FREE RADICAL SCAVENGING AND NOS ACTIVATION PROPERTIES OF WATER SOLUBLE CRUDE POLYSACCHARIDE FROM PLEUROTUS OSTREATUS

PAYEL MITRA, SOMANJANA KHATUA, KRISHNENDU ACHARYA*

Molecular and Applied Mycology and Plant Pathology Laboratory, Department of Botany, University of Calcutta, Kolkata, West Bengal - 700019, India. Email: krish_paper@yahoo.com

ABSTRACT

The aim of this study was to investigate the antioxidant activity using different *in vitro* assays and nitric oxide synthase activation property of crude water soluble polysaccharide from *P. ostreatus*. The polysaccharide was isolated by hot water extraction and physico-chemical investigation revealed that it contained high amount of carbohydrate (mostly β-glucan) and low amount of protein and phenolic compounds. EC_{50} values for scavenging of hydroxyl radical and superoxide radical as well as chelating ability of ferrous ion were 665, 390 and 370 μg/ml respectively. The polysaccharide also showed potential nitric oxide synthase activation properties. This mushroom might be a good source of bioactive compounds.

Keywords: Nitric Oxide, Polysaccharide, Physico-Chemical Parameters, Reactive Oxygen Species.

INTRODUCTION

Reactive oxygen species (ROS) including superoxide anion radical, hydroxyl radical and hydrogen peroxide are produced through oxidative process within the mammalian body. They are degraded by many defence mechanisms against oxidative stress, including antioxidant enzymes and non-enzymatic compounds [1]. Under certain circumstances including synthetic (xenobiotic) chemicals, radiation, x rays, pollution, stress etc the natural antioxidant defence mechanisms become insufficient and then the uncontrolled production of reactive oxygen species can damage both the structure and function of cell membrane in a chain reaction leading to degenerative diseases and conditions such as Alzheimer, arteriosclerosis, cardiovascular disease, nephritis, diabetes mellitus, acute liver toxicity, rheumatism, inflammation, ageing process, cataracts and carcinogenesis [2,3]. In recent years, interest has augmented considerably in finding naturally occurring antioxidants for use in foods, cosmetics or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity [4].

Nitric oxide (NO), which is biosynthesized at the cellular level from L-arginine catalyzed by nitric oxide synthase (NOS), is a very important signalling molecule from the ground of pathophysiologic condition of living entities. The omnipresence of NO in the living body suggests that NO plays an important role in the maintenance of health [5]. It is well studied in mammalian system and has been found to have numerous roles in pathophysiology [6,7]. Cellular production of NO below physiologic level causes initiation of different diseases like hypertension, atherosclerosis, diabetes mellitus, ischemia, stroke, myocardial infraction, heart failure, hypoxia, Alzheimer disease, fibrosis, cancer, renal failure, etc. [8]. In recent years, the NO signalling pathway has become a target for new drug development. The high level of flavonoids, catechins, tannins and other polyphenolic compounds present in vegetables, fruits, mushrooms, soy, tea and even red wine is believed to contribute to their beneficial health effects [5]. Thus, NOS activation by supplementation of food would find a new route of therapy.

Now-a-days, mushrooms have become attractive as functional foods and as a source of physiologically beneficial medicines, while being devoid of undesirable side effects [9]. Edible mushrooms are highly nutritious and having therapeutic potentiality for the treatment of cancer, heart ailments, diabetes, inflammation, hepatic damage, high blood pressure, microbial pathogens etc [10-17]. Recent investigations revealed that polysaccharides and extracts of mushrooms had strong antioxidant and NO synthase activation properties [18-24].

Therefore, the primary aim of this study was to isolate crude polysaccharide (POCP) from *P. ostreatus* basidiocarps and to investigate its antioxidant activity and NOS activation property.

MATERIAL AND METHODS

Collection of Mushroom

Mushrooms, *P. ostreatus*, were purchased from mushroom cultivation unit of Narendrapur Ramakrishna Mission Ashrama, Narendrapur, West Bengal, India. Fruit bodies were dried in the oven for few days until all became desiccated.

Chemicals

Ferric chloride, L-methionine, nitro-blue tetrazolium (NBT), riboflavin, 2-Deoxy-D-ribose, hydrogen peroxide (H_2O_2), ferrozine, ferrous chloride, L-arginine, trichloro acetic acid (TCA), thiobarbituric acid (TBA), ascorbic acid, standards such as, ethylenediaminetetraacetic acid (EDTA), butylated hydroxyltoluene (BHT), butylated hydroxyanisole (BHA), gallic acid, bovine serum albumin (BSA) were purchased from Sigma chemicals Co. (St. Louis, MO, USA). An analytical mushroom β-glucan kit was obtained from Megazyme Int. (Wicklow, Ireland). All other chemicals and reagents used were of analytical grade.

