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In-vitro antibacterial and antioxidant properties of some non-cultivated and cultivated edible mushrooms

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ABSTRACT

Three species of cultivated edible mushrooms *Pleurotus ostreatus, Pleurotuspulmonarius, Agaricusbisporus*, and four species of non-cultivated edible mushrooms *Termitomycesmicrocarpus, Termitomyces heimii, Termitomyceseurhizus* and Phallus indusiatus were selected for the present study. Antioxidant activity was evaluated by using free radical scavenging activity and antimicrobial activity was performed by agar-well diffusion technique. The antimicrobial ability of mushroom extracts was compared with the standard antibiotics(streptomycin) against Staphylococcus aureus (MTCC-96), Escherichia coli (MTCC-739), and Bacillus sp. (MTCC-5981). The scavenging effects of mushroom species and standards were assessed through DPPH. The extracts of the mushroom fruiting body showed potential antimicrobial activities (Inhibition zone between 3.1–12.2 ±0.5 mm) against the selected strains. The scavenging activity of mushroom extracts as measured through percentage inhibition, decreased in the order of *T.heimii>P. indusiatus> T.eurhizus> T.microcarpus > P.ostreatus > P.pulmonarius > A.bisporus* and were93.32%,92.6 %,90.51 %, 78.12 %, 42.89 %,33.27 % and 30.58 % respectively. The present study shows that tested mushroom species demonstrate antioxidant and antimicrobial activities.

Keywords: Mushroom, Antioxidant activity, Antibacterial activity, Inhibition zone, Scavenging activity

INTRODUCTION

Mushrooms are seasonal fungi that live in a variety of ecological niches (Venkatachalapathi and Paulsamy,2016).Mushrooms possess high contents of qualitative proteins, crude fibers, minerals, and vitamins(Lindequist *at el.*,2005). Apart from their nutritional potential, mushrooms are also sources of physiologically beneficial bioactive substances that promote good health (Valverde*at el.*,2015).There are supposed to be 1,40,000 species of mushrooms on earth, but only 22,000 are known, and only a small portion (5% of them) have been

studied (Alves et al., 2012). Therefore, there is much to understand about mushrooms' properties and potential uses. Numerous plants, lichens, and mushroomshave been demonstrated with potential antioxidant activities in the last few decades (Marijana, *et al.,* 2012). Wild non-cultivated mushrooms are traditionally used in many countries both for food and medicine (Isildak Ömer, *et al.*2004). The mycelium and fruiting bodies both contain compounds with a mixture of antioxidant and antibacterial properties (Oyetayo *et al.,*2009; Jeng-Leun *et al.,*2004; Lillian *et al.,*2007; Isabel *et al,*2007). The regular use of mushrooms is dependent on three basic tenets: first, they are used as a staple food because of their nutritional benefits. Second, fruiting bodies are recognized for their complex nature andthirdly, therapeutic benefits for mushrooms are quite popular (Poucheret *et al.,*2006; Mehmet *et al.,* 2011; Xiaofei *et al.,*2011).

Mushrooms are rich sources of natural antibiotics; in these, the cell wall glucans are well known for their immunomodulatory properties, and many of the extracellular secretions by the mycelium secondary metabolites combat bacteria (Benedict and Brady, 1972; Barros *et al.,* 2007). Antioxidants are substances that protect biological systems from the highly damaging effects of actions or reactions that lead to excessive oxidation (Krinsky,1989). Many synthetic antioxidants have adverse effects (Grice, 1988) and are assumed to be the cause of liver damage and carcinogenesis, examples include butylated-hydroxy-anisole and butylated-hydroxy-toluene. As a result, in nutrition applications, natural antioxidants are preferable. Natural components, such as vitamins A, C, and E, carotenoids, flavonoids, and other simple phenolic compounds, have been shown to defend the body against oxidative damage (Dorothy, 1995).

The majority of rural tribal communities depend on wild mushrooms as a source of nutrition. Thus, the present study aims to screen mushrooms with antibacterial and antioxidant properties so asto identify a potent nutrient food supplement.

METHOD

Collection of wild mushrooms (the Dang region of South Gujarat)

Selected areas of forests were visited for three consecutive years (2019–21) during rainy and winter sessions (June to December). The samples were collected in sterile paper bags and brought into the laboratory (Passari *et al.,* 2016). Digital pictures of mushrooms were taken in natural habitat as well as under laboratory conditions. All collected specimens were characterized morphologically for proper identification.

