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Antioxidant potentiality of *Pleurotus ostreatus* (MTCC142) cultivated on different agro wastes

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ABSTRACT

Pleurotus ostreatus (MTCC142) was cultivated on three different agro wastes viz: Wheat straw, Soybean straw and Chick pea straw. Total antioxidant, reducing power and total phenolic content effect of the extracts was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and Folin-Ciocalteu method. The aqueous extract of P.ostreatus cultivated on soybean straw exhibited good antioxidant activity (230 µg equivalent of BHT/g), and also contain high phenolic content (6.25 mg gallic acid/g of dry extract). P. ostreatus cultivated on Wheat straw, had the lowest IC50 value 15.6µg/ml against DPPH radical while P. ostreatus cultivated on chickpea straw had the highest IC50 value 42.9µg/ml. The results provided evidence that the aqueous extracts of P. ostreatus might indeed be potential sources of natural accepted antioxidant and the study revealed that different agro wastes used in cultivating P. ostreatus has a significant effect and positive consequence on its antioxidant properties.

Keywords: Agro Wastes, Antioxidant activity, *P. ostreatus* aqueous extract.

INTRODUCTION

Edible mushrooms have been ingredient of human diet for centuries. The nutritional value of mushrooms is twice that of any vegetable or fruit [1]. Oyster Mushrooms are known to be medically active in several therapies, such as antitumour, antibacterial, antiviral, haematological and immunomodulating treatments [2]. The therapeutic effect had been linked to the presence of bioactive compounds in mushrooms. Some of these bioactives include glycolipids, compounds derived from shikimic acid, aromatic phenols, fatty acid derivatives, polyacetylamine, polyketides, sesquiterpenoids, and many other substances of different origins [3]. Moreover, mushrooms are recognized as a respectable source of amino acids which play an essential role in their nutritional property.

Pleurotus is an important genus of edible basidiomycetes which are commonly called oyster mushroom [4]. All known species are edible, with several being commercially cultivated. The popularity of this genus is on the increase, especially because of its flavor and texture [5].
Pleurotus mushrooms grow in the wild in tropical, subtropical and temperate regions and are easily artificially cultivated [6]. They are healthy foods, low in calories and in fat, rich in protein, chitin, vitamins and minerals [7]. Extract of *P. ostreatus* had been reported to alleviate hepatotoxicity induced by CCl4 in rats [8]. The ability of extract from *P. ostreatus* to protect major organs such as the liver, heart, and brain of aged rats against oxidative stress had also been reported [9].

The cultivation of *Pleurotus spp.* is an economically and cost-effective efficient food industry worldwide which has expanded in the past few years .*P.ostreatus* is the third most important cultivated mushroom for food

purposes[10]. One of the most important aspects of *Pleurotus spp.* is related to the use of their ligninolytic system for a variety of applications, such as the bioconversion of agricultural wastes into valuable products for animal feed and other food products and the use of their ligninolytic enzymes for the biodegradation of organopollutants, xenobiotics and industrial contaminants [11].

Several lignocellulosic wastes such as corncobs, sawdust, straws etc had been used in cultivating *Pleurotus* species [12]. Preference for the wild mushrooms by some consumers is based on flavor, texture, nutritional, and pharmacological characteristics of these mushrooms [13]. The different substrates used in cultivating mushrooms do have effect on the functional, organoleptic and chemical properties of mushrooms [14]. It is therefore pertinent to investigate the effect of such substrates on some functional properties of *Pleurotus* species cultivated on such substrates. A preliminary investigation of present study therefore seeks to assess antioxidant properties of *P. ostreatus* cultivated on three different substrates.

Antioxidants play an important role in maintaining human health due to their ability to scavenge free radicals in the bodies [15]. Fortunately, human body is designed to have its own defense system such as superoxide dismutase enzyme, glutathione enzyme and catalase to fight harmful substances and prevent cell damages[16].However, the consumption of supplemented antioxidants present in our diets such as in fruits, vegetables and mushrooms is very important to offer adequate security. Hence, the objectives of the present study are to conclude the antioxidant activities, total phenolic content extract of selected mushrooms, *P. ostreatus* cultivated on different agro wastes as substrates.

MATERIALS AND METHODS

Strains of *P. ostreatus* MTCC 142 were grown at Lab of Microbial Tec., Dept. of Botany, Dr. Hari Singh Gour Central University, Sagar (M.P.). The pure fungal Species procured from IMTECH, Chandigarh, India.

