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Proximate and Antioxidant Activity of Mycelia of *Termitomyces microcarpus* and *Amanita loosii*

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Abstract

In the present investigations two mushrooms i.e. *Amanita loosii* and *Termitomyces microcarpus* was collected from the Similipal biosphere reserve of Odisha (India), identified and cultured *in vitro* culture conditions. Malt extract medium was used to develop these mushroom mycelia and subjected for their proximate nutritional composition along with the antioxidative characterization. Protein content was high in the mycelia of *T. microcarpus* whereas comparatively low in *A. loosii*. Carbohydrate concentration was also high in *T. microcarpus* (32.27±4.03 gm/100gm) when compared with *A. loosii* (6.94±0.39 gm/100gm). During the antioxidant analysis high radical scavenging effects was recorded in the *T. microcarpus* along with its corresponding AEAC i.e 74.92 % and 0.29 mg/gm respectively which is expected on account of high amount of antioxidant component like phenolics (1.02±0.16 mg/gm) and carotenoids. In all the aspect the comparative study between the mycelia of both the mushrooms reveals that *T. microcarpus* (2.09±0.32 mg/gm) proves to be a good source for nutritional as well as antioxidant components. This study can provide a basic knowledge for the development of therapeutically important compounds.

Keywords: Nutritional; Antioxidant; Mycelia; *Amanita; Termitomyces*

Abbreviations: DPPH: 2, 2-Diphenyl-1-picryl hydrazyl; AEAC: Ascorbic acid Equivalent Antioxidant Capacity; RSA: Radical Scavenging Activity

Introduction

Several edible mushrooms were studied by the researchers, in the search of novel therapeutic alternatives, and its results proved their potential bioactivity [1]. Mushrooms are rich sources of nutraceuticals [2-4] responsible for their antioxidant [5,6] antitumor [7], and antimicrobial properties [8,9] along with their other pharmacological characteristics [1,10], people are now paying much attention towards wild edible mushrooms since they are important dietary supplement due to their high nutritional value, including high protein and low fat contents [2]. Since last two decades scientists have spent much time in culturing the mushroom mycelium in order to get large amount of industrially important components [11].

Amanita species counts about 100 in number in Indian context. Mycorrhizal fungi in plant roots have indeed shown strong stimulation of the growth of their hosts [12,13]. Wild edible macrofungi plays an important ecological role, many of the leading species thrives symbiotically with trees and this mycorrhizal association sustains the growth of native forests and commercial plantations in temperate and tropical zones [14]. Many of the *Amanita* sp. are ectomycorrhizal in nature and are important as a food source for local people [15-17]. *Termitomyces* species, contribute as a significant food to tribals in the Western Ghats as well as from other regions of India along with west coast region [17,18]. A recent report by our research group suggests that *Amanita loosii* selected for the present studies possess appreciable source for the dietary supplements as well as antioxidants [17,19].

This is for the first time that the fruit body of *T. microcarpus* and *A. loosii* was collected, their corresponding mycelia was developed and subjected for the nutritional and antioxidant analysis. Reports regarding the mycelial characterization as presented in the present studies are very less among the scientific communities however no reports are available regarding this from the Odisha (India).

Materials and Methods

Collection and identification of mushrooms: Fully succulent *Amanita loosii and Termitomyces microcarpus* was collected from forest divisions of Odisha and brought to the site of identification, all macroscopic characteristics was taken into consideration like pileus, stipe, veil, ring, volva, lamellae and gills etc. Macro morphological as well as ecological characteristics of both the species were recorded and photographed in their natural habitats.

Preparation of master plate: Mushroom sample was cleaned manually and by using a dissecting blade which has been flame sterilized a segment of mushroom was taken out from the untouched parts carefully and placed in the malt extract agar media aseptically in laminar air flow. After the inoculation, it was incubated at 28°C for the proliferation of mushroom mycelia, subsequent cultures were done in order to get pure culture.

Inoculation: Agar discs of both mushrooms cultured on malt extract agar served as inoculum, they were taken out by cutting with sterilized cork borer (Figure 1). The inoculum was transferred to the malt extract broth media in triplicates. The inoculated culture flasks were incubated at 28°C for 10 days. After the complete growth of mushroom mycelia in culture flasks, mycelial mats were filtered and subsequently washed many a times with sterilized distilled water and air dried.

