



International Journal of Applied Sciences and Biotechnology

A Rapid Publishing Journal

ISSN 2091-2609

Indexing and Abstracting

CrossRef, Google Scholar, Global Impact Factor, Genamics, Index Copernicus, Directory of Open Access Journals, WorldCat, Electronic Journals Library (EZB), Universitätsbibliothek Leipzig, Hamburg University, UTS (University of Technology, Sydney): Library, International Society of Universal Research in Sciences (EyeSource), Journal Seeker, WZB, Socolar, BioRes, Indian Science, Jadoun Science, Jour-Informatics, Journal Directory, JournalTOCs, Academic Journals Database, Journal Quality Evaluation Report, PDOAJ, Science Central, Journal Impact Factor, NewJour, Open Science Directory, Directory of Research Journals Indexing, Open Access Library, International Impact Factor Services, SciSeek, Cabell's Directories, Scientific Indexing Services, CiteFactor, UniSA Library, InfoBase Index, Infomine, Getinfo, Open Academic Journals Index, HINARI, etc.

CODEN (Chemical Abstract Services, USA): IJASKD

Vol-3(3) September, 2015

Available online at:

<http://www.ijasbt.org>

&

<http://www.nepjol.info/index.php/IJASBT/index>



Impact factor*: 1.422
Scientific Journal Impact factor#: 3.419
Index Copernicus Value: 6.02
IBI Factor 2015:** 4.19

*Impact factor is issued by Universal Impact Factor. Kindly note that this is not the IF of Journal Citation Report (JCR).

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Research Article

ESTABLISHMENT OF ENZYME INHIBITORY ACTIVITIES OF LOVASTATIN, ISOLATED FROM PLEUROTUS OSTREATUS

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Abstract

Enzyme inhibition is significant biological process to characterize the enzyme reaction, extraction of catalysis parameters in bio-industry and bioengineering. The enzyme inhibitors are low molecular weight chemical compounds. Inhibitor can modify one amino acid, or several side chain(s) required in enzyme catalytic activity. To protect enzyme catalytic site from any change, ligand binds with critical side chain in enzyme. Safely, chemical modification can be done to test inhibitor for any drug value. In the present day world they are known for culinary values due to their high-quality proteins, vitamins, fibers and many medicinal properties and accordingly they are called nutraceuticals. Overproduction of ROS can damage cellular biomolecules like nucleic acids, proteins, lipids, carbohydrates, proteins and enzymes resulting in several diseases. The chemical nature of the bioactive compounds present in this mushroom includes: polysaccharides, lipopolysaccharides, proteins, peptides, glycoproteins, lectins, lipids and their derivatives. We report the presence of Statins in fruiting bodies and fermentation processes from *Pleurotus ostreatus*. Lovastatin extracted from the *Pleurotus ostreatus* showed Anti-oxidant Activity when compared with Standard Quercetin, IC₅₀ values of 3.5µg/ml and 7.5 µg/ml respectively, amylase Assay the percentage inhibitions of 35% and 70% with 1 and 3 mg/ml respectively. In Membrane Stability assay, the percentage inhibitions of 47.06% and 50%. Acid Phosphatase Assay: percentage inhibitions of 82.78% and 76.11% with 2mg/ml and 4mg/ml respectively. *Pleurotus* possess a good antioxidant and enzyme inhibitions for Alpha Amylase, Acid Phosphatase and membrane stability studies, hence as health promoter and environmental restorer is gaining more importance.

Key words: *Pleurotus ostreatus*; Lovastatin; DPPH assay; Amylase Assay; Acid Phosphatase Assay; Membrane Stability assay

Introduction

Pleurotus mushrooms, commonly known as oyster mushrooms, grow wild in tropical and subtropical areas, and are easily artificially cultivated. They are healthy foods, low in calories and in fat; rich in protein, chitin, vitamins and minerals (Akindahunsi and Oyetajob 2006; Manzi *et al.*, 1999; Mattila *et al.*, 2002). They also contain high amounts of γ -amino butyric acid (GABA) and ornithine. GABA is a nonessential amino acid that functions as a neurotransmitter whereas ornithine is a precursor in the synthesis of arginine (Jayakumar *et al.*, 2006). The extract of *P. ostreatus* was able to alleviate the hepatotoxicity induced by CCl₄ in rats, also reported to protect major organs such as the liver, heart, and brain of aged rats against oxidative stress (Jayakumar *et al.*, 2007).

