Assessment of *in vitro* antioxidant and antimicrobial properties of cultivated *Pleurotus ostreatus*: An edible mushroom

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ABSTRACT

Objectives: In this study, *Pleurotus ostreatus* was cultivated under the laboratory condition and investigated for its *in vitro* antioxidant and antimicrobial property. **Materials and Methods:** To study the total antioxidant activity we performed 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfdonic acid, 1,1-diphenyl-2-picrylhydrazyl, superoxide anion, hydroxy radical-scavenging assays. **Result:** The *P. ostreatus* ethanolic extract (POEet) exhibited potent scavenging activity there by Posses increased antioxidant capacity when compared with positive control ascorbic acid. Moreover, we extended our work to investigate the antimicrobial activity of POEet which showed increasing zone of inhibition of extract with *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia.* **Conclusion:** Our finding confirms that *P. ostreatus* is a potent natural antioxidant and act as prevailing antimicrobial agent to promote public health by making it as a part of normal diet to reduce the risk of various diseases.

Keywords: Antimicrobial activity, antioxidant property, lingocellulosic substances, *Pleurotus ostreatus*

INTRODUCTION

Mushrooms have been used as an important nutritional or therapeutic item throughout world since ancient times.[1] Among the various edible species, the *Pleurotus ostreatus* (oyster mushroom) is one of the most important production after *Agaricus bisporous*.[2] The fruiting body of *P. ostreatus* contains approximately 100 different bioactive compounds, which mainly considered to be a potential new source of dietary fibers, proteins and an abundance of essential amino acids, minerals (Ca, P, K, Fe, Na) and also contain vitamin C, B-complex- thiamine, riboflavin, niacin and folic acid.[3,4] Due to its documented probiotic properties and relatively high nutritive value, they are recommended in numerous countries as functional foods.[5] Moreover mushrooms have been eaten and appreciated for their flavor, economic and ecological values and medicinal properties for many years.[6] They have ability to reproduce by the recycling of certain agricultural wastes therefore several *Pleurotus* species are cultivated commercially in various part of world.[7]

Cultivation of edible mushrooms is a biotechnological process for lignocellulosic organic waste recycling. It might be the only current process that combines the production of protein-rich food with the reduction of environmental pollution.[8] The origin for cultivation of oyster mushroom *P. ostreatus* was initiated on experimental basis in Germany by Flack during the year 1917 on tree stumps and wood logs. Conversely, growing technology was perfected in USA by Block, Tsao and Hau. In India, Cultivation of different varieties of oyster mushroom was initiated in the early sixties and commercial cultivation began in mid-seventies. The main substrate used for cultivation of *P. ostreatus* are any type of lignocellulose material like paddy straw, wheat straw, corn cobs and hardwoods sawdust, rice hull, etc.[9] The substrate used for the harvesting of the mushroom is valuable as a fertilizer or it could be used as animal feed after cultivation.[10] Thus, the cultivation process of mushroom can solve one of the most important problems in soil waste disposal, economical gain, and protect environment.

Mushrooms have powerful antioxidant properties derived from compounds such as selenium, ergothioneine and

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DOI: 10.5530/fra.2014.2.6

phenolics.[11] In order to utilize this valuable bioresource better, it is desired to evaluate the antioxidant activity of *P. ostreatus* systematically. Although research was focused on the therapeutic effects of the mushroom, little information is available about their antioxidant and antimicrobial properties. Our objective of the current study is undertaken to predict an easy, quick and reproducible protocol for cultivation of *P. ostreatus* and determining its scavenging effects on free radicals and the antibacterial activity.

MATERIALS AND METHODS

Chemicals

2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), phenazinemetho sulphate, nicotinamide adenine dinucleotide were purchased from Sigma Aldrich (MO, USA). All other chemicals and antibiotic discs were of analytical grade and procured from Sigma Aldrich (India) or Merck (India) unless otherwise stated.