2.1 Preparation and cultivation of *Pleurotus ostreatus* on substrates

Substrates used in cultivating *P. ostreatus* were prepared from straws of different cereal plants. Wheat straw, soybean straw and chick pea straw mixed with water. The substrates were filled into polyethylene bags (800g/bag). The bags were then sealed, autoclaved, cooled and inoculated with the spawn. Spawn of *P. ostreatus* collected from MTCC142, MTCC Chandigarh. substrate in bags were inoculated with approximately 2 gm of spawn using surface spawning technique under laminar flow and incubated in a dark chamber .The growth of mycelium in each bag was observed. When the mycelium fully covered the substrate, bags were kept open in the growing house for fruit body formation. The harvested mushroom sporocarps were air-dried after that they were grind to powder using grinding machine.

2.2 Preparation of Mushroom Extracts

The extracts from the oyster mushrooms cultivated on the different substrates were obtained using 95% distilled water, following the method of Tsai, [17]. Fruiting bodies were dried in oven at 40 ^{o}C for eight hours, crushed and grind to fine powder using a REMI electronic blender. 50 grams of each mushroom powder was reflexes with 500 ml water in a soxhelet apparatus at 100 ^{o}C for 16-18 hours to ascertain excellent extraction of bioactive metabolites. The liquid extracts were then rotavaporised (Rota vapor R-114, Buchi) at 40 °C under pressure to obtain aqueous extract of the mushroom species and then subjected to freeze drying (LYOVAC, GEA), finally stored at 4 ^{o}C for further analysis.

2.3 Drugs and chemicals

1,1, diphenyl picryl hydrazyl (DPPH), Folin ciocalteau reagent, were purchased from Biochem. Pharmaceuticals, (Mumbai). Ascorbic acids and other standards were procured from Sigma Co (Mumbai). All other reagents purchased from Hi-Media were of analytical grade.

2.4 Total Phenolic Contents (TPC)

The total phenolic content was determined by Folin- Ciocalteu method [18]. Sample solution (50 µl) was added to 50 µl of 7.5% sodium carbonate, 50µl distilled water mixed thoroughly and allowed to stand for 2 min. Then, 500 µl of 10% Folin-Ciocalteu reagent (Folin: Methanol, 1:1, v/v) was added and the mixture was mixed well. After incubation for 40 min. Absorbance was then measured at 765nm using the Perkin Elmer Lamda 25 UV-

spectrophotometer. A calibration curve was obtained using various concentrations of gallic acid. The total phenolic content of the sample was expressed as mg of gallic acid equivalents (GAEs) per gram of dry sample.

Absorbance = 0.0009 × gallic acid (μg)

2.5 Total Antioxidant Activity

Total antioxidant activities of crude extracts were determined according to the method of Prieto et al., [19]. Briefly 0.3 ml of samples was mixed with 0.3 ml reagent solution (0.6m sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). Reaction mixture was incubated at 95^0C for 90 min under water bath. Absorbance of all the sample mixture was measured at 695nm. Total antioxidant activity is expressed as the number of equivalence of ascorbic acid.

2.6 Scavenging Effect of Extracts on DPPH Radicals

Radical scavenging potential of extracts obtained from *P. ostreatus* cultivated on different substrates was assessed using an aqueous solution of the "stable" free radical, DPPH. The method of Blois, [20] was used in studying the effect of these extracts on DPPH radicals. A solution of DPPH (0.5 mmol/L) in ethanol and 0.05 mol/L acetate buffer (pH 5.5) was prepared. Extracts in solution (0.1 ml) at different concentrations was mixed with 2 ml of acetate buffer, 1.9 ml of absolute ethanol and 1 ml DPPH solution. The mixture was shaken immediately after adding DPPH and allowed to stand at room temperature in dark for 30 min.

The decrease in absorbance at 517 nm was measured using a UNICO 2100 spectrophotometer. Ascorbic acid was used as positive control and the sample solution without DPPH was used as blank. The radical scavenging activity was measured as a decrease in absorbance of DPPH. Absorbance was measured at 517 nm (Spectra Max Plus384, United States) and IC50 value (concentrations of each sample required to give 50% of the optical density shown by control) was calculated. Inhibition of DPPH free radicals was calculated by using following formula:

Inhibition of DPPH radical (%) = 100 × (A control – A sample)/A control

Where,
A control = Absorbance of the control solution (containing all reagents except the test extract)
A sample = Absorbance of the test extract.