Lovastatin is also naturally produced by certain higher fungi, such as *Pleurotus ostreatus* (oyster mushroom) and closely related *Pleurotus* sp. Research into the effect of oyster mushroom and its extracts on the cholesterol levels of laboratory animals has been extensive, although the effect has been demonstrated in a very limited number of human subjects. The primary uses

of lovastatin are for the treatment of dyslipidemia and the prevention of cardiovascular disease. It is recommended to be used only after other measures, such as diet, exercise, and weight reduction, have not improved cholesterol levels. *Pleurotus* used as a health promoter and environmental restorer is gaining more importance as compared to other medicinal mushrooms resulting in an upsurge in their R & D activities during the past two decades (Patel *et al.*, 2012).

Reported therapeutic values of *Pleurotus* explored to combat simple and multiple drug resistant isolates of *Escherichia coli*, *S. aureus* (Akyuz *et al.*, 2010), antimicrobial and antifungal activity of oyster mushrooms depended upon the nature of the solvent, ether extract were more active against Gram negative bacteria as compared to acetone extract (Iwalokun *et al.*, 2007). Ether and acetone extracts of oyster mushrooms were effective against *B. subtilis*, *E. coli* and *S. cerevisiae*. Later, not only intracellular proteins of *P. ostreatus* but its extracellular extract also contains polysaccharides that have immunomodulating effects (Selegan *et al.*, 2009).

Another ribonuclease, pleuteregine, was also isolated from both fresh and dried sclerotia of *P. tuber-regium* (Wang *et*

al., 1998) Later in the fruiting bodies of oyster mushrooms, they observed an ubiquitin-like protein having HIV-1 reverse transcriptase inhibitory activity. Similarly hot water extracts of *P. sajor-caju* and *P. pulmonarius* inhibit HIV-1 reverse transcriptase activity by SU2 molecule having 4.5 kDa transcriptase activities by SU2 molecule having 4.5 kDa mol. Wt (Wang *et al.*, 2007).

A lectin isolated from fresh fruiting bodies of *P. citrinopileatus* also inhibited HIV-1 reverse transcriptase (Li *et al.*, 2008). Preliminary reports indicated that diet containing 4-10 % dried fruiting body of *Pleurotus* species show more reduction in the arterial pressure and blood cholesterol level when compared to normal diet in rabbits and rats (Bobek *et al.*, 2001). Lovastatin, a drug, used in the lowering blood cholesterol level, produced by *P. ostreatus* was approved by FDA in 1987. When dried mushroom mixed in the diet of experimental animal acted as accelerator of HDL (high density lipoprotein), reduced production of VLDL (very low density lipoprotein). LDL (low density lipoprotein), cholesterol, reduced cholesterol absorption and reduced HMG-CoA reductase activity in the liver (Hossain *et al.*, 2003).

Statins are found to be an inhibitor of the enzyme hydroxymethyl glutaryl coenzyme A (HMG CoA) reductase that catalyzes the reduction of HMG-CoA to mevalonate during synthesis of cholesterol (Kurashige *et al.*, 1997; Bobek *et al.*, 1997).

In the present study Isolation of the enzyme lovastatin from *Pleurotus ostreatus*, determination of the total phenolic content & the total flavanoid content, pharmacological Assay of amylase, acid phosphatase and membrane stability, determination of lovastatin from High Performance Liquid Chromatography were carried out.

Materials and Methods

Chemicals

All of the chemicals used in this work were purchased from Merck, Loba Chem. 1,1-diphenyl-2-picryl-hydrazil (DPPH) S. D. Fine Chem. Ltd - (Mumbai - India), solvents and other reagents of analytical grade were from E.Merk (Mumbai, Maharashtra, India).

Selection of Microbial Material

Microbial material collected from BIOCENTER, Hulimavu, Bengaluru, India, in March 2015. Voucher specimens were deposited in the Herbarium of R&D, C.D. Sagar Centre Department of P.G. Studies Biotechnology Department, Dayananda Sagar College, Bengaluru.