Cultivation of *P. ostreatus*

Oyster mushroom (*P. ostreatus*) can be grown on wide range of substrates containing lignin, cellulose and hemicelluloses. Substrate should be fresh and dry. Substrates exposed to rain or harvested premature (green color) are prone to various weed molds which may result in failure of the crop. It can be grown on straw of paddy, wheat, ragi, maize/bajra/cotton stalks and leaves, sugarcane bagasse, cotton and jute wastes, dehulled maize cobs, tea/coffee waste etc., However cereal straw (paddy/wheat) easily available in abundance, is being widely used. For substrate preparation paddy straw is chopped in small pieces (2-4 cm size) and soaked in fresh water for 8-16 h. The substrate is pasteurized by addition of boiling water or steam. The purpose of pasteurization is to kill harmful microbes. After reducing temperature the excess of water drained off. The preserved substrate is inoculated by the spawn. The polypropylene bags (60 cm × 30 cm, holding 2-3 kg wet substrate) and sterilized at 15lb psi for 1 h.

Freshly prepared (20-30 days old) grain spawn is best for spawning. The spawning should be done in a pre-fumigated room (48 h with 2% form aldehyde). The bottom of the bag is tied with thread and the mushroom spawn is taken. The straw bits are placed in bottom of bags and the spawn is sprinkled over the entire surface. The second layer is sprayed over first layer to a height of 10 cm. Then, the second layer of straw arranged and the spawn is sprinkled. The third, fourth and fifth layer are spread in the same way. Finally, the fifth layer is covered with straw and the top of the bag is tied with thread. Thus, the bag looks like a cylinder. The cylindrical beds are kept in mushroom shed. The mushroom shed were maintained under optimal conditions that are favorable for fruiting bodies, i.e., enough of light, temperature 22 ± 1°C humidity in the mushroom room 85-90% airflow and low carbon dioxide content were secured. The mushroom are harvested from the substrate approximately 3-4 weeks after spawning depending on strain, amount of supplement used and temperature of spawn run.[12]

The mushroom was taxonomically identified and authenticated by Dr. V. Venkatesalu, Professor, Department of Botany, Annamalai University. A voucher specimen (No.: 233) was deposited in the Herbarium of Botany, Department of Botany, Annamalai University.

Preparation of ethanolic extraction

The harvested fresh fruiting bodies of *P. ostreatus* were dried in shade conditions and the dried materials were pulverized in a blender to get coarse powder. For *P. ostreatus* ethanolic extract (POEet), 5 g of the powder was extracted with 100 mL of 95% ethanol using a soxhlet apparatus. The solvent was evaporated on a rotary evaporator (Buchi Rotarvapor, Switzerland) under reduced pressure and controlled temperature (40-50°C).[13] A dark semisolid material (6% yield) thus obtained was stored at 4°C until use.

In vitro antioxidant assay

The *in vitro* antioxidant activities of POEet was estimated by $ABTS^{\bullet+}$, 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$), hydroxy radical and superoxide anion scavenging assay were determined by Wolfenden and Willson (1982), Williams *et al.* (1995), Halliwell *et al.* (1987), Nishikimi *et al.* (1972) respectively.[14-17] The scavenging percentage was calculated using the formula given below:

$$\% \text{ Scavenging} = \frac{\text{A Control - A Sample}}{\text{A Control}} \times 100$$

Where, A Control = Absorbance of the control and A Sample = Absorbance of the extract.

Antibacterial assay

Antibacterial activities of medicinal compounds were treated through disc diffusion assay by most widely used Kirby-Bauer method.[18] The following bacterial strains were used in this study *viz.*, *Escherichia coli*, *Staphylococcus aureus*, *Psedomonas aeuroginosa*, *Klebsilla pneumonia*.

RESULTS

Cultivation aspects

The *P. ostreatus* mushroom required high temperature and high humidity along with good light and aeration. Yield is adversely affected when these conditions are not provided. While good mycelia growth occurs between 10 and 33°C. For fruiting the temperature requirement is from 22 to 26°C. The spawn and cultivated bags of *P. ostreatus* are showed in (Figure 1a and b). After 2nd and 3rd day mycelium will start spreading and 20th day complete mycelium spreading will occur. On 21 day the small mushroom has been developed and after 25-45 days the mushroom growth is increased and it is ready to harvest (Figure 1c). Finally, the harvest mushrooms are used to carry out further antioxidant and antibacterial activity.

