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Research Article

Antioxidant activity of some wild mushrooms from southern Western Ghats, India

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Abstract

Objective: To investigate the antioxidant activities of crude methanol extracts of 10 southern Western Ghats wild mushrooms. **Methods:** Crude methanol extracts from 10 mushrooms from southern Western Ghats, India were evaluated for their antioxidant activity by Nitric Oxide scavenging assay and DPPH scavenging assay. **Results:** The methanol extract of *Trametes versicolor* recorded maximum percentage of NO activity of 94% followed by *Tricholoma equestre* 92% at the concentration 50 µg/ml. However, the extract of *Gymnopilus junonius* (80.33%) and *Tricholoma equestre* (79%) tested against DPPH stable radicals reveals that the radical scavenging activity of wild mushrooms possessed excellent antioxidant capacity which increased with the increasing concentration of the extract. **Conclusions:** *Gymnopilus junonius, Tricholoma equestre* and *Trametes versicolor* have higher antioxidant activity showed both in Nitric Oxide scavenging assay and DPPH scavenging assay.

Key words: Antioxidant activity, macrofungi, Western Ghats, *Gymnopilus junonius, Tricholoma equestre*

INTRODUCTION

Natural products are chemical compounds derived from living organisms. The most prominent producers of natural products can be found within different groups of organisms including plants, animals, marine macro-organisms (sponge, corals and algae), and microorganisms (bacteria, actinomycetes, and fungi). The discovery of natural products involves isolation, structural elucidation and establishment of the bio-synthetic pathway of the secondary metabolites[1]. Alexander Fleming published his observation on the inhibition of growth of *Staphylococcus aureus* on an agar plate contaminated with *Penicillium notatum* in 1929 and penicillin was finally exploited and its clinical application was achieved during World War II, which is perhaps the most important discovery in the history of therapeutic medicine. This has shown an important route for the discovery of modern antibiotics. However, the first fungal-derived secondary metabolite in the history of natural product research is mycophenolic acid from *Penicillium glaucum* discovered in 1896 by Gosio. On the basis of these antibiotics discovery, the secondary metabolites of higher fungi have received an increasing attention worldwide since the 1940s[2].

The secondary metabolites are an area of considerable interest to scientists due to the structural diversity, complexity and various bioactivities of isolated compounds. Crude natural products had been used directly as drugs which were of low cost and important sources of traditional medicines. They also provided the basic chemical architecture for deriving semi-synthetic natural products [3].

Fungi is the second most diverse of all groups and is considered as a prime member of the other "mega-diverse" groups like insects, bacteria, arachnids and nematodes[4-5]. The number of fungal species that exist is estimated to be around 5.1 million[6-8]. The number of fungi recorded in India ex-

ceeds 27,000 species[9]. Fungi are among the most important groups of eukaryotic organisms that are well known for producing many novel metabolites which are directly used as drugs or function as lead structures for synthetic modifications[10-16].

Numerous reports made on the biodiversity of mushrooms in South India have opened up an avenue to study their biochemical composition and the bioactive compounds responsible for the healing potential. Several mushrooms are known to be the sources of various bioactive substances possessing antibacterial, antifungal, antiviral, antiparasitic, antioxidant, antiinflammatory, antiproliferative, anticancer, antitumour, cytotoxic antiHIV, hypocholesterolemic, antidiabetic, anticoagulant, hepatoprotective properties among others[17-20]. These mushrooms have been used as ethnomedicines by tribals for treatment of various diseases. Many mushrooms still remain unreported and their nutritional as well as health benefits are unknown to us. If discovered, some of them may have high clinical potential and serve as valuable sources of bioactive drugs with various applications. Hence, a detailed study on the biomolecules produced by the mushrooms is highly essential to ease the diseases of the suffering humanity.

Materials and Methods

Collection and preparation of mushroom species: Macrofungal species were collected from November 2013 to October 2014 from from southern Western Ghats in the Fingerpost Reserve Forest of North Zone of Nilgiri, Tamilnadu, India. In order to have wide range of species as possible, specimens were collected from forests, fields and woods. Identification of the mushrooms was done by comparing their morphological, anatomical and physiological traits with the standard description and the keys provided by the Directorate of Mushroom Research, Solan, India. The mushrooms were dried in the shade to prevent mushroom cells from sun light which destroy the cell and ground to powder using mortar and pestle.

Determination of antioxidant activity

Extraction: A quantity of 300mg of the dried powder of mushroom was extracted with 95% methanol (Merck). After that, the extract was evaporated in water bath at 50°C to obtain crude extract for antioxidant assay.

Reagents and chemicals: 5mM Sodium Nitroprusside, Griess reagent (1% sulphonil amide, 0.1% N 1-naphthylethylenediamine, 2% orthophosphoric acid), Phosphate buffer (pH- 7.4) and 0.5mM methanolic solution of DPPH.

