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Research Article
In vitro Antioxidant and Antimicrobial Activity of Polysaccharides Extracted from Edible Mushrooms *Pleurotus florida* and *Agrocybe cylindracea*

[1]S. Selva Kumar, [2]S. Shankar and [3]Shanmugavadivelu Chandra Mohan

[1]Department of Chemistry, Rajah Serfoji Government Arts College, 613 005 Thanjavur, Tamil Nadu, India
[2]Department of Chemistry, A.V.V.M. Sri Pushpam College, Poondi, 613 503 Thanjavur, Tamil Nadu, India
[3]Division of Phytochemistry, Shanmuga Centre for Medicinal Plants Research, 613007 Thanjavur, Tamil Nadu, India

Abstract

Background and Objective: Mushrooms are potent source of biologically active substances which have beneficial effect on human health. Many mushrooms contain polysaccharides which may boost human immune system. In the present study, polysaccharides were extracted and purified from two edible mushroom species, *Pleurotus florida* and *Agrocybe cylindracea* and their *in vitro* antioxidant activity and antimicrobial activity were investigated. **Materials and Methods:** Mushrooms samples were collected at Ooty, Tamil Nadu. The bacterial strains of *Staphylococcus aureus*, *Klebsiella pneumonia* and *Escherichia coli*, obtained from Microbial Type Culture Collection (MTCC) centre, Chandigarh. **Results:** The antioxidant activities of extracted polysaccharides were evaluated by Fe^{2+}-chelating ability and hydroxyl radical scavenging assay. The Fe^{2+}-chelating activity and hydroxyl radical scavenging assay of *P. florida* polysaccharides (P1) and *A. cylindracea* polysaccharides (P2) increased gradually with the increasing polysaccharide concentrations. The extracted polysaccharides showed stronger antioxidant activity compared to butylhydroxytoluene (BHT). **Conclusion:** The results of the present investigation clearly demonstrated that *P. florida* and *A. cylindracea* polysaccharides could be used as a good food supplements that would support for both natural antioxidant and antimicrobial action. The synthetic antioxidants such as BHT need to be replaced because of their potential health risks and toxicity . Therefore, the attention now has been shifted towards naturally occurring antioxidants. Results from this study indicated that extracted polysaccharides could be potentially used as a natural antioxidant.

Key words: Edible mushrooms, polysaccharide, *in vitro* antioxidant, BHT, antimicrobial

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Corresponding Author: Shanmugavadivelu Chandra Mohan, Division of Phytochemistry, Shanmuga Centre for Medicinal Plants Research, 613007 Thanjavur, Tamil Nadu, India Tel: +917871411720

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antioxidants which scavenge Reactive Oxygen Species (ROS) are found in a variety of foodstuff and are commonly referred to as scavengers. The ROS is thought to be a major factor in aging, hardening of arteries, diabetes, cancer and tissue injury of skin[1]. Antioxidants that scavenge free radicals play an important role in cardiovascular disease, aging, cancer and inflammatory disorders. In addition, these naturally occurring antioxidants can be formulated to give nutraceuticals, which can help to prevent oxidative damage from occurring in the body[2]. Plants are rich source of antioxidants and considerable amount of data have been generated on antioxidant properties of food plants around the globe[3,4]. However, traditionally used mushroom species await such screening, so we aimed to evaluate the antioxidant potential of polysaccharides extracted from edible mushrooms *Pleurotus florida* and *Agrocybe cylindracea*. Many synthetic antioxidant components have shown toxic and/or mutagenic effects. There is a widespread need to be replaced because of their potential health risks and toxicity[5,6]. Therefore, the attention now has been shifted agreement that the synthetic antioxidants such as butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) towards naturally occurring antioxidants. The present study was conducted to investigate the antimicrobial and antioxidant potential of polysaccharides extracted from mushroom species *Pleurotus florida* and *Agrocybe cylindracea.*

MATERIALS AND METHODS

The fruiting bodies of *Pleurotus florida* and *Agrocybe cylindracea* (Fig. 1) were collected from Ooty, Tamil Nadu and dried soon after harvest in a convection dryer.