In vitro antioxidant assays

The free radical-scavenging activity of the extract was tested through ABTS method and the results were depicted at various concentrations of POEet (10-50 μg/mL) (Figure 2). The mean values across the concentration range indicate that the POEet was potent in neutralizing ABTS cation radicals and was comparable with that of Ascorbic acid. The scavenging capacity of POEet extract exhibit the percentage of inhibition in a dose dependent manner and showed maximum inhibition of 86% at 50 μg/mL. The inhibitory concentration 50 (IC_{50}) value of *P. ostreatus* and standard ascorbic acid were found to be 25.78 μg/mL and 24.05 μg/mL respectively. The antioxidant capacity of POEet was determined by the DPPH method and the results were presented in different concentrations (10-50 μg/mL) (Figure 3). The DPPH assay method is based on the reduction of DPPH˙, a stable free radical. The mean values across the concentration range indicate that the POEet was potent free radical-scavenging activity, which was comparable with that of Ascorbic acid. The scavenging capacity of POEet was showed maximum 76% inhibition at 50 μg/mL. Results showed the percentage of inhibition in a dose dependent manner. The IC_{50} value of *P. ostreatus* and standard ascorbic acid were found to be 27.96 μg/mL and 25.48 μg/mL respectively (Figure 4), summarized the hydroxyl radical-scavenging activity of the extract using the deoxyribose assay. The various concentrations of POEet (10-50 μg/mL) elucidating the mean values across the concentration range, indicates that the POEet was potent in scavenging the hydroxyl radicals generated *in vitro*, when compared with the standard ascorbic acid. The scavenging capacity of POEet was showed 60% maximum inhibition. The IC_{50} value of *P. ostreatus* was found to be 36.04 μg/mL. As positive controls, IC_{50} of ascorbic acid (IC_{50} = 34.08 μg/mL). The following graph revealed for superoxide anion scavenging activity and the results were depicted at various

Figure 1: (a and b) Spawns and cultivation bag of *Pleurotus ostreatus*, (c) fruiting bodies of *P. ostreatus*

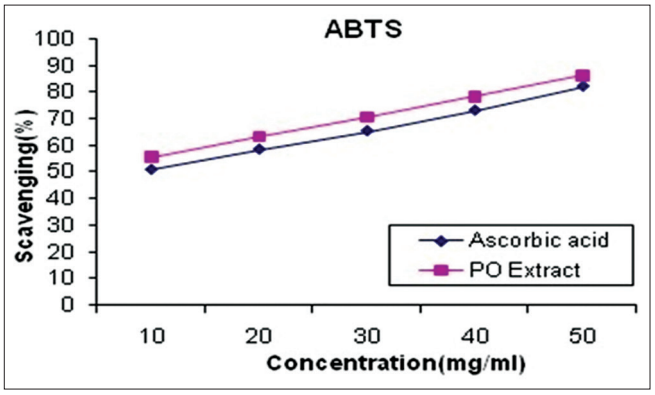

Figure 2: Scavenging effect of an ethanolic extract of *Pleurotus ostreatus* on 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid radical compared to that of ascorbic acid