Sample Preparation

Dried and powdered fruit bodies of *P. ostreatus* were extracted with ten volume of 99% ethanol for two days at room temperature to remove phenolic compounds and lipid. Residue was filtered and re-extracted with ethanol. The air dried filtrate was suspended and refluxed with distilled water. It was maintained at boiling condition for eight hours. The extract was filtered through gauze and was concentrated to one-tenth of the volume with a rotary evaporator at 80°C under vacuum. Four volumes of absolute alcohol were added to precipitate polysaccharide and was left at 4°C overnight. After centrifugation, the pellets were washed with 70% (v/v) ethanol and successively by ethyl acetate and acetone. The washed pellets were dissolved in water. At last it was lyophilized to yield crude polysaccharide. POCP was then redissolved in distilled water for further works.

Physico-Chemical Characterization

Determination of Polysaccharide Content

The total polysaccharide content was determined using phenol-sulphuric acid method with D-Glucose as a reference [25]. Total

reaction mixture (4 ml) consisted of 100 µg of POCP, 0.5 ml of 6% phenol and 2.5 ml of concentrated sulphuric acid. It was incubated for 30 min at room temperature and absorbance was taken at 490 nm. Results were expressed as g of glucose equivalents/100 g of the dry polysaccharide.

Determination of Glucan Content

Contents of total and α-glucan were determined using the Mushroom and Yeast β-glucan Assay Procedure (Megazyme Int.) kit. The enzyme kit contained exo-1, 3-β-glucanase, amyloglucosidase, invertase, β-glucosidase, glucose determination reagent and glucose standard solution. To estimate the total glucan content, HCl hydrolysed polysaccharide was subjected to enzymatic degradation by exo-β- (1-3) –β-glucanase and β-glucosidase. Free glucose was determined by addition of glucose oxidase/peroxidase reagent spectrophotometrically. The α glucan content was estimated upon enzymatic hydrolysis with amyloglucosidase plus invertase yielding glucose. Again glucose oxidase/peroxidase reagent was added to determine α-linked glucose. The β glucan content was calculated by subtracting α glucan from total glucan content according to kit`s manual. All values of glucan content were expressed as g/100 g of dry weight of the polysaccharide.

Measurement of Protein Content

Protein content was measured according to the method of Bradford [26]. BSA (0.01 – 0.1 mg/ml) was used to produce standard calibration curve. Total protein content of the extracts was expressed as g of BSA equivalents/100 g of dry polysaccharide.

Determination of Phenol Content

The total phenolic compounds present in the crude polysaccharide were estimated according to the method given by Ziestin and Ben-Zaker [27]. Gallic acid (0.01 – 0.1 mg/ml) was used to draw the standard curve. Total phenol was expressed as g of gallic acid equivalents/100 g of dry polysaccharide.

Free Radical Scavenging Assays

Superoxide Radical Scavenging Activity

According to Martinez et al. [28] each 3 ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.8), 13 mM methionine, various concentrations (100 – 400 µg/ml) of POCP, 100 µM EDTA, 75 µM NBT and 2 µM riboflavin. Reaction started by illuminating sample with light and the absorbance was measured at 560 nm after 10 min of illumination. Identical tubes with the reaction mixture were kept in the dark and served as blank. BHA was used as a positive control. The degree of scavenging was calculated by the following equation:

Scavenging effect (%) = $\{(A_0-A_1)/ A_0\} \times 100$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of sample

Hydroxyl Radical Scavenging Activity

The method used by Halliwell et al. [29] was followed in this study. The reaction mixture (1ml) consisted of KH_2PO_4 – KOH buffer (20 mM, pH 7.4), 2-deoxy-D-ribose (2.8 mM), variable concentration (100 – 600 µg/ml) of POCP, $FeCl_3$ (100 mM), EDTA (104 µM), ascorbate (100 µM) and H_2O_2 (1 mM). It was incubated at 37°C for 1 h. 2ml thiobarbituric acid (TBA) and trichloroacetic acid (TCA) solution (0.375 w/v TBA, 15% TCA and 0.25 N HCl) was added and

incubated at boiling water bath for 15 min. After cooling, absorbance was measured at 535 nm. EC_{50} value in µg/ml expressed the effective concentration at which the It will be scavenging activity of free radical is 50%