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Anti-Oxidative Stress Enzyme from *Pleurotus sp*

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Abstract

Pleurotus sp. known for its medicinal values and considered as king of all edible mushrooms. Three species of oyster mushrooms (Pleurotus spp.) that are cultivated mostly throughout the year in the plains of India were studied for their antioxidant properties with few modifications like ultra-sonication and standardizing various factors like solvent, buffer, pH, time, incubation time and enzyme concentration. Using buffer extraction/homogenization, there was remarkable increase (increase of two-fold) in enzyme activity from 13.35 to 26.70U/mg in Pleurotus djamor var. roseus, 8.47 to 19.05 U/mg in P. ostreatus and 11.74 to 22.12U/mg in P. florida after ultrasonication which has not been reported earlier while using different solvents i.e. Methanol, ethanol, and water. Maximum inhibition was observed in natural extract (96.38%), followed by buffer (91.84%) and ethanolic extract (91.74%). Comparative efficiency of homogenization of mushroom samples in buffer system with or without ultrasonication was evaluated, that has not been reported earlier for study of antioxidative stress enzymes in *Pleurotus species*. Maximum SOD (26.70U/mg) and POX (0.51U/ml) was recorded at pH 6.0 (potassium phosphate buffer, 1M) with P. djamor var. roseus and (22.12U/mg) and (0.39U/ml) for P. Florida (phosphate citrate buffer, pH 6.5). Optimum SOD (29.95U/ml) and POX (0.63 U/ml) activity for P. djamor var. roseus was reported at 40 °C and 37 °C respectively at different time of incubation. Though the anti-oxidative stress enzymes could make a significant contribution to the antioxidant activity in these edible mushroom extracts, yet the chemical characteristics of these components are required to be investigated.

Keywords: Pleurotus; Antioxidant; Superoxide dismutase; Peroxidase; Ultra-sonication; Mushrooms; *Pleurotus species*; P Florida; P djamor var; Homogenization; Methanol; Ethanol; Water; Superoxide dismutase; Ostreatus

Mini Review

Pleurotus (edible mushroom) widely consumed globally has been reported to possess good antioxidant activity [1]. *Pleurotus* are reported to be a good source of cysteine, methionine and aspartic in comparison to Agaricus bisporus (brown, white) and Lentinus edode [2] and bioactive compounds (Phenolic, terpenes, polyketides) [3]. In addition, *Pleurotus species* reported to possess excellent free radical scavenging and therapeutic potential (Levostatin) for treating hypercholesterolemia [4]. *Pleurotus* extracts could be used in the treatment of infections commonly associated with the micro -organisms and in treatment of skin diseases. Among various species of *Pleurotus*, *Pleurotus* djamor var. roseus has remained less studied till now, definite its use as potent mushroom. Efforts made in past to study the anti-oxidative stress enzymes from microbial as well as plant sources. The present investigation was carried out to test the anti-oxidative potential of medicinally important species of *Pleurotus* growing in the region.

Materials and Methods

Collection and processing of mushroom samples

Different *Pleurotus* sp viz *Pleurotus* ostreatus, *Pleurotus* djamor var. Roseus and *Pleurotus florida* and *Pleurotus* djamor var. roseus were procured from vikas benal mushroom farm, thakur mushroom farm and Directorate of mushroom research (DMR, Solan) respectively located in Himachal Pradesh, India. *Pleurotus*samples were morphologically analysedfor differences in colour, presence of scaling on their surface and characterized for anti-oxidative stress enzymes; SuperOxide Dismutase (SOD) and Peroxidase (POX).