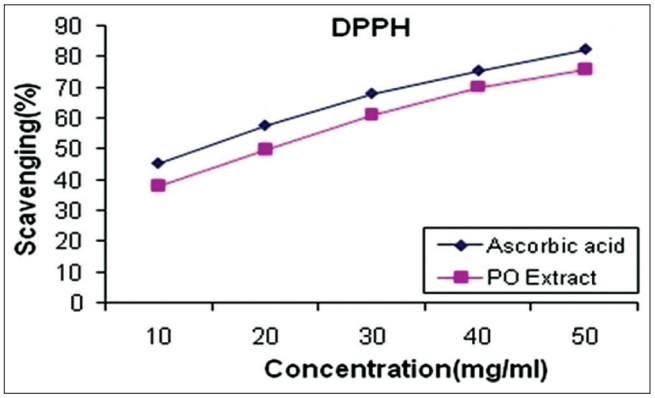

Figure 3: Scavenging effect of an ethanolic extract of *Pleurotus ostreatus* on 1,1-diphenyl-2-picrylhydrazyl radical compared to that of ascorbic acid

concentrations of POEet (10-50 μg/mL) (Figure 5). The scavenging capacity of POEet was showed 67% inhibition at the concentration of 50 μg/mL. The IC_{50} value of *P. ostreatus* and standard ascorbic acid were found to be IC_{50} = 33.46 μg/mL and IC_{50} = 29.77 μg/mL respectively.

Antibacterial activity

The antibacterial activity of *P. ostreatus* against Gram-positive (*S. aureus*) and Gram-negative (*E. coli, P. aeruginosa*) bacteria were evaluated by using standard zone of inhibition (Table 1 and Figure 6).

Table 1: Zone of inhibition produced by A-PO and POEet against Gram-positive and Gram-negative bacteria

Name of the test organisms	Zone of inhibition (mm)			
	A-PO	POEet	Standard drug (vancomycine)	Standard drug (amicosin)
S. aureus (Gran-poitive)	3	8	10	-
E. coli (Gram-negative)	4	6	-	13
K. pneumonia (Gram-negative)	5	6	-	14
P. aeruginosa (Gram-negative)	5	9	-	16

K. pneumonia: Klebsiella pneumonia, E. coli: Escherichia coli, P. aeruginosa: Psedomonas aeruginosa, S. aureus: Staphylococcus aureus, A-PO: Aqueous PO, POEet: *Pleurotus ostreatus* ethanolic extract

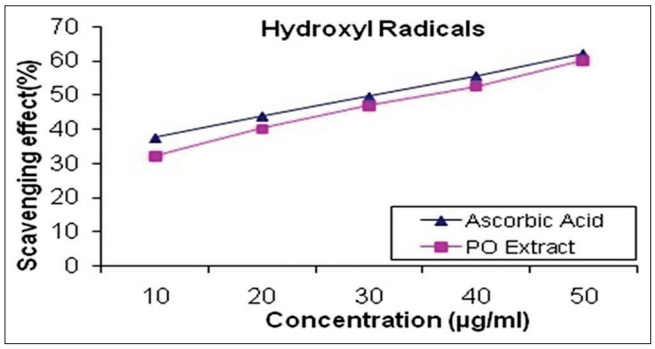

Figure 4: Scavenging effect of an ethanolic extract of *Pleurotus ostreatus* on hydroxye radical compared to that of ascorbic acid

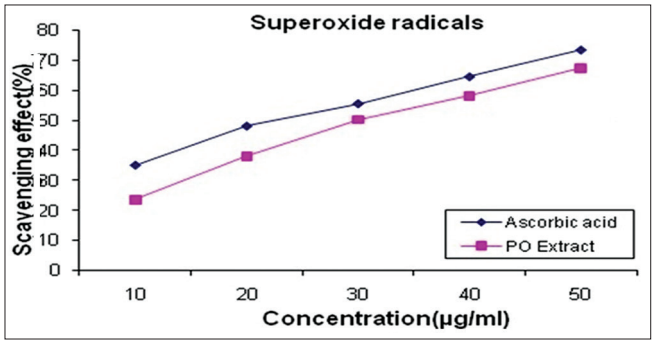

Figure 5: Scavenging effect of an ethanolic extract of *Pleurotus ostreatus* on superoxide radical compared to that of ascorbic acid

Vancomycin in Gram-positive bacteria and amicosin in Gram-negative bacteria were used as a standard reference antibacterial drug. Our results showed that POEet showed maximum zone inhibition on (Gram-positive bacteria) *S. aureus* - 8 mm, when compared with aqueous PO (A-PO) shows 3 mm zone of inhibitions. Whereas, in Gram-negative bacteria (*E. coli, K. pneumonia, P. aeruginosa,)* it also shows maximum inhibitory zone on POEet as 6 mm, 6 mm, 9 mm when compared with A-PO 4 mm, 5 mm, 5 mm zone of inhibition.