Nitric Oxide scavenging assay procedure: Mushroom extract was dissolved in distilled water for this quantification. Sodium Nitroprusside (5mM) in standard phosphate buffer saline (0.025m, pH 7.4) was incubated with different concentration of methanol extract and the tubes were incubated at 29°C for 3 hours. Control experiment was conducted in an identical manner without the test compounds but with same amount of buffer. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates Nitric oxide which interacts with oxygen to produce Nitrite ions. After 3 hours, the incubated samples were diluted with 1 ml of Griess reagent. The absorbance of the colour developed during diazotization of Nitrite with sulphanilamide and its subsequent coupling with Napthylethylenediaminehydrochloride was observed at 550nm on spectrophotometer. Same procedure was done with ascorbic acid which was standard in comparison to methanol extract. The percentage inhibition was calculated using following formula and graph was plotted compared to standard.

Formula:

$$\% \text{ of inhibition} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

DPPH scavenging assay procedure

DPPH radical scavenging activity of extract was determined following the standard method[21]. An aliquot of 0.5ml of sample solution in methanol was mixed with 2.5ml of 0.5mM methanolic solution of DPPH. The mixture was shaken vigorously and incubated for 30 min in the dark at room temperature. The absorbance was measured at 517nm using UV spectrophotometer. Ascorbic acid was used as a positive control. DPPH free radical scavenging ability (%) was calculated using the formula,

Formula:

$$\% \text{ of inhibition} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

Results

Antioxidant power of the extracts

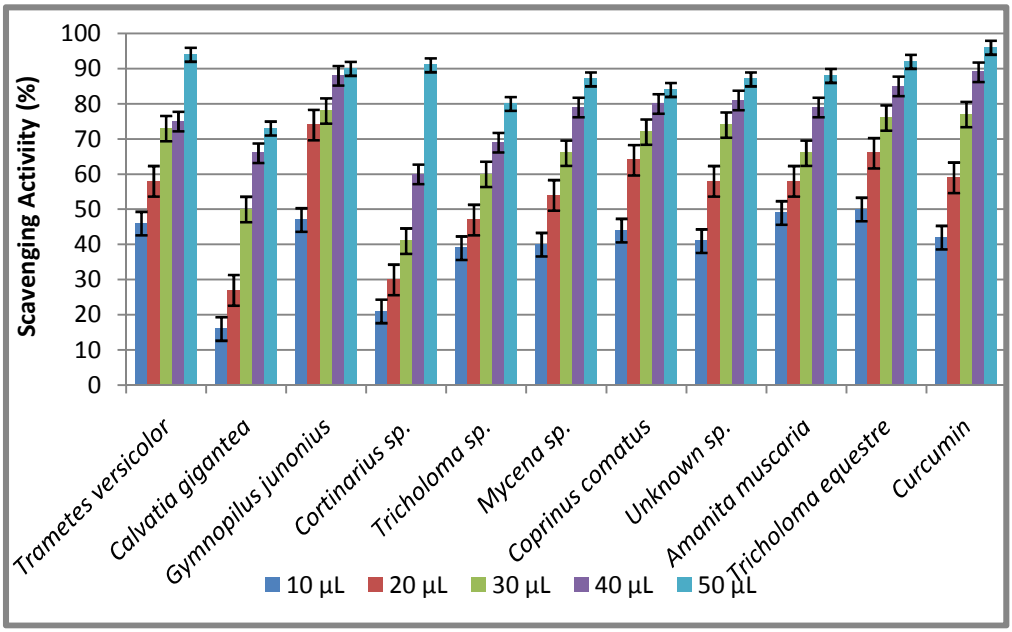
Antioxidant power of selected mushroom species (10 species) was evaluated using nitric oxide radical scavenging assay and DPPH radical scavenging activity.

Nitric oxide radical scavenging assay

The methanolic extract of wild mushrooms effectively reduced the generation of nitric oxide from sodium nitroprusside. Half maximal effective concentration (EC_{50}) value of methanol extract of mushrooms showed higher nitric oxide scavenging activity comparable with that of standard Curcumin (Table 1). Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured using Griess reagent. The methanol extract of *Trametes versicolor* recorded maximum percentage of NO activity of 94% at the concentration 50 µg/ml (Fig. 1).