Extracts and Solutions

Oyster mushrooms were air dried at room temperature and finely grounded. Each sample (100 g) was macerated with ethanol 90% (500 ml) three times. Solvent was evaporated under reduced pressure at approximately 40°C. The dried extracts were dissolved in ethanol 90% to a final

concentration of 1000µ/ml -1 (sample stock solution), then the different concentrations of the sample (100, 50, 40, 30, 20, 10,) µg/ml were used.

Antioxidant Assay

Sample preparation: - Add 1gm dried powder of *pleurotus ostreatus* in 10 ml of methanol and shaken vigorously then filtered the mixture. Filtrate was taken and used as sample.

DPPH solution: - 5mg in 2ml methanol. Assay: - Three tubes were taken and marked as blank, test tube 1, test tube 2. 0.5 ml of sample was poured in test tube 1 and 1ml of sample was poured in test tube 2, test tube blank was kept empty. 3ml of DPPH was poured in all three test tubes and kept for incubation for 15 minutes and spectrophotometric reading was taken at 510 nm against suitable blank

Total Flavanoids

Sample:-Aqueous extracts of *pleurotus ostreatus* was prepared by adding 1 gm dried powdered oyster mushroom in 10 ml distilled water NaNO₂ (5%):- dissolve 5 gms of NaNO₂ in distilled water and make up the volume to 100 ml. AlCl₃.H₂O (10%):- dissolve 10 gms in distilled water and make up the volume to 100 ml NaOH (1M) 10 ml: - dissolve 0.4 gms in distilled and make the volume up to 10 ml.

Method -Take three test tubes and mark them 1, 2 and 3. Pour 0.2 ml, 0.4ml, 0.6ml sample in test tube 1,2 and 3 respectively. Then add 4 ml distilled water to all the tubes. Then add 0.3ml NaNO₃

to all tubes and after five minutes add 0.3ml AlCl₃ to all tubes. Then add 2 ml NaOH to all the test tubes. Pour distilled water to make up the volume to 10 ml. Measure O.D. at 510 nm.

Total Phenol Content

Four test tubes were taken and marked 1,2,3,4. 0.5 ml and 1 ml of methanolic extract of oyster mushroom was poured into test tube 1 and test tube 2. 0.5 ml and 1 ml of standard was poured into test tube 3 and test tube 4. To each test tubes 1 ml of Folin-Ciocalteu reagent was poured. Then 0.5 ml of 7.5% Sodium Carbonate solution was poured into each tube. The tubes were kept for incubation at room temperature for 30 minutes and the absorbance was measured at 765 nm.

Membrane Stability Assay

Preparation of Drugs

Standard drug (Ibuprofen 2.5 mg per ml). Standard drug extract was prepared in isosaline (0.85% w/v of NaCl) to final concentration of 3mg/ml and 5mg/ml. Preparation of Sheep Red Blood Cell: - Fresh Sheep blood samples were collected in an anticoagulant. Blood samples were centrifuged at 3000 rpm for ten minutes at room temperature. The supernatant was carefully removed while packed RBC was washed in fresh normal saline (0.85%

NaCl). The process was repeated till the supernatants were clear.

Method

The membrane stabilizing activity was carried out using sheep's erythrocyte suspension while ibuprofen was used as the standard drug. The assay consisted of 2ml hypo saline (0.25% NaCl). 1 ml of 0.15 M phosphate buffer pH 7.4, 0.5 ml of sheep's erythrocyte suspension. 0-1 ml drugs and final volume of the mixture was made up to 4.5 ml with isosaline. Drugs were not added in the blood control while the drug control did not contain erythrocyte suspension. The reaction mixture was incubated at 56°C for 30 minutes on a water bath followed by centrifugation and the absorbance of the released hemoglobin was read at 560 nm.

