Standard Deviation.

RESULTS AND DISCUSSION

The fruiting bodies of *Schizophyllum commune* are leathery, pileus 1-5 cm. wide, fan shaped when laterally attached, saucer shaped when centrally attached, white to grey colored; stipe absent; gill folds hairy, split length wise radiating from the point of attachment and rolling back to cover the space between gills; the flesh is yellow and tough; spore print is white. It is generally saprobic in nature and found on branches of dead wood (Fig. 1). Based on 3 years of study, occurrence of *S. commune* is reported throught the year. The maximum frequency of occurrence reported in rainy season (Table-1).

Fig. 1: *Schizophyllum commune* Fr. collected from study site (Ayodhya)

Table-1: Seasonal Occurrence of *Schizophyllum commune*

Year	Rainy Season				Winter Season				Summer Season			
	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
2020	+	+	+	+	+	+	+	+	+	+	+	+
2021	+	+	+	+	+	+	+	+	+	+	-	-
2022	+	+	+	+	+	+	+	+	+	+	+	-

The biochemical analysis shows that *Schizophyllum commune* is highly nutritional and exhibits antioxidant activity (**Table-2**). Mushrooms are popular for their rich nutritional content like high protein, fibers, and minerals etc. Moreover, the edible mushrooms collected from the wild are considered nutritionally richer than the commercially available mushrooms (Barros *et al*., 2008). The above table shows the nutritional composition of *Schizophyllum commune* from Ayodhya (Ao *et al*., 2019).

Table-2: Biochemical analysis of *Schizophyllum commune* (at Dry Weight)

Moisture (%)	68.60±0.12	**Dry matter (%)**	30.20±0.24
Total Protein (g/100g)	25.12±0.22	**Total Carbohydrate (g/100g)**	06.31±0.17
Reducing sugar (g/100g)	02.13±0.04	**Non-reducing sugar (g/100g)**	03.02±0.02
Crude fiber (%)	11.74±0.06	**Ash (%)**	05.07±1.06
Total phenolic content (GAE/100g)	16.41±0.11	**Total flavonoid content (QE/100g)**	05.01±0.62
IC50 (µg/ml)	152.10±0.07		

Ash is referred to the inorganic residue or substance resulting from the incineration of dry matter or oxidation of organic matter in the powdered sample. Even though minerals represent only a small proportion of dry matter, sometimes less than 7% of the total, they play a significant role from a physicochemical and nutritional point of view. The value of ash content is important because it determines the minerals present in the food. In mushrooms, the mineral constituents are Cu, K, Zn, P, Fe, Na, Mo, Ca, Cd and Mg (Bano *et al*., 1981; Chang, 1982). It is reported that Na, K, Mg and P constitute

more than 56% of the ash content in mushrooms while potassium constitutes around 45% of the ash content (Li and Chang, 1982). The mineral composition of mushrooms varies according to species type, age, size of the basidiocarps and the substratum (Demirbas, 2001). The mineral content of WEM has been reported to be significantly more than the cultivated varieties (Mattilla *et al*., 2001; Rudawska and Leski, 2005). The presence of fibers is also an important characteristic in mushrooms as fibers are necessary in maintaining healthy and balanced diet in humans.

Generally, the presence of phenols and flavonoids indicates mushrooms as source of biologically active compounds and exhibits antioxidant, anticancer, anti-inflammatory, immuno-modulatory, anti-tumor, anti-viral, hypocholesterolemic and hypoglycemic properties which are important in pharmacology. The experiment performed during the study showed that the content of flavonoids was lower in comparison to phenolics. The methanolic extracts at different extract concentrations showed significant antioxidant activity. During the antioxidant activity experiment, the lower absorbance indicated that the mushroom specimen studied exhibited higher scavenging activity of the free radicals. The IC50 value of the mushroom sample was calculated for assessing the inhibition ability against DPPH free radicals. The IC50 is the exact amount of sample which is required to scavenge or destroy 50% of the DPPH radicals. It is expected that lower the value of IC50 higher is the ability of the samples to inhibit the free radicals. During the study, the mushroom *Schizophyllum commune* showed the presence of significant antioxidant activity. The current interest in natural antioxidant substances is due to restriction or ban in the usage of manufactured antioxidants (Branen, 1975). Antioxidant supplements or foods containing antioxidants helps the human body reduce oxidative damage. Antioxidants play an enormous role in managing the human health due to their scavenging ability (Elmastas *et al.*, 2007; Ferreira et al. 2007; Ao *et al*., 2019). Thus, mushrooms are considered as potential sources of natural antioxidants (Puttaraju *et al*., 2006; Oyetayo 2007). Many workers have already reported the presence of phenolics, flavonoids and antioxidant properties of mushrooms (Ramesh and Pattar 2010; Keles *et al*., 2011; Boonsong *et al*., 2016; Sanchez 2017; Ao *et al*., 2019). Moreover, it is said that the antioxidant potential of mushrooms is higher than most vegetables and fruits (Sanchez 2017).

CONCLUSION

The study brings to light the medicinal value of *Schizophyllum commune* apart from its rich nutritional value. Mushrooms are nature's gift to humans and has a limitless scope. The present study throws light on the potentiality of mushrooms in the study area (Ayodhya). With proper infrastructural development and awareness, these mushrooms can be judiciously exploited for the greater good of the society. The ethnic people can be encouraged to work in this field by making them aware of the nutritional and medicinal benefits of mushrooms and how it can be an income generator for them.

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