Table 1: Nitric oxide scavenging activity of wild mushrooms

Concentration	10 µL	20 µL	30 µL	40 µL	50 µL	EC_{50} µL
Mushroom sample	**% INHIBITION**					
Trametes versicolor	46	58	73	75	94	13.33333
Calvatia gigantea	16	27	50	66	73	30.00
Gymnopilus junonius	47	74	78	88	90	11.11111
Cortinarius sp.	21	30	41	60	91	34.73684
Tricholoma sp.	39	47	60	69	80	22.30769
Mycena sp.	40	54	66	79	87	17.14286
Coprinus comatus	44	64	72	80	84	13.00
Unknown sp.	41	58	74	81	87	15.29412
Amanita muscaria	49	58	66	79	88	11.11111
Tricholoma equestre	50	66	76	85	92	10.00
Curcumin	42	59	77	89	96	14.70588

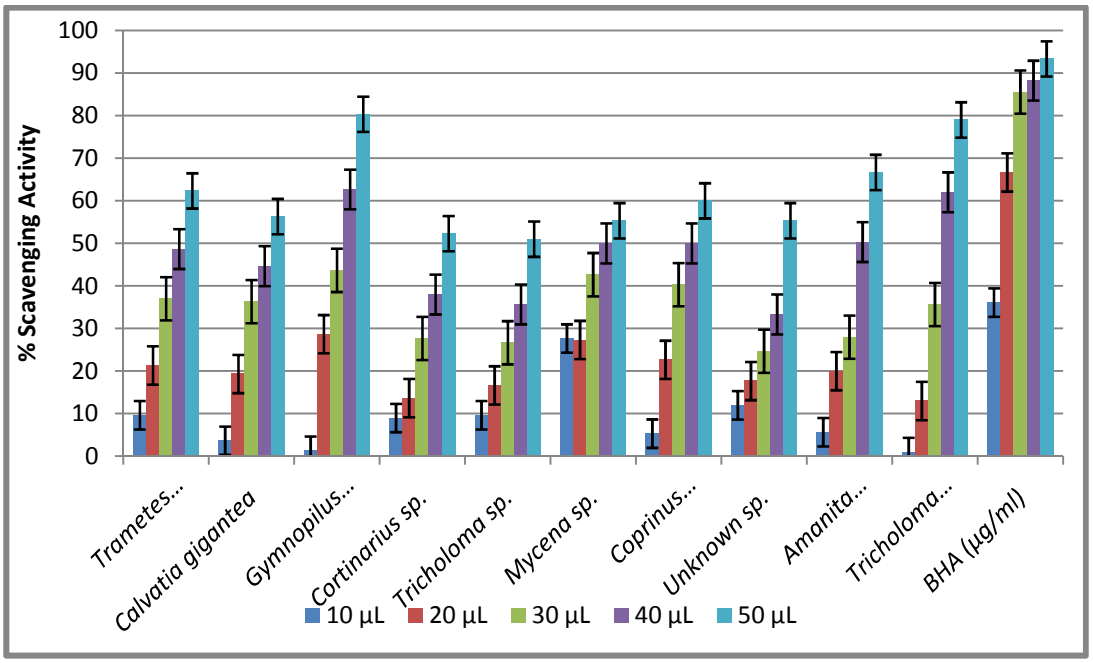


*Error bars with standard error

Fig. 1: Nitric oxide scavenging activity (µL / ml)

Table 2: DPPH radical scavenging activity of wild mushrooms

Concentration	10 µL	20 µL	30 µL	40 µL	50 µL	EC_{50} µL
Mushroom sample	**% INHIBITION**					
Trametes versicolor	9.67	21.33	37.00	48.67	62.33	40.81
Calvatia gigantea	3.67	19.33	36.33	44.67	56.31	44.66
Gymnopilus junonius	1.33	28.67	43.67	62.67	80.33	33.26
Cortinarius sp.	9.00	13.67	27.67	38.00	52.3	48.73
Tricholoma sp.	9.67	16.67	26.67	35.67	51.00	49.01
Mycena sp.	27.67	27.33	42.67	50.00	55.33	39.38
Coprinus comatus	5.33	22.67	40.33	50.00	60.00	40.29
Unknown sp.	12.00	17.67	24.67	33.33	55.33	47.79
Amanita muscaria	5.67	20.00	28.00	50.33	66.67	40.05
Tricholoma equestre	1.00	13.00	35.67	62.00	79.00	35.43
BHA (µg/ml)	36.11	66.67	85.56	88.23	93.33	25.78



*Error bars with standard error

Fig. 2: DPPH radical scavenging activity (µL / ml)

DPPH RADICAL SCAVENGING ACTIVITY

2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical compound has been widely used to test the free radical scavenging ability of various food samples; the antioxidant present neutralizes the DPPH by the transfer of an electron or hydrogen atom. The reduction capacity of DPPH could be determined by colour changes from purple to yellow read at 517 nm. The methanolic extract of wild mushrooms demonstrated H-donor activity in the present study. The DPPH radical scavenging activity of extracted material was detected and compared with standard antioxidant – butylated hydroxytoluene (BHA). The extract of *Gymnopilus junonius* and *Tricholoma equestre* tested against DPPH stable radicals spectrophotometrically which reveals that the radical scavenging activity of the methanol extract of wild mushrooms possessed excellent antioxidant capacity which increased with the increasing concentration of the extract (Fig. 2). The EC_{50} value of methanol extract

of *Gymnopilus junonius* was found at the concentration of 33.26 μg/ml (Table 2).