Calculations were done using the expression

$$\text{Percentage Haemolysis} = \frac{\text{O.D. of Test}}{\text{O.D. of Drug Control}} \times 100$$

$$\text{Percentage Inhibition} = 100 - \text{Percentage Haemolysis}$$

Alpha Amylase (saliva)

Preparation of the Enzyme

a) Salivary amylase:-10 ml of saliva was collected and diluted to 100 ml with cold phosphate buffer of pH 7.1. The solution was centrifuged at 5000 rpm for 20 minutes and the clear supernatant was used.

b) Extraction of the crude inhibitor:-500 mg of dried powder of *pleurotus ostreatus* was extracted with 2.5 % of cold TCA with magnetic stirrer for 45 minutes. The solution was centrifuged to get clear supernatant and was used as for assay of enzyme inhibition. This is referred to as crude inhibitor.

Amylase Assay

1 ml of the enzyme solution was added to 2 ml of phosphate buffer pH 7.1 and reaction started with addition of 2ml of 1% starch solution. The tubes were incubated for 20 minutes at 37°C. The reaction was arrested by addition of DNS colour reagent. The tubes were kept in boiling water bath for ten minutes, cooled and diluted to final volume of 10 ml with distilled water. The absorbance was read at 530 nm.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Acid Phosphatase Assay

Extract preparation

Aqueous extract of *pleurotus ostreatus* was prepared by adding 500mg of dried *pleurotus* powder to 10 ml of distilled water.

For the assay 6 tubes were taken and marked from 1 to 6. To all the six tubes 1 ml of citrate buffer was poured. Test tube 4 was marked F (Presence of fluoride inhibitor). 0.5 ml of enzyme was poured to the tubes 2 and 4. 1 ml of enzyme was poured to tube 3. To tube 1 enzyme will not be poured. 0.5 ml and 1 ml of aqueous extract of *pleurotus ostreatus* was

poured to tube 5 and tube 6 respectively. Volume of all the tubes were made to 1 ml by adding distilled water except tube 4 where volume was made up by adding 0.5 ml sodium fluoride. Tubes were kept for incubation at 37°C in water bath for 5 minutes. After the temperature was equilibrated 5 ml of PNPP was added to each tube and kept for incubation at 37°C for 10 minutes. After incubation 3ml of 0.1 M NaOH was poured to all the tubes. Measure the absorbance of each tube at 530 nm

HPLC

Mobile Phase Preparation

0.68g of potassium dihydrogen ortho phosphate was weighed and stirred in 100 ml of HPLC grade distilled water, sonicated and pH was adjusted to 3 by orthophosphoric acid. This solution was filtered using a 0.45 micron Millipore filter paper. Combination of buffer (30%) and Methanol (70%) was taken and made up to 1000ml using HPLC grade water (Ratio of mobile phase: 70 : 30).

Standard Stock Solution

10 mg of provasatin (standard) was taken in 10 ml volumetric flask and methanol was added up to mark and sonicated for 3 min then further diluted to 10 folds and the final conc. of this solution is 100 µl/ml from this 20micro liter were injected and chromatogram was recorded.

Sample Preparation

10 mg of extract was taken in 10ml volumetric flask to this 10ml of methanol was added (the conc. of this solution 1000 µl/ml) and sonicated Then the solution was filtered using 0.45micron Millipore filters from this 20micro liter were injected and chromatogram was recorded.

Optimized Chromatographic Conditions

Detector : Shimadzu spd10A uv-vis, Japan

Pump: LC-10ATVP, Japan

Column: Phenomenex 5 µm C18 (2) 100 Å, LC Column 250 x 4.6 mm, Ea

Elution A: Methanol

Elution B: Potassium di hydrogen ortho phosphate pH 3, ratio of 70:30

Flow Rate: 1ml/min

Col. Temp: ambient

Detection: UV-Vis Abs.-Variable Wave. (UV) @ 235 nm

Result and Discussions

Antioxidants interfere with the production of free radicals and play a key role to inactivate them. Phytochemicals like carotenoids, tocopherols, ascorbates and phenols present in plants are strong natural antioxidant and have an important role in health care system. Since it is ideal to evaluate an alternative microbial source like *Pleurotus ostreatus*, we

report the presence of statins in fruiting bodies and fermentation processes from *Pleurotus ostreatus*.