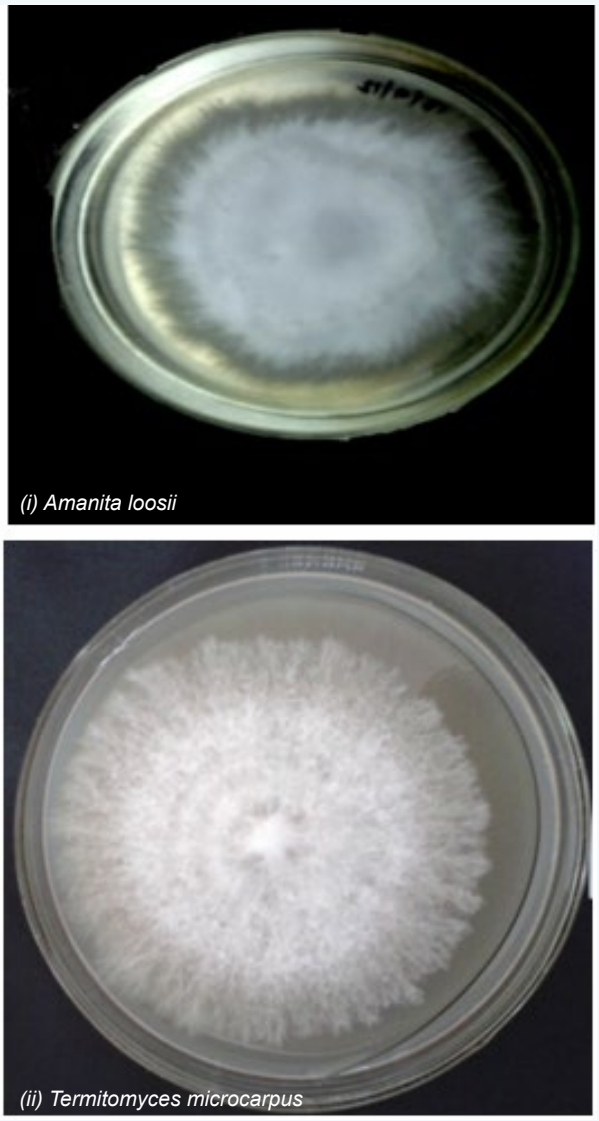

Figure 1: Representing Mycelial culture of Amanita loosii and Termitomyces microcarpus in Malt extract agar medium.

Nutritional analysis: Protein estimation in the mycelium was done by following the method given by Bradford. Estimation of total carbohydrate content was carried out by following phenol sulphuric acid method. Reducing sugars content was done by following DNS method. Non reducing sugars were calculated by subtracting the value of reducing sugars from total carbohydrate content.

Antioxidant analysis: One gm of fresh mycelium was extracted with 10 ml of methanol, for effective extraction it was stirred for 15 minutes and finally centrifuged at 3000 rpm for 20 minutes. Supernatant thus obtained was served as methanolic extract and kept at 4°C until further analysis. The DPPH free radical scavenging activity of methanolic extracts was estimated by spectrophotometric method. Ascorbic acid Equivalent Antioxidant Capacity (AEAC) value was estimated by calibrating the value of absorbance in standard ascorbic acid curve and expressed in terms of milligram per gram of the sample. The total phenolic content in the mycelium were determined through Folin-phenol method with slight modifications. Carotenoid content in the mycelia of both the mushrooms was estimated by following the method of Arnon.