Discussion

Nitric oxide (NO) which is produced in mammalian system by an enzyme nitric oxide synthase (NOS) was first identified as an endothelium derived relaxing factor (EDRF)[22] and identified from vascular endothelium cells. It is now clear that NO is a widespread intracellular and intercellular signaling molecule involved in the regulation of diverse physiological and pathophysiological mechanisms in the cardiovascular system and the central and peripheral nervous systems and in immunological reactions[23-26] and has numerous roles in biological systems including vasodialation, regulation of blood pressure, inhibition of platelet aggregation and adhesion, inhibition of of neutrophil adhesion, and neuromodulation in the CNS. It is antioxidant, antithrombotic and a second messenger of insulin[27-28].

The Radical scavenging activity of methanolic extracts of mushrooms was tested using a free radical, DPPH. DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition[29]. It was reported that *Lactarius deliciosus* revealed better antioxidant properties than *Tricholoma portentosum* (lower EC_{50} values), which is in agreement with the higher content of phenols found in the *Lactarius deliciosus*[30].

Some work has been done regarding antioxidant activity and NOS activation properties of mushrooms[31-32]. Several species of mushrooms contain a wide variety of antioxidant molecules which have made mushrooms attractive as nutritionally beneficial foods and as a source for drugs development[33]. Mushroom flavonoids can act as free radical scavengers to terminate the radical chain reactions that occur during the oxidation of triglycerides in the food system[34]. Free radical scavenging activity of methanolic extract of *Pleurotus florida* mushroom has significant antioxidant activity[35]. A large number of antioxidant components such as phenolic compounds, flavonoids, carotenoids and vitamins C and E have been isolated from the fruit bodies of *Pleurotus* mushrooms[36].

Mycelia of a number mushrooms have recently been successfully used for the development of novel pharmaceutical products. It was examined that the antioxidant potential of aqueous-ethanol extract of cultured mycelia of the morel mushroom, *Morchella esculenta* (Morchellaceae) by super oxide, hydroxyl, nitric oxide, 2,2'-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS)[37]. Radical assays as well as inhibition of lipid peroxidation tests showed that the extract efficiently scavenged all these radicals and also inhibited lipid peroxidation. Antioxidant activity of methanolic extract of *Hypsizygus ulmarius* shows superior activity against free radicals and it may serve as a good pharmacological species[38].

Radical scavenging activity as well as the polyphenolic content and anthocyanins content of five edible mushrooms (*Boletus edulis, Agaricus bisporus, Agaricus bisporus* var. *avellaneous, Pleurotus ostreatus, Lentinula edodes*) was investigated. Various levels of DPPH scavenging activity was observed in the studied five edible mushroom species[39]. The potential of methanol and chloroform extracts of *Pleurotus citrinopileatus* mushroom by DPPH radical scavenging assay showed higher percentage of inhibition for methanol and moderate inhibition for chloroform depending on concentration of antioxidant molecules[40]. Similarly, the methanolic extract of 24 wild edible mushroom species showed significant antioxidant activity against various *in vitro* antioxidant systems[41].

It was also found that the methanolic extract from *Agaricus blazei* showed a high scavenging ability[42]. Likewise it was mentioned that the methanolic extract from *Agrocybe cylindracea* strain B scavenged DPPH radicals[43]. Wild mushrooms like *Coprinus comatus*[44], *Pleurotus citrinopileatus*[45], *Hypsizigus marmoreus*[46], *Agaricus blazei*, *Agrocybe cylindracea*, and *Boletus edulis*[43] showed a moderate scavenging ability.

The bioactive and antioxidant potential of 20 wild culinary mushroom species being consumed by the people of northern Himalayan regions have been evaluated. *Gymnopilus junonius* is reported to have higher antioxidant FRAP activity with least EC_{50} values (0.78 mg/mL)[47]. In the present study the same mushroom showed EC_{50} values 11.11111 ◎L (0.01111 mL) with Nitric Oxide scavenging activity and EC_{50} values 33.26 ◎L (0.033 mL) with DPPH scavenging assay.

Conclusion

The antioxidant potential of 10 selected mushroom species were evaluated using nitric oxide radical scavenging assay and DPPH radical scavenging activity. 2 species - *Gymnopilus junonius* and *Tricholoma equestre* showed better antioxidant activity. *Trametes versicolor* showed better antoxidant activity in NO scavenging assay but showed comparatively poor activity with DPPH scavenging assay. Thus, further study on these two wild mushroom species (*Gymnopilus junonius* and *Tricholoma equestre*).

Conflict of interest statement

The authors declare no conflict of interest.

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