Reactive oxygen species (ROS) are produced in the cells by cellular metabolism and other exogenous environmental agents. They are generated by a process known as redox cycling and are catalyzed by transition metals, such as Fe²⁺ and Cu²⁺ (Halliwell *et al.*, 1996). Overproduction of ROS can damage cellular biomolecules like nucleic acids, proteins, lipids, carbohydrates, proteins and enzymes resulting in several diseases. Living systems have specific pathways to overcome the adverse effects of various damages. However, sometimes these repair mechanisms fail to keep pace with such deleterious effects (Nilsson *et al.*, 2004).

Antioxidants scavenge free radicals and are associated reduced risk of cancer and cardiovascular diseases. Statins are found to be an inhibitor of the enzyme hydroxymethylglutaryl coenzyme A (HMGCoA) reductase that catalyzes the reduction of HMG-CoA to mevalonate during synthesis of cholesterol (Endo, 1992; Bobek *et al.*, 1997). Antioxidants interfere with the production of free radicals and also play a key role to inactivate them (Lobo *et al.*, 2010). Phytochemicals like carotenoids, tocopherols, ascorbates and phenols present in plants are strong natural antioxidant and have an important role in health care system. Phenols, a major group with antioxidant properties, comprise subclasses such phenolicacids, flavonoids, biflavonoids, anthocyanins and isoflavonoids and act against allergies, ulcers, tumours, platelet aggregation, cardiovascular diseases and can reduce the risk of cancer (Block, 1992; Bingham *et al.*, 2003.)

Therefore, the great interest has been recently focused on the natural foods, medicinal plants and phytoconstituents due to their well-known abilities to scavenge free radicals (i.e. antioxidant power) (Hou *et al.*, 2003; Galvez *et al.*, 2005; Kukic *et al.*, 2006). Polyphenols possess many biological effects. These effects are mainly attributed to their antioxidant activities in scavenging free radicals, inhibition of peroxidation and chelating transition metals. In generally, polyphenols all share the same chemical patterns, one or more phenolic groups for which they react as hydrogen donors and in that way neutralize free radicals (Heinonen *et al.*, 1998; Parejo *et al.*, 2002; Lee *et al.*, 2003; Miliauskas *et al.*, 2004; Atoui *et al.*, 2005; Galvez *et al.*, 2005; Melo *et al.*, 2005).

Statin is a well-known anti-hyperlipidemic agent that inhibits 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase that catalyzes the conversion of HMGCoA to mevalonic acid in the cholesterol synthesis pathway. Statins have also pleiotropic actions in the cardiovascular, immune and nervous systems (Liao *et al.*, 2005). Lovastatin is a prodrug that is reversibly converted into active [beta]hydroxyacid form by carboxyesterase enzyme

(Jadhav *et al.*, 2006). There are studies which reported that statins were able to increase bone formation and has potential for the treatment of osteoporosis and its complications by stimulation of bone morphogenetic proteins-2 (BMP-2) (Mundy *et al.*, 1999). However, the oral dose of statins given to lower serum cholesterol may unlikely have any action on bones as they are metabolized in the liver, resulting into much lower concentration reaching the fracture site (Hamelin *et al.*, 1998). Therefore, high doses of oral statins are needed to reap the benefit of their anabolic actions on bone. However, high doses of statins were associated with liver failure, kidney disease and rhabdomyolysis (Guyton, 2006).

In recent years one of the areas which attracted a great deal of attention is the possible therapeutic potential of antioxidants in controlling degenerative diseases associated with marked oxidative damage. The anti-oxidant assay of lovastatin extracted from *Pleurotus ostreatus* when compared with quercetin gave IC₅₀ values of 3.5µg/ml and 7.5 µg/ml respectively. (Table: 1) Phenolic compounds are a large group of structurally diverse naturally occurring compounds that possess atleast a phenolic moiety in their structures. Most of these compounds possess various degrees of antioxidant or free radical scavenging properties as well as medicinal properties and have long been used as drugs. For total phenol content 1ml of lovastatin extracted from *Pleurotus ostreatus* when compared with 1ml of quercetin (standard) gave a percentage response of 43.5 ± 0.07 and 40.0 ± 0.058 respectively (Table:2).