Extraction and purification of polysaccharides: The extraction and purification of fruiting body polysaccharides were carried out according to the method of Yan *et al.*[7]. In short, fruiting bodies powder of mushrooms suspended in distilled water at the established volume and then stirred for extraction at the established temperature and time. The mixture was then centrifuged at 5000 rpm for 20 min. The supernatant was concentrated to 1/5 of the original volume by evaporation at 45°C. Three volumes of absolute ethanol were added into the filtered solution and produced polysaccharide precipitate. The precipitated materials were collected by centrifugation at 5000 rpm for 20 min and then purified using the classic Sevag method[8]. Extraction process shown in flow chart (Fig. 2). Under these conditions, the maximal polysaccharide yield was 8.45 g/100 g.

Assessment of antioxidant activity of polysaccharides

Assay of Fe^{2+}-chelating activity: The chelating activities of polysaccharides and BHT on Fe^{2+} were determined as reported by measuring the formation of ferrous iron-ferrozine complex[9]. Different concentrations of polysaccharides or BHT (0.2, 0.4, 0.6, 0.8 and 1.0 mg mL^{-1}) were mixed with 3.7 mL of deionized water and then reacted with $FeSO_4$ (2 mM, 0.1 mL). The reaction was allowed to proceed for 30 sec. After 0.2 mL of 5 mM ferrozine was added, the solution was mixed, left to stand for 10 min at room temperature and then the mixture absorbance was determined at 562 nm. The chelating activity of Fe^{2+} was calculated by using the following equation:

$$\text{Chelating ability (\%)} = \frac{(A_c - A_s)}{A_s} \times 100$$

where, A_c was the absorbance of the control (deionized water, instead of sample) and A_s was the absorbance of the test sample mixed with reaction solution.

Fig. 1(a-b): Images of (a) *Pleurotus florida* and (b) *Agrocybe cylindracea*

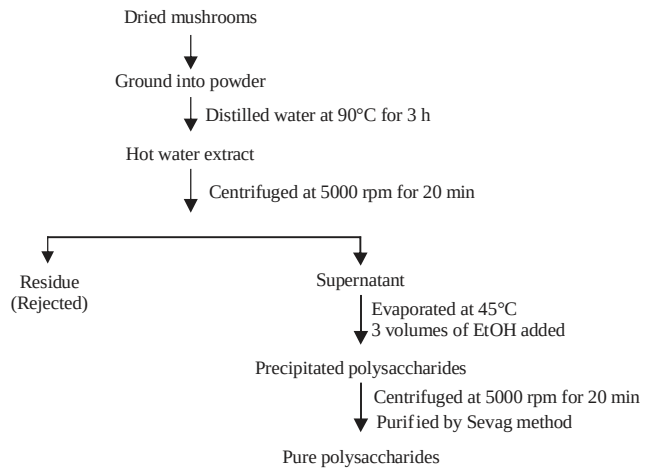

Fig. 2: Flow chart for the isolation and purification of polysaccharides

Assay of hydroxyl radical scavenging activity: The scavenging activity of polysaccharides on hydroxyl radical was determined by the method previously reported by Sun and Kennedy[10]. Reaction mixtures in a final volume of 1.0 mL contained deoxyribose (60 mM), phosphate buffer (pH 7.4, 20 mM), ferric trichloride (100 μM), ethylene diamine tetraacetic acid (100 μM), H_2O_2 (1 mM) and different concentrations of polysaccharides or BHT (0.2, 0.4, 0.6, 0.8 and 1.0 mg mL^{-1}). The reaction solution was incubated for 1 h at 37°C and then 1 mL of 1% thiobarbituric acid and 1 mL of 20% (v/v) HCl were added to the mixture. The mixture was boiled for 15 min and cooled on ice. The absorbance of the resulting mixture was measured at 532 nm. The scavenging activity of hydroxyl radical was calculated according to the following equation:

$$\text{Scavenging activity (\%)} = \frac{(A_c - A_S)}{A_c} \times 100$$

where, A_c was the absorbance of the control (deionized water, instead of sample) and A_S was the absorbance of the test sample mixed with reaction solution.

Statistical analysis: The experimental results were expressed as Means±Standard Deviation (SD) of triplicates. Statistical analysis was performed using Fisher's F-test and $p<0.05$ was regarded as significant.