DISCUSSION

All over the world and especially in developing countries, there is a problem of shortage of protein. Producing mushrooms can be one suitable solution to this problem. Due to rapid industrialization, the amount of waste materials has increased and utilization of the waste is very important for government economy and natural balance.[19] Mushrooms have the ability to degrade several lignocellulosic substances and can be produced on natural materials from agriculture, woodland, animal husbandry and manufacturing industries.[20] *P. ostreatus* is a prospective source of valuable food protein and an organism with the ability to utilize various lignocellulosic materials.[10] It is possible to provide additional income to people living in the rural areas particularly working on wheat, hazelnut and rice agriculture. This will provide an economical gain and protect the environment while providing a nutritious food source such as mushrooms.[7] According to the results of the present study indicate that when utilizing agricultural lignocellulosic wastes represents the ideal and most

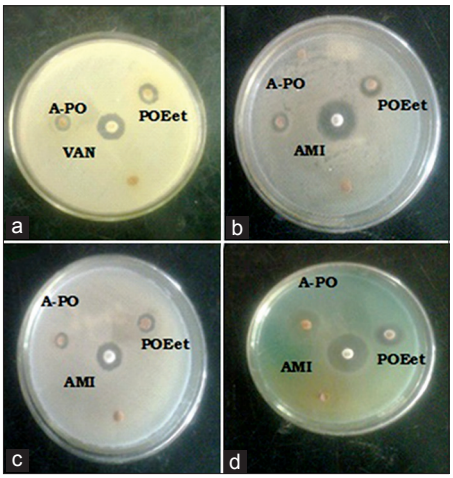

Figure 6: Antimicrobial activity of aqueous PO and *Pleurotus ostreatus ethanolic* extract (POEet) assayed by agar diffusion method on, (a) *Staphylococcus aureus* (Gram-positive), (b) *Escherichia coli* (Gram-negative), (c) *Psedomonas aeruginosa* (Gram-negative), (d) *Klebsiella pneumonia* (Gram-negative)

promising substrate for cultivation of *P. osteratus*. Where the spawn run completion took nearly 3 weeks in paddy straw, were in agreement with the findings reported by Shah *et al.*, 2004 and Tan 1981.[21,22] Utilization of these lignocelluloses waste for the production of oyster mushroom could be more economically and cost-effective organic recycling processes.[12]

In order to confirm the antioxidant potential of the cultivated ethanolic extract of *P. ostreatus* mushroom, the present study employed four different antioxidant testing systems such as ABTS, DPPH, hydroxyl radical scavenging, and superoxide anion scavenging activity. The ABTS assay is based on the inhibition of the absorbance of the radical cation $ABTS^{\bullet+}$, which has a characteristics long wavelength absorption spectrum.[23] The concentration dependent curve of ABTS radical scavenging activity of *P. ostreatus* compared well with ascorbic acid as standard drug. The result obtained in our study revealed that the activity of the extract either by inhibiting or scavenging the $ABTS^{\bullet+}$ radical. Both inhibition and scavenging properties of antioxidant towards $ABTS^{\bullet+}$ radical has been reported earlier.[24] These results are in agreement with the antioxidant effect generated by polysaccharides, including those obtained by the polysaccharides from medicinal plants.[25]

DPPH being a stable free radical accepts electrons or hydrogen radicals to become stable diamagnetic molecules.[26] The reaction of POEet with purple colored DPPH radical, is convert the radical α,α diphenyl-β-picrylhydrazine due to the extract antioxidant property. The degree of discoloration indicates the potential of the POEet to scavenge free radical due to its ability to donate hydrogen proton.[27] The concentration dependent curve of DPPH radical scavenging activity of *P. ostreatus* compared well with ascorbic acid as standard drug. The results obtained from this study in line with the finding of Iwalokun *et al.*[28]