Test mushrooms

Threespecies of cultivated edible mushrooms *Pleurotus ostreatus, Pleurotus pulmonarius*, *Agaricus bisporus,* and four species of non-cultivated edible mushrooms *Termitomyces microcarpus, Termitomyces heimii, Termitomyces eurhizus and Phallus indusiatus* were selected for the present study.

Preparation of mushroom extracts

Five grams of each mushroom were extracted by stirring and heating with 100 ml of methanol at 60°C and filtering through Whatman's No.1 filter paper. The residue was re-extracted twice. The residual solvent extract was removed under reduced pressure at 4°C using a rotary evaporator (Sudha *et al.,* 2008). The resulting organic extracts were stored under refrigeration for antimicrobial and non-enzymatic antioxidant properties.

Study of Antimicrobial activity of mushrooms

Test microorganisms *Staphylococcus aureus (*MTCC-96*), Escherichia coli (*MTCC-739*)*and *Bacillus sp. (MTCC-5981)* were used to determine the antibacterial properties of the organic extracts of all test mushrooms. All the tested pathogens were obtained from the Microbial Type Culture Collection (MTCC).

Mushroom extracts were tested for antibacterial activity by agar-well diffusion technique (Mondal *et al.*,2013) with little modifications. An overnight culture of each bacterial isolate was inoculated into 20ml nutrient broth and incubated for 6 hours at 37˚C. The nutrient agar was then poured to the sterile petri dishes and allowed to stand for 10 minutes. Then 0.5ml of inoculum was added on to the agar plate so as to attain a confluent growth. The wells were filled with 100 µl of cell free extracts of mushrooms. The petridishes were incubated for 24hrs. at 37˚ C. A standard antibacterial antibiotic streptomycin served as positive control. All experiments were carried out in triplicates and the zone of inhibition was measured in mm.

Study of Antioxidant activity of mushrooms

The antioxidant assay was carried out by using microdilution method (Ren *et al.,*2014), and modified as follows. Methanolic extract (2ml) was mixed with solution containing 2 ml of 0.05 mM DPPH. The reaction was allowed to proceed for 30 min in the dark. The reduction of DPPH radicals was evaluated by measuring the absorption at 515 nm. Radical scavenging activity (RSA) as evidenced by DPPH discoloration was calculated using the equation below:

$$RSA = [A_{DPPH} - (A_M - A_E)] / A_{DPPH} \times 100$$

where A_{DPPH} is the absorbance of the DPPH solution; A_M is the absorbance of the mixture consisting of both DPPH and extract at a particular concentration; and A_E is the absorbance of the corresponding extract solution.

RESULTS AND DISCUSSION

Antibacterial activity of mushroom extract based on agar-well diffusion technique

Preliminary antibacterial testing of the above mushrooms produced a zone of inhibition (2.1-16 mm). The results are shown in Table 1. *Pleurotusostreatus* showed the highest zone of inhibition for all tested organisms, followed by *Pleurotus pulmonarius* against Staphylococcus aureus, *Bacillus sp.*. However, *phallus indusiatus* was more potent against *E.coli.*

Antioxidant activity

All mushroom extracts showed scavenging activity of DPPH radicals (Fig. 1). *A. bisporus* demonstrated the lowest ability to reduce DPPH at 30 %. The maximum scavenging percentage for *T. heimii* was 93 %. The scavenging percentages of *P. ostreatus and P. pulmonarius* extracts were less than 40%. The scavenging percentage for *P. indusiatus, T. eurhizus and T. microcarpus* was 92 %,90 % and 78 % respectively.

The scavenging effects of methanolic extracts from mushroom species and standards on the DPPH radical decreased in the order of *T.heimii*> *P. indusiatus*> *T. eurhizus*> *T.microcarpus*> *P.ostreatus*> *P. pulmonarius*> *A.bisporus and were* .93.32%,92.6 %,90.51 %, 78.12 %, 42.89 %,33.27 % and 30.58 % respectively. Results indicated that methanolic extracts of mushroom species have a noticeable effect on scavenging free radical.

Table 1. Antibacterial properties of mushrooms.