Table 1: Antioxidant Assay

S.N.	Samples	IC ₅₀ (µg/ml)
1	Quercetin (Standard)	7.5
2	Lovastatin (From <i>Pleurotus ostreatus</i>)	3.5

Table 2: Total Phenol Content

S.N.	Extract	Vol. of extract (mg/ml)	% Response
1	Lovastatin (from <i>Pleurotus ostreatus</i>)	0.5	30.21±0.17
2	Lovastatin (from <i>Pleurotus ostreatus</i>)	1	43.5±0.07
3	Quercetin (standard)	0.5	34.0±0.12
4	Quercetin (standard)	1	40.0±0.058

Flavonoids are a class of natural phenolic compounds with several biological activities. For total flavanoid content 0.2 µg/ml lovastatin extracted from *Pleurotus ostreatus* when compared with 0.2 µg/ml of quercetin gave IC₅₀ values of 10 ± 0.12 and 6.2 ± 0.068 respectively whereas when 0.6 µg/ml lovastatin extracted from the same source was compared with 0.6 µg/ml of quercetin IC₅₀ values of 26 ± 0.22 and 10.0 ± 0.057 respectively were obtained (Table: 3).

Table 3: Total Flavanoid Content

S.N.	Extract	Conc. of extract(µg/mg)/Ic ₅₀		
1	Lovastatin (from <i>Pleurotus ostreatus</i>)	0.2	0.4	0.6
		10 ± 0.12	21 ± 0.17	26 ± 0.22
2	Quercetin (standard)	6.2 ± 0.068	8.2 ± 0.042	10.0 ± 0.057

Enzyme inhibition studies with the help of acid Phosphatase assay using the organic extract of *Pleurotus ostreatus* showed percentage inhibitions of 82.78% and 76.11% with 2mg/ml and 4mg/ml of Lovastatin (extracted from the same source) respectively. Membrane Stability assay showed percentage inhibitions of 47.06% and 50% with 2 and 3 mg/ml concentration of the extract of *Pleurotus ostreatus*, amylase assay revealed percentage inhibitions of 35% and 70 % with 1 and 3 mg/ml of Lovastatin (extracted from the same source) respectively (Table 4, 5, 6).

Table 5: Amylase Assay

S.N.	Extract	Conc./O.D.					
1	Lovastatin (standard)	0.5mg/ml	1 mg/ml	1.5 mg/ml	2 mg/ml	2.5 mg/ml	3 mg/ml
		0.10	0.20	0.25	0.30	0.35	0.40
2	Lovastatin (From <i>Pleurotus ostreatus</i>)	0.012	0.014	0.016	0.019	0.022	0.028

Table 6: Membrane Stability Assay.

S.N.	Samples	Conc./O.D.	
1	(Drug - Control)	2 mg/ml	3 mg/ml
		0.17	0.12
2	(Drug + Erythrocyte)	0.21	0.20
3	(Drug + Sample)	0.09	0.06

Note: Test Drug: Ibuprofen. Test Sample: *Pleurotus ostreatus*

Fruiting bodies of *Pleurotus* possessed higher concentration of antioxidants than other commercial mushrooms (Mau *et al.*, 2001; Yang *et al.*, 2002; Lo, 2005). This activity was mainly due to presence of polysaccharide pleuran (β -glucan) that has been isolated from *P. ostreatus* showing a positive effect on rat colon with pre-cancerous lesions (Bobek *et al.*, 2001). *P. ostreatus* increased the activities of important antioxidant enzymes (*viz.* superoxide dismutase, catalase and peroxidase) thereby reducing oxidative damage in humans (Yang *et al.*, 2002; Keyhani *et al.*, 2007). Oyster mushrooms are now widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases due to their higher free radical scavenging activities. These free radical scavenging activities of oyster mushrooms depend upon the colour of fruiting bodies (Yang *et al.*, 2002).