Assay of antibacterial activity

Disc preparation: Six millimeters (diameter) discs were prepared from Whatmann No. 1 filter paper. The discs were sterilized by autoclave at 121°C. After the sterilization the moisture discs were dried on hot air oven at 50°C. Then discs were mixed with chemical compounds separately and control discs were prepared.

Collection of test microorganisms: The bacterial strains of *Staphylococcus aureus*, *Klebsiella pneumonia* and *Escherichia coli*, obtained from Microbial Type Culture Collection (MTCC) centre, Chandigarh.

The dried polysaccharides extract (20 mg) was dissolved in 1 mL of 20% DMSO (Dimethyl sulphoxide). From this stock solution 10 μL of respective extracts were added to the disc (0.2 mg $disc^{-1}$) individually and aseptically. Each disc contained 0.2 mg of extract. Then the discs were allowed for drying at room temperature. After drying they were used for screening the antibacterial activity.

Antibacterial activity test was carried out following the modification of the method originally described by Bauer *et al.*[11]. Muller Hinton agar was prepared and autoclaved at 15 lb pressure for 20 min and cooled to 45°C. The cooled media was poured on to sterile petri plates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The various solvents/extract prepared discs were placed individually on the each petri plates and also placed control and standard discs. Gentamicin antibiotic discs (30 mg $disc^{-1}$) were used as standard. The plates were incubated at 37°C for 24 h. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in millimeter.

RESULTS AND DISCUSSION

Fe^{2+}-chelating activity of polysaccharides: The previous study reported some transition metals, such as Fe^{2+}, Cu^{+} and

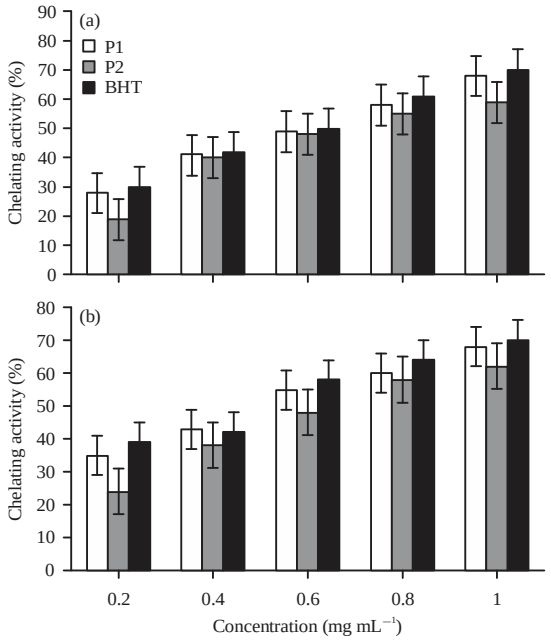

Fig. 3(a-b): (a) Fe^{2+}-chelating activity and (b) Hydroxyl radical scavenging activity of *Pleurotus florida* polysaccharides (P1) and *Agrocybe cylindracea* polysaccharides (P2). Values are Means±SD of three independent determinations

Co^{2+} could trigger process of free radical reaction to magnify the cellular damage[12]. Among these metal ions, Fe^{2+} was known as the most powerful pro-oxidant due to its high reactivity, which accelerated lipid oxidation by breaking down hydrogen and lipid peroxidase to reactive free radicals via the Fenton reaction[9]. In this study, ferrozine could react with Fe^{2+} to form red complexes of ferrozine-Fe^{2+}. When there was other chelating agent, the ferrozine-Fe^{2+} formation was disrupted which resulted in decrease of red complexes. The Fe^{2+}-chelating activity of antioxidant could be estimated by measuring absorbance of reaction solution at 562 nm. The Fe^{2+}-chelating activity of *Pleurotus florida* polysaccharides (P1) and *Agrocybe cylindracea* (P2) polysaccharides are shown in Fig. 3a. The Fe^{2+}-chelating activity of P1 and P2 ranged 28.06-68.44 and 19.42-59.24% at 0.2-1.0 mg mL^{-1}, respectively. Polysaccharides possessed stronger ($p<0.05$) Fe^{2+}-chelating activity, which is nearly equal to BHT in a dose-dependent manner.