Name of the tested organism	Mushroom	Inhibition zone (mm)
Staphylococcus aureus	*Pleurotus ostreatus*	12.2 ±0.5
	Pleurotus pulmonarius	10.2 ±0.5
	Termitomyces microcarpus	6.1 ±0.5
	Termitomyceseurhizus	4.1 ±0.5
	Agaricus bisporus	4.0 ±0.5
	Termitomycesheimii	3.8 ±0.5
	Phallus indusiatus	3.4 ±0.5
Bacillus sp.	*Pleurotus ostreatus*	10.2 ±0.5
	Pleurotus pulmonarius	8.8 ±0.5
	Termitomyces microcarpus	5.1 ±0.5
	Agaricus bisporus	4.0 ±0.5
	Termitomyceseurhizus	3.2 ±0.5
	Termitomycesheimii	3.1 ±0.5
	Phallus indusiatus	3.1 ±0.5
E. coli	*Pleurotus ostreatus*	9.8 ±0.5
	Phallus indusiatus	8.2 ±0.5
	Pleurotus pulmonarius	7.2 ±0.5
	Termitomyces microcarpus	5.2 ±0.5
	Agaricus bisporus	4.2 ±0.5
	Termitomyceseurhizus	4.2 ±0.5
	Termitomycesheimii	3.9 ±0.5

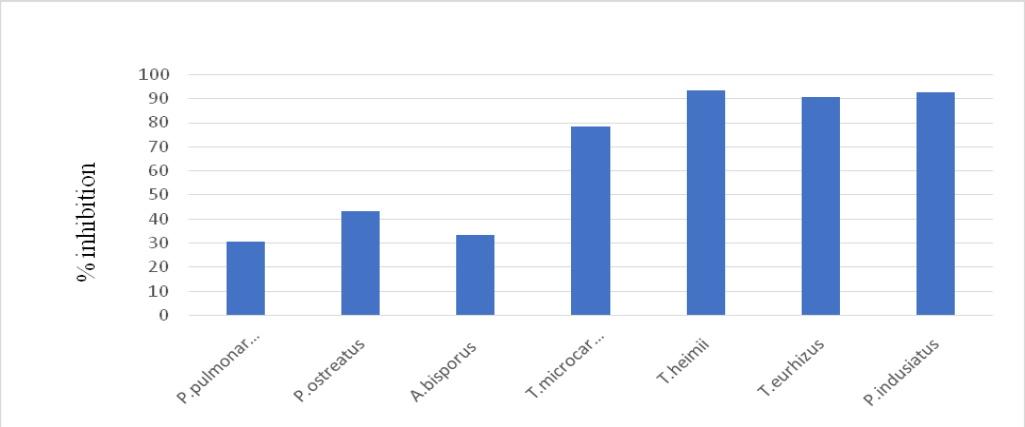

Fig.1 DPPH scavenging activity of different mushroom extracts.

The extracts of seven mushroom were screened for antibacterial activity against three bacteria using agar-well diffusion technique. All mushroom extracts at the concentrations of 1 mg/mL demonstrated anti-bacterial activity by the agar-well diffusion technique against all tested organisms (*E. coli,* B. subtilis, *and S. aureus)*. However, the standard antibiotic (streptomycin)at the concentration of 1 mg/mL, was more effective in generating inhibition zones around agar-well for all tested bacteria. *Pleurotus ostreatus* showed greater inhibition zone with an average diameter of 12.2 mm and was observed to be the most potent to kill the gram-positive bacteria. The smallest inhibition zones with an average diameter of 3.1 mm were produced by *T. heimii and P. indusiatus* on agar inoculated with the strain of *B. subtilis*.

The intensity of the antimicrobial effect depends on the species of mushroom, its concentration and the tested organism (Hugo, *et al,* 1987). The sensitivity of Gram-positive bacteria to mushroom extracts agrees with previous studies. Other researchers looked into some mushroom extracts' antimicrobial properties. Similar results were reported for some other mushroom. For example, (Mondal, *et al,* 2013) found similar antimicrobial activity for methanol extracts from *Pleurotus ostreatus* and *Agaricus bisporus.*

The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in methyl alcohol was at 517 nm. The decrease in absorbance of DPPH radical is caused by antioxidants, because the reaction between antioxidant molecules and the radical progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants (Duh *et al,*1999; Chang *et al,* 2002).

These results indicated that methanolic extracts of mushroom species were capable of acting as primary antioxidants and free radical scavengers. The main catalyst for the autoxidation chain of fat, free radicals, may react with methanolic extracts of wild edible mushroom species, inhibiting the chain reaction. (Frankel1991; Gordon 1990).

According to the results of this study, the tested mushroom species can be used as an easily accessible source of natural antioxidants and as a possible food supplement in pharmaceutical industry. Further it can be stated that tested mushroom extracts have a strong antimicrobial activity in-vitro. Additional studies should be done on the isolation and characterization of new compounds from mushrooms, which are responsible for antioxidant and antimicrobial activity.

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