Results and Discussion

Thousands of years ago, the fruiting body of higher fungi has been used as a source of food. The results of the proximate composition and estimated antioxidant value (expressed on fresh weight basis) of the mycelium of two wild edible mushrooms are shown in Table 1. Protein is considered as the building block of the organism, in the present findings *T. microcarpus* showed higher protein (5.26mg/gm) content than *A. loosii* (0.80 mg/gm). Carbohydrates, including polysaccharides are infact considered as the principal substrates of energy as well as they play important roles nutrition and therapeutics [20-22]. Investigations by [23] Jagadeesh et al. found that carbohydrate content ranges from 34.75 and 38.9% in mycelia and fruit body of *V. bombycina*. In the present studies carbohydrate content was recorded highest in *T. microcarpus* whereas *A. loosii* showed lesser carbohydrate content (Table 1). [24] Manjunathan and Kaviyarasan and found 58.05 % and 55.8% carbohydrate in cultivated variety and in wild variety of mushrooms respectively which was higher than the carbohydrate in the two mushroom mycelia studied. However the amount of carbohydrate in the fruit body of *A. loosii* was found to be 23.61 % as reported in our previous report which is more than the mycelium of same organism in present report [17]. Whereas the amount of carbohydrate in the mycelia of *T. microcarpus* is comparable with the results of [25] Atri et al. where investigations covered fruit body of the same. Reducing sugars was found much more in *T. microcarpus* (44.30±4.75mg/gm) than *A. loosii* (13.88±3.09 mg/gm). *T. microcarpus* showed higher non reducing sugar content (27.84gm/100gm) than *A. loosii* (5.84gm/100gm).

Radical generated by DPPH is reduced by antioxidant components and shows direct relation with the antioxidative

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effects [26]; therefore for studying the radical scavenging activity (RSA) this assay is widely used even in regard of mushrooms [27-29]. DPPH free radical scavenging activity along with its corresponding AEAC value was highest in *T. microcarpus* than *A. loosii* (Table 1). DPPH free radical scavenging effects of the mycelia of the present mushrooms are found to be satisfactory when compared with the results of [30] Kalyoncu et al. Phenolic compounds form a major class of phytochemicals, which are responsible for inhibiting the oxidative damage caused by accumulation of free radicals generated [31]. Analysis of phenolic content revealed highest amount in *T. microcarpus* (1.02±0.16 mg/gm) as compared to *A. loosii* i.e. 0.45±0.05 mg/gm, which agree the results obtained by [32] Kim et al. and [4] Ribeiro et al. Carotenoids are responsible for various bioactivities which in turn provide many health enhancing properties [33-35] Carotenoid content was more in *T. microcarpus* (0.44±0.18 mg/gm) than the *A. loosii* (2.09±0.32 mg/gm).

Table 1: Nutritional and Antioxidant analysis mycelium of A. loosii and T. microcarpus

Sl. no	Parameters	*Amanita loosii*	*Termitomyces microcarpus*
1	Protein (mg/gm)	0.80±0.00	5.26±0.52
2	Carbohydrates (gm/100gm)	6.94±0.39	32.27±4.03
3	Reducing- sugars (mg/gm)	13.88 ±3.09	44.30±4.75
4	Non reducing sugars (gm/100gm)	5.84 ±0.57	27.84±3.78
5	DPPH scavenging (%)	36.19 ±1.34	74.92±3.87
6	AEAC value (mg/gm)	0.04±0.01	0.29±0.02
7	Phenolics (mg/gm)	0.45±0.05	1.02±0.16
8	Carotenoids (mg/gm)	0.44±0.18	2.09±0.32

Note: DPPH: 2, 2-Diphenyl-1-picryl hydrazyl; AEAC: Ascorbic acid Equivalent Antioxidant Capacity; ± is the average and standard deviation of three replicates.

Conclusion

According to the results of this study, it is clearly indicated that the methanolic extract of mushroom species has significant antioxidant activity against various antioxidant systems *in vitro*. Good extractability of therapeutically important antioxidants i.e., Phenolics and carotenoids contents in methanol and acetone respectively was also established in the present studies. Mushroom mycelia hence can be served as a good dietary supplement for providing adequate amount of proteins as well as cost effective and easily accessible source for natural antioxidants for multiple health benefits. Culturing mushroom mycelia in the submerged process of mushroom mycelia is very critical, some reports suggests that the culture broth becomes viscous due to mycelial growth, this causes obstructions in the diffusion of bioactive metabolites, but this case was not seen in the present media selected. Present findings might provide a reasonable description for the proximate composition and antioxidants in edible mushroom during submerged culture conditions. Further work is necessary on the isolation, purification of and mode of action of the active components. To the best for our knowledge, this is the first report of the proximate and antioxidant activity of these mushroom species from Odisha (India).

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