Briefly, the collected *Pleurotus* samples were washed under tap water, surface sterilized (70% ethanol) and stored at -20 °C for one week. The freeze-dried samples were homogenized in liquid nitrogen using mortar and pestle in three different freshly prepared buffer solutions (Phosphate citrate, Potassium

phosphate, Tris-HCl; 0.1 M) having pH 5.0 to 9.0 respectively in the ratio of 1:10 (w/v). Ultra -sonication of the homogenised samples was done (PUL 59, AMP1 32%, Temp. 8 °C) to obtain the maximum disruption of cells. Post-ultracentrifugation, the colloidal solution was centrifuged (15,400rpm for 30 min. at 4 °C) to remove cell debris and the supernatant was used as crude enzyme solution for carrying out the enzyme assay.

All the chemicals were of high quality analytical grade and procured from Sigma (USA), Merck (Germany) and Hi-Media (India).

Preparation of solvent extracts and quantitative determination of anti-oxidative stress enzymes

Pleurotus samples were dried at 27 °C for 24 hrs. and grounded to powder using mortar and pestle. The extraction was done using different mushroom sample with three different solvent (methanol, ethanol and water) under the shaking conditions (150 rpm) in the ratio of 1:10 (w/v) for 24 to 48 hrs. at 30 °C. The mixture was filtered and the assay of anti-oxidative stress enzymes i.e. superoxide dismutase (SOD) and (POX) was performed in solvent extracts, supernatant and pellet.

Super Oxide Dismutase (SOD) assay based on the generation of the superoxide radicals in PMS-NADH systems by oxidation of NADH [5]. Briefly, one-unit SODenzyme activity (U) is that amount of enzyme required for 50% inhibition of NBT reduction in one minute at 560 nm under assay conditions. Similarly, one-unit peroxidase (POX) activity isthe amount of peroxidase required for the conversion of substrate (1µM) per minute under assay conditions [6] and involved O-PhenyleneDiamine (OPD) (chromogen) and hydrogen peroxide (H2O2) as substrate.

Optimization of assay conditions for intracellular superoxide dismutase and peroxidase

Optimization of buffer and pH for homogenization

The most suitable buffer and pH was determined by utilizing three different buffer systems namely phosphate citrate (1M; pH 5.0-8.0), potassium phosphate (1M; pH 6.0-8.0) and Tris-HCl (1M; pH 8.0-9.0) for homogenization of sample and measuring enzyme activity in each case.

Effect of incubation temperature, time and enzyme concentration

The effect of incubation temperature and time on the enzyme activity was evaluated by varying the range of temperature (20, 25, 30, 35, 40 and 45 °C) for SOD, (25-45 °C) for POX and incubation time (80-105 second) for SOD, incubation time (5-30 min) and enzyme was assayed in both cases. The influence of reaction volume on enzyme activity was observed by varying the volume of crude enzyme (100µl-500µl) and enzyme assay done in each case.

Results and Discussion

Three different species of *Pleurotus* i.e. *Pleurotus* ostreatus, *Pleurotus florida* and *Pleurotus* djamor var. roseus showed distinct morphological characteristics of whitish color, white grey color and pink color respectively. Samples were processed using different solvent extracts and screened for two intracellular anti-oxidative enzymes superoxide dismutase (SOD) and peroxidase (POX).

Solvent extract of enzyme

The different samples subjected to various solvent extracts showed maximum SOD activity and POX activity. In ethanolic extract, *Pleurotus* djamor var. Roseus showed maximum (23.71U/mg) activity, followed by *Pleurotus florida* and (22.09 U/mg) in methanolic extract. Ramkumar et al. [7] reported that the methanol extraction of *Pleurotus* strains had the highest antioxidant activity. Free radical scavenging potential of ethanolic extract recorded for *Pleurotus* djamor var. roseus (90.74%) was found to be comparable to *Pleurotus* pulmonarius extract (90%) as reported earlier [8]. Similarly, ethanolic extract of *Pleurotus* djamorvar. Roseus and methanolic extract of *Pleurotus florida* showed (0.47U/ml) and (0.35U/ml) POX activity respectively. However, aqueous extract emerged as poorest SOD and POX activity for *Pleurotus* djamorvar. roseus and *Pleurotus florida*. Methanolic extraction of *Pleurotus florida* exhibited (84.97%) potent superoxide radical scavenging activities which is similar to that reported earlier by Jose and Janardhanan [9]. Antioxidant mechanisms of the *Pleurotus species* extract; Pleurtus ostreatus (84.52%), *Pleurotus* djamor var. roseus (90.74%) and *Pleurotus florida* (84.97%) attributed by hydrogen-donating and superoxide, free radicals scavenging ability [10].