The hydroxy radical is the most reactive radical known which can attack and damage almost every bio-macro molecules in living cells. The best characterized biological damage caused by hydroxyl radical is, its capacity to stimulate lipid peroxidation, which occur when hydroxyl radical is generated close to membranes and attack the fatty acid side chains of the membrane phospholipids.[29] In our present study, the POEet also showed the percentage of inhibition in a dose-dependent manner. These result correlate with the previous report, which also pointed out that *P. ostreatus* polysaccharide showed lower antioxidant activity than vitamin C and their scavenging effects increased with increasing concentration.[30]

Superoxide anion is one of the precursors of the single oxygen and hydroxyl radicals; therefore it indirectly inhibits lipid peroxidation. Apart from that the presence of superoxide anion can magnify cellular damage because it produces other kinds of free radicals and oxidizing agent.[31] In biochemical systems, superoxide radical can be converted into hydrogen peroxide by the action of superoxide dismutase and the H_2O_2 can subsequently generate extremely reactive hydroxyl radicals in the presence of certain transition metal ions. In the present study, we investigated the scavenging or preventive capacity of *P. ostreatus* extracts against the superoxide anion free radical it clearly showed that the extract has the ability to decrease the absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. Zhang, *et al.* (2012) also suggested that the superoxide anion radical scavenging effects of the crude polysaccharides increased with increasing concentration.[32] Therefore, we speculate that the basic phytochemicals like polysaccharides, alkaloids, steroids and terpenoids might be the key factor for the antioxidant activity of POEet.

Moreover, the secondary metabolites from *P. ostreatus* exert antimicrobial activity through different mechanism. Nowadays, the use of antibiotics increased significantly due to infections causing life-threatening illness and the pathogenic bacteria becoming resistant to drugs in common due to its discriminate use of antibiotics.[33] It becomes a greater problem of giving treatment against resistant pathogenic bacteria. While decreased efficiency and resistance of pathogen to antibiotic has necessitated the development of new alternatives.[34] The present study observed that POEet showed antibacterial activity against *E. coli*, *S. aureus*, *P. aeuroginosa*, *K. pneumonia*. The variation in the effectiveness of the extract against different microorganism depends upon the chemical composition of the extract and membrane permeability of the microbes for the chemicals and their metabolism. The data obtained from the disc diffusion method revealed that *P. ostreatus* exhibit antimicrobial activity this could be due to presence of polysaccharides, alkaloids, steroids and terpenoids. The results also lined up with Karaman *et al.*, postulated that organic extract (methanol and chloroform) of *P. osteatus* has been manifested as effective against Gram-positive bacteria which showed to be a potential source of antibacterial agents.[35]

CONCLUSION

P. ostreatus mushroom able to grow under different climatic conditions on cheap, readily available waste materials. They require shorter growth time when compared to other edible mushrooms and the substrate used for its

cultivation do not require sterilization, only pasteurization, which is less expensive. This mushroom demands few environmental controls for cultivation and its fruiting bodies are not often attacked by diseases and pest, and it can be cultivated in a simple and cheap way. Another advantage of growing oyster mushrooms is that a high percentage of the substrate is converted to fruiting bodies, increasing profitability when compared to other mushrooms, making *P. ostreatus* an excellent choice for mushroom cultivation. Owing to the presence of the active secondary metabolites such as terpenoids, polysaccharides, alkaloids, steroids and proteins in *P. ostreatus* may be useful as a naturally potential antioxidant and antimicrobial agent for application in food and medicinal fields. Further, studies to elucidate the exact mechanical pathway and isolation of various active compounds responsible for the chemotherapeutic potentials of *P. ostreatus* on various diseases and malignances.

ACKNOWLEDGMENTS

The authors would like to acknowledge for the Financial Support from (UGC) - Major Research project (F. NO: G7/17342/2012 (SR)), New Delhi, India to carry out the work successfully.

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