Hydroxyl radical scavenging activity of FB polysaccharides: Among all reactive oxygen radicals, hydroxyl radical was known as the most powerful radical. It could induce severe damage to adjacent biomolecules in the body, which result in cell damage that caused ageing, cancer and several other diseases[13]. The removal of hydroxyl radical was probably one of the most effective ways to defence oxidative damage of human body. Therefore, hydroxyl radical scavenging activity was considered to be one of the most important antioxidant mechanisms. Hydroxyl radical scavenging activity of *Pleurotus florida* polysaccharides and *Agrocybe cylindracea* polysaccharides are shown in Fig. 3b. With the increase of concentration, the scavenging abilities of FB polysaccharides and BHT on hydroxyl radical also increased. Hydroxyl radical scavenging activity of P1 and P2 ranged 35.14-68.32 and 24.02-62.54% at 0.2-1.0 mg mL^{-1}, respectively. At the concentration range of 0.2-1.0 mg mL^{-1}, polysaccharides showed stronger scavenging activities nearly equal to BHT. These results suggested that polysaccharides were better natural antioxidant than BHT in scavenging hydroxyl radical.

Table 1: Antibacterial activity of extracted polysaccharides and standard (Gentamicin)*

	Zone of inhibition (mm in diameter)			
Bacteria	C	S*	P1	P2
Escherichia coli	-	15	17	20
Klebsiella pneumonia	-	17	21	25
Staphylococcus aureus	-	08	07	08

Antibacterial activity: Polysaccharides are composed of repeating monosaccharide units linked by glycosidic bonds. They function primarily as structural storage compounds in plants and algae. Algal polysaccharides and sulphated polysaccharides have been used successfully for pharmaceutical and dietary applications. Their mechanism of antibacterial action is proposed to be due to glycoprotein-receptors present on the cell-surface of polysaccharides which bind with compounds in the bacterial cell wall, cytoplasmic membrane and DNA. This results in increased permeability of the cytoplasmic membrane, protein leakage and binding of bacterial DNA[14-16]. Polysaccharides, such as fucoidan and laminarin have been successfully used in drug delivery as oral antibiotics to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* and to prevent the adhesion of *Helicobacter pylori* biofilms in gastric mucosa.

Disk diffusion method (Kirby-Bauer method) allowed for preliminary evaluation of antibacterial activity of both polysaccharide fractions. The results of antibacterial tests for polysaccharides extracts P1 and P2 are presented in Fig. 4 and Table 1. Both polysaccharide extracts were demonstrated to possess antibacterial activity against Gram-positive bacterial strains *Staphylococcus aureus* and Gram-negative bacterial strains *Klebsiella pneumonia* and *Escherichia coli*. The