All test analyses were run in at least three replicates and averaged.

2.7 Statistical Data Analysis

All experiments were carried out in triplicates. Data obtained were analyzed by one way analysis of variance and means were compared by Duncan's multiple range tests (SPSS 16 version). Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

P. ostreatus is one of the more commonly sough wild mushrooms, though it can also be cultivated on straw and other media. The present study reports the antioxidant properties of *P. ostreatus* cultivated on different substrates.

3.1 Total Phenolic Content (TPC)

The phenolic content of three aqueous extract of *P. ostreatus* cultivated on agro wastes substrates, (soybean straw, wheat straw, chickpea straw) was evaluated using Folin ciocalteu method (Table.1.). Among the different substrates, aqueous extract of soybean straw showed high phenolic content (6.24 mg gallic acid/g of dry extract) followed by wheat straw (4.17 mg gallic acid/g of dry extract) and chickpea straw (3.15 mg gallic acid/g of dry extract). Antioxidant effects of biological materials are known to be contributed by various secondary metabolites preferentially phenolic compounds [21]. Phenolics have been reported as strong antioxidants capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes reduce α-tocopherol radicals and inhibit oxidases [22]. Furthermore, phenolics may also elicit antibacterial activity as found in many medicinal plants with mechanisms of action characterized by cell membrane lyses, inhibition of proteisynthesis, proteolytic enzymes and microbial adhensins. [23].

Phenolic compounds such as phenolic acids and tannins are known as major components of antioxidant in plants and mushrooms. Previous literatures reported that genus Pleurous contained several types of phenolic compounds such as vanillic acid [24] , myricetin, naringin, homogentisic acid, 5-O-caffeoylquinic acid [25], chrysin, rutin [26], gentisic acid, gallic acid, protocatechuic acid caffeic acid, tannic acid. syringic acid, cinnamic acid and p-coumaric acid [27]. In addition, Ferreira, [28] stated that most antioxidant properties that can be found in mushrooms are mainly in the form of phenolic acids and flavonoids, followed by tocopherols, ascorbic acid and carotenoids..

3.2 Scavenging Effect of Extracts on DPPH Radicals

P. ostreatus extracts displayed a good DPPH radical scavenging ability thereby expanding its nutraceutical values. The results revealed that all the extracts were able to scavenge DPPH radical. (Fig.1). *P. ostreatus* cultivated on Wheat straw, had the lowest IC50 value 15.6µg/ml against DPPH radical while *P. ostreatus* cultivated on chickpea straw had the highest IC50 value 42.9µg/ml. The DPPH scavenging effect of *P. ostreatus* cultivated on soybean straw was comparability good IC50 value 34.6µg/ml.(Table.1) DPPH scavenging ability of aqueous extract were significantly different at $p<0.05$. Antioxidants are important compounds that defend our body against free radicals and mushrooms are rich sources of these antioxidants [29]. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells [30].

DPPH radical is a stable free radical and possess a characteristic absorbance at 517 nm, which decreases significantly on exposure to radical scavengers by providing hydrogen atom or electron to become a stable diamagnetic molecule. The use of stable DPPH radical has the advantage of being unaffected by side reactions, such as enzyme inhibition and metal chelating. Using the DPPH free radicals is a common practice in order to assess the scavenging activity of antioxidant extracts, because it is a fast and reliable method to detect the hydrogen-donating ability of the different alcoholic extracts at low concentration [31]. The decrease in absorbance is due to the reaction between the antioxidant components of the extract and the stable radical. The scavenging effect of *P.ostreatus* extracts on DPPH radicals increased with the increase in sample concentration.