Effect of ultra-sonication on SOD activity

After 5 cycles of ultra-sonication, *Pleurotus* djamor var. Roseus reported two-fold increases in SOD activity to maximum 26.70U/mg protein.

Standardization of different reaction parameter for SOD and POX

Maximum enzyme activity was observed in *Pleurotus* djamor var. roseus and *Pleurotus florida* whereas *Pleurotus* ostreatus showed minimum activity. Therefore, these were selected for the optimization of reaction parameters for superoxide dismutase (SOD) and peroxidase (POX) enzymes.A comparison of the results of the previous and the present investigations on *Pleurotus species* showed that the intracellular antioxidative stress enzymes are variably secreted among the different species[11].

Buffer system and pH

Among the three-buffer system (1M), namely potassium phosphate, phosphate citrate and Tris-HCl with varied pH [5-9], maximum SOD activity was recorded at pH 6.0 with potassium phosphate buffer in case of P. djamor var. Roseus (26.70 U/mg) while for optimum SOD activity for P. *florida*, pH 6.5 (phosphate citrate buffer) resulted best with 22.12 activity units. Ramkumar et al. [7] has also recorded 20.29U/mg of SOD and 4U/mg of POX in dried samples of P. djamor. Increasing pH beyond 6.5 for all three buffers, showed a sharp decline in SOD activity.

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For POX activity in three different buffer systems with pH ranging from 5-10, *Pleurotus* djamor var. Roseus reported maximum POX activity (0.51U/ml) with potassium phosphate buffer (1M, pH 6.0), followed by phosphate citrate0.39U/ml at pH 6.5. The activity of peroxidase enzyme in case of P. *florida* was recorded highest (0.39U/ml) at pH 6.5 with phosphate citrate buffer. Tris-HCl showed least activity SOD and POX activity (0.22U/ml) in the three buffer systems with both samples of *Pleurotus* tested.

Effect of incubation temperature and time

Pleurotus spp. was subjected to varying temperature ranged from 20-45 °C to estimate the SOD activity. Temperature range from 30-45 °C was found to be suitable for optimum SOD enzyme activity, with maximum at 40 °C for P.djamor var. roseus and P. *florida* i.e. 29.95U/ml and 22.74U/ml respectively. However, any variation in temperature (below and above 40 °C) resulted in decline in enzyme activity. *Pleurotus* djamor var. Roseus and *Pleurotus florida*exhibit maximum POX activity of 0.63U/ml and 0.46U/ml respectively at 37 °C when subjected to temperature range of (25-45 °C). A sharp decline in POX activity was recorded beyond 37 °C. Different time intervals for SOD (80-105 sec) and POX (5-30 min) were tested to determine the time dependent activity of SOD and POX. The maximum SOD activity for *Pleurotus* djamor var. roseus (27.24U/mg) and *Pleurotus florida* (23.29U/mg) was recorded at 90 seconds and 100 seconds respectively. However, *Pleurotus* djamor var. Roseus showed maximum POX activity (0.59U/ml) at 15 min and *Pleurotus* florida gave maximum activity of 0.42U/ml after 20 minutes of incubation. Further decrease in SOD and POX activity was reported with increase in incubation time.