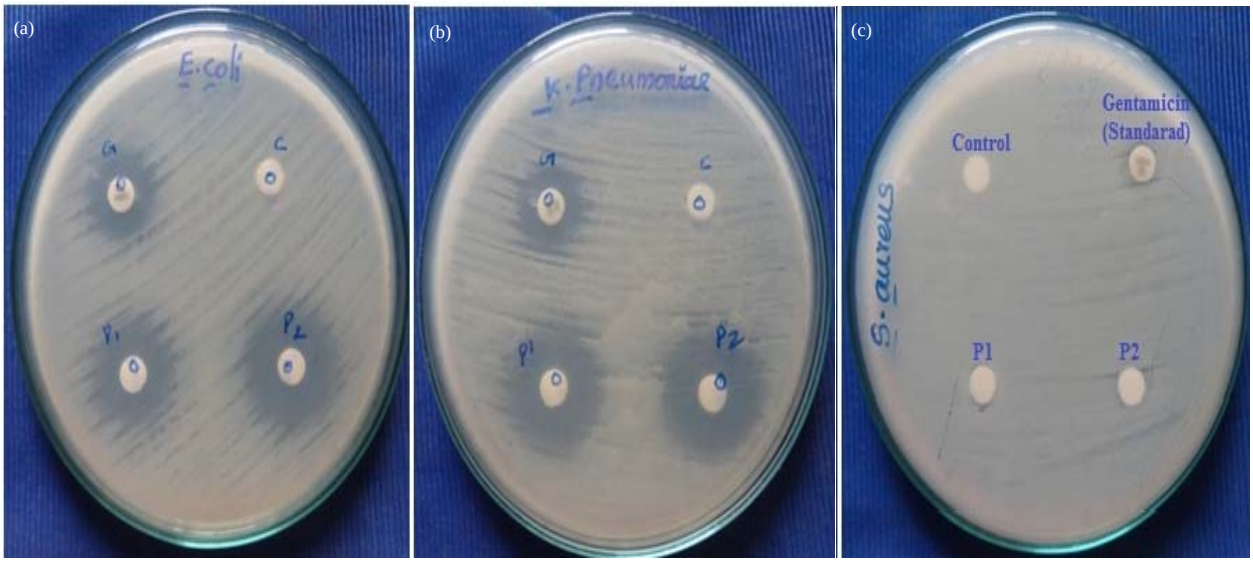

Fig. 4(a-c): Antimicrobial activity of polysaccharides against common pathogens, (a) *E. coli*, (b) *K. pneumonia* and (c) *S. aureus*. P1: *Pleurotus florida* polysaccharides, P2: *Agrocybe cylindracea* polysaccharides, G: Gentamicin, C: Control

polysaccharide extracts had greatest activity against Gram-negative bacterial strains *Klebsiella pneumonia* and *Escherichia coli*. Gram-positive bacterial strain *Staphylococcus aureus* exhibited weak growth-inhibiting effect on both polysaccharides extract. *Staphylococcus aureus* is a Gram-positive cocci, catalase and coagulase positive bacterium. Staphylococci have a record of developing resistance quickly and successfully to antibiotics. This defensive response is a consequence of the acquisition and transfer of antibiotic resistance plasmids and the possession of intrinsic resistance mechanisms. The importance of *Staphylococcus aureus* as a persistent nosocomial and community acquired pathogen has become a global health concern. It has a remarkable capability of evolving different mechanisms of resistance to most antimicrobial agents. The emergence of antibiotic resistant bacteria constitutes a major problem in antibiotic therapy. This could be attributed to unrestricted use of antibiotics in a particular environment. Previous study showed that vancomycin, levofloxacin and ofloxacin are recommended as first line antibiotics in the management of *Staphylococcus aureus* infections[17].

CONCLUSION

The antioxidant activities of extracted polysaccharides from edible mushrooms *Pleurotus florida* and *Agrocybe cylindracea* were evaluated by Fe^{2+}-chelating ability and hydroxyl radical scavenging assay. Polysaccharides exhibited stronger antioxidant activity compared to BHT. Results from this study indicated that polysaccharides could be potentially used as a natural antioxidant. Also the results in this study confirmed polysaccharides to possess antibacterial potency by displaying high zones of inhibition that are higher than the standard antibiotics employed in this study.

SIGNIFICANT STATEMENTS

- Active oxygen and free radicals are increasingly being recognized as being responsible for the pathogenesis of certain human diseases, including cancer, aging and chronic arterial disease. In order to reduce the oxidative damage of active oxygen and free radicals, some synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are widely used in food industry
- Nowadays, natural antioxidants have become increasingly popular among consumers because synthetic antioxidants are often perceived as undesirable or harmful. Therefore, there is an increasing interest in studying natural antioxidants that can be used in food processing to improve body's antioxidant defences and reduce the oxidative stress to human body
- The edible mushroom not only is a good source of nutrients, including protein, vitamin and dietary fiber but also contains a variety of bioactive substances, such as polysaccharides, cordycepin and lectin. Previous studies indicated that some mushroom polysaccharides, isolated from *Lentinus edodes*, *Agaricus nevoi*, *Coprinus comatus* and *Daedalea quercina* are found to have strong antioxidant activity. In addition, most of mushroom

polysaccharides are relatively non-toxic and do not cause significant side effects. Thus, mushroom polysaccharides have great development potential as a natural antioxidant

- In this study, we extracted polysaccharides from two mushroom species, *Pleurotus florida* and *Agrocybe cylindracea*. The antioxidant abilities of polysaccharides were then analyzed by *in vitro* systems including ferrous ion chelating activity and hydroxyl radical scavenging assay. The results suggest that extracted polysaccharides could be a promising source of natural antioxidant and be contributor to the health benefits of *P. florida* and *A. cylindracea*

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