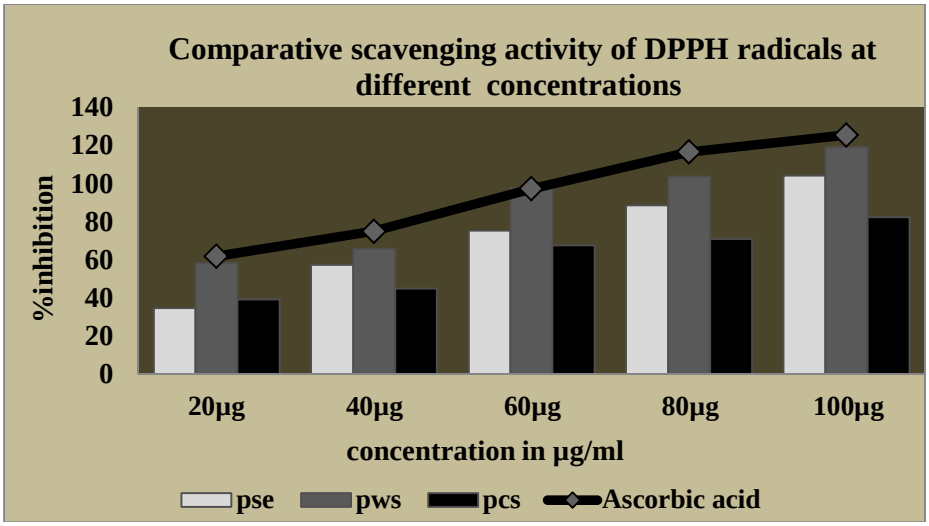
3.3 Total Antioxidant Activity

The results indicated that the good antioxidant activity was observed at aqueous extract of *P. ostreatus* on soybean straw (230 µg equivalent of BHT/g). *P. ostreatus* chickpea aqueous extract also showed good antioxidant activity (200 µg equivalent of BHT/g) next to aqueous extract of *P. ostreatus* on wheat straw exhibited least antioxidant activity (90 µg equivalent of BHT/g). This was agreed with the result of Vamanu et al., [32] reported that water was the most appropriate solvent. If aqueous was used *P. ostreatus* strain had an antioxidant activity of 94.54%, it was higher than that of other extracts.

The reducing power of a compound is known to be associated with the presence of certain antioxidant agents and reductions such as ascorbic acid [33]. The extracts acted as electron donor to reduce the ferricyanide (Fe^{3+}) to ferrocyanide (Fe^{2+}) which turned the yellow solution of test compound containing ferric ion to Pearl's Prussian blue or green to intense blue colour solution (depending on the extracts ability) as the reduced form. As reported by Harborne, [34] we assumed that reducing power in the mushrooms extract might be due to their hydrogen-donating ability that stabilized the corresponding molecules by accepting hydrogen ions from the extracts and terminating the radical chains.

In this study, the trend lines for anti oxidant potential is differed between one species to another, which indicates that every species has their own unique strategies in phenolic synthesis and metabolic reaction. The previous literatures stated that the exposure of living cells to variety sources of radicals such as sunlight and chemicals may lead the organisms to develop their own protection systems in both enzymatic and non enzymatic reaction [35]. Thus the production of phenolic compounds in fungi is believed to provide adequate defensive mechanisms towards radicals and reactive species of certain chemicals.

Figure1. % inhibition of DPPH Radical scavenging activity (RSA) of *Pleurotus ostreatus* cultivated on the different agro wastes at different concentrations



Bars are significantly different *(P≤0.05)*. pse*: Pleurotus ostreatus* cultivated on soybean straw, pws: *Pleurotus ostreatus* cultivated on wheat straw, pcs: *Pleurotus ostreatus* cultivated on chickpea straw.

Table 1: Total phenolic content (TPC), IC50 and Total antioxidant Activity values of *P.ostreatus* cultivated on different agro wastes

Samples	TPC (mg gallic acid/g of dry extract)	IC50 DPPH	Total Antioxidant Activity (µg equivalent of BHT/g)
Soybean straw	6.24±0.057[a]	34.354[a]	230[a]
Wheat straw	4.17±0.135[b]	15.699[b]	90[b]
Chickpea straw	3.15±0.077[c]	42.986[c]	200[c]
Ascorbic acid	-	8.350[d]	

For TPC, values are mean ±S.E. of triplicate data. Different superscript [a, b] are significantly different (P≤0.05)

CONCLUSION

Conclusively, this study established the effect of different agro-wastes as substrates on the antioxidant of *P.ostreatus*. *P. ostreatus* cultivated on soybean straws exhibited better antioxidant activities when compared with *P. ostreatus* cultivated on the other substrates, wheat straw and chick pea straw. Hence, soybean straw will be a good substrate that will enhance the nutraceutical properties of *P. ostreatus*. Nutraceutical industries can therefore exploit straw from straw mills as substrates for the cultivation of *P. ostreatus*. The results revealed that straw from soybean showed good potential as substrate for cultivation based on higher and significantly different *(P≤0.05)* antioxidant contents found in *P. ostreatus* cultivated on it. Straw from soybean and wheat which most straw mill dispose into the environment could be a good substrate for pharmaceutical prospect and antioxidant nature of *P. ostreatus*.

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