Table 4: Acid Phosphatase Assay

S.N.	Extract	Conc. of extract (mg/ml)/O.D.(nm)			
1	Lovastatin (from <i>Pleurotus ostreatus</i>)	0.5	1	2	4
		0.022	0.044	0.062	0.086
2	Lovastatin (standard)	0.01	0.021	0.041	0.062

Fruiting bodies of oyster mushrooms have higher phenol concentration when compared with mycelium and fermentation broth filtrate of *P. citrinopileatus* (Lee *et al.*, 2000; Jose *et al.*, 2002 & 2004; Mau *et al.*, 2002). These phenolic compounds have free radical scavenging property that reduces inhibitory effects of mutagens and carcinogens (Gezer *et al.*, 2006). Recently a very surprising result was shown by the juvenile bud stage (one day stage) contained highest amount of phenols (2.79 mg/g) and antioxidants that gradually decreased (1.27 mg/g), but upon maturity (four day stage), the total concentration of total phenol was again increased (2.08 mg/g) (Saha *et al.*, 2012) Similar result was found when they compare the *in vitro* antioxidant capacity of acetone extracts and petroleum ether extract (Iwalokun *et al.*, 2007).

The extract from *P. ostreatus* inhibited the growth of HL-60 cells by cell cycle arrest i.e. by the induction of apoptosis by their experiments due to the presence of flavonoid (quercetin equivalent) and phenolics components (catechin equivalent) in fruiting bodies (Venkatakrishnana *et al.*, 2010). Methanolic extract of *P. ostreatus* significantly enhanced the activity of antioxidant enzymes (Ramkumar *et al.*, 2010) these results were comparable to our present study.

Pleuran, isolated from fruiting bodies of *OM* possesses anti-inflammatory activity (Bobek *et al.*, 2001; Nosál'ová *et al.*, 2001). Extracts of many of them e.g. *P. florida*, *P. pulmonarius* etc. give a lowering response in both acute as well as in chronic inflammation (Jose *et al.*, 2002 & 2004) and when oral or percutaneous administration of extract of *P. eryngii* was done, it suppress the inflammation in delayed type (type IV hypersensitive) allergy response in mice (Sano *et al.*, 2002; Jedinak *et al.*, 2011) in their experiments also observed that anti-inflammatory activity of Oyster mushrooms that was mediated through the inhibition of NF-kB and AP-1 signalling. Another potent anti-inflammatory agent, a polysaccharide has been extracted from the *P. pulmonarius* that acted against carrageenan and formalin-induced paw edema in rats (Adebayo *et al.*, 2012).

Extracts of *P. abalonus* elevated levels of vitamin C and E, increased activities of catalase, superoxide dismutase and glutathione peroxidase in aged rats (Shashoua *et al.*, 2004). Similar results were obtained with the extracts of *P. ostreatus* (Jayakumar *et al.*, 2007). These enzymes are known potent antioxidant enzymes (Bobek *et al.*, 2001; Keyhani *et al.*, 2007). The levels of malondialdehyde, a polyunsaturated lipid and an electrophilic mutagen, was lowered on administration of mushroom extract to aged rats (Buddi *et al.*, 2002), that reacted with deoxyadenosine and deoxyguanosine in DNA, forming a DNA adduct. Different extracts (methanol, ethanol, acetone or water extract) of *Pleurotus* can improve the antioxidant status during ageing leading reducing the occurrence of age-associated disorders

like stroke, Parkinson's disease, atherosclerosis, diabetes, cancer and cirrhosis.

Conclusion

The mushroom fruiting body, its mycelium and their extracts or concentrates have been considered a functional food as it has the potentiality to control many human ailments. Though in most cases biological activity is better understood but in many cases there is need to identify the active principle to understand the exact mechanism(s) for its exploration in right perspective. Availability of high-tech methods should allow the researchers to explore novel metabolites from Oyster mushrooms, standardize there *in vitro* pharmacological effectiveness and go for clinical trial with a goal to meet international standards, to study the mechanism of action in Nutrigenomics with a biomarker-based approach for up-grading *Pleurotus* from functional food to holistic medicine.

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APPENDIX

HPLC analysis of Std: Provastatin



HPLC analysis of Std: Rosovastatin



HPLC analysis of Sample: *Pleurotus ostreatus*



HPLC

Content of provastatin in the extract

Purity	99.3
Sample area	109373.031
Standard area	1615787.25
Sample weight	10
Standard weight	10
Sample dilution ml	10
Standard dilution ml	100
%content of provastatin in the extract	0.672164109