Effect of enzyme concentration

The concentration of enzyme used in the reaction mixture has its own significance in converting the substrate into the product and therefore influence the activity of SOD and POX. The crude enzyme extract from *Pleurotus*djamorvar.roseus showed 27.59U/mg SOD activity at concentration of 300 µl whereas*Pleurotusflorida* showed 24.09U/mg activity with 400µl crude enzyme extract. Further purification/processing may attribute to higher enzyme activity. The crude enzyme from *Pleurotus*djamor var. roseus showed 0.72U/ml POX activity at 70µl concentration followed by 0.59 activity units in *Pleurotusflorida*at 90µl. A further increase in volume of crude mushroom extract resulted in abrupt decline in the POX activity. The high inhibition value of *Pleurotus* may be due to the high phenolic residues in the extracts [12].

Conclusion

In this work, variable results were observed in *Pleurotus species* (*Pleurotus*o streatus, *Pleurotus* djamor var. roseus and *Pleurotus* florida) for the extraction of intracellular anti-oxidative enzymes (Superoxide dismutase and Peroxidase) using different parameters. Solvent extracts, homogenization with buffer systems of *Pleurotus* spp. with or without ultrasonication showed different levels of SOD activity and inhibition patterns of NBT reduction causing variable inhibition. Anti-oxidative stress enzymes (SOD and POX) revealed maximum activity in ethanolic extract of *Pleurotus* djamorvar. roseus with potassium phosphate buffer followed by methanolic extract of *Pleurotus florida* with phosphate citrate buffer. Maximum SOD (26.70U/mg) and POX (0.51U/ml) was recorded at pH 6.0 (potassium phosphate buffer, 1M) with P. djamor var. roseus and (22.12U/mg) and (0.39U/ml) for P. *Florida* (phosphate citrate buffer, pH 6.5). The levels of anti-oxidative enzymes as reported in the present work are quite encouraging and indicate their usefulness for such enzymes, but more efforts and extensive studies are needed to derive and to reach a definite conclusion.

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References

1. Yang JH, Lin HC, Mau JL (2002) Antioxidant properties of several commercial mushrooms. J Agric Food Chem 50(21): 6072-6077.
2. Mattila P, Salo V, Ananen P, Onko OK, Aro H, et al. (2002) Basic composition and amino acid contents of mushrooms cultivated in Finland. J Agric Food Chem 50(22): 6419-6422.
3. Kues U, Liu Y (2000) Fruiting body production in basidiomycetes. Applied Microbiology and Biotechnology 54(2): 141e152.
4. Endo A (1988) Chemistry, biochemistry, and pharmacology of HMGCoA reductase inhibitors. Klinische Wochenschrift. 66(10): 421-427.
5. Kakkar P, Das B, Viswanathan PN (1984) A modified spectrophotometer assay of superoxide dismutase. Indian J Biochem Biophys 21(2): 130-132.
6. Mahadevan R, Sridhar (1982) Methods in Physiological Plant Pathology, (2nd edn), Sivakami Publications, Madras, India.
7. Ramkumar L, Ramanathan T, Thirunavukkarasu P, Arivuselvan N (2010) Antioxidant and radical scavenging activity of nine edible mushrooms extract. International journal of Pharmacology 6(6): 950-953.
8. Adebayo EA, Oloke JK, Ayandele AA, Adegunlola CO (2012) Phytochemical, antioxidant and antimicrobial assay of mushroom metabolite from *Pleurotus* pulmonarius –LAU09 (JF736658) Journal of Microbiology and Biotechnology Research 2(2): 366-374.
9. Jose N, Janardhanan KK (2000) Antioxidant and antitumour activity of *Pleurotus florida*. Curr Sci 79 (7): 941-943.
10. Keles A, Koca Í, Genccelep H (2011) Antioxidant properties of wild edible mushrooms. J Food Process Technol 2: 130.
11. Yim H S, Chye FY, Ho SK, Ho CW (2009) Phenolic profiles of selected edible wild mushrooms as affected by extraction solvent, time and temperature. Asian Journal of Food and Agro-Industry 2(03): 392-401.
12. Turkoglu A, Mercan D, Nazime K, İbrahim G (2007) Antioxidant and antimicrobial activiti es of *Laetiporus sulphureus* (Bull.) Murril. Food Chemistry 101(1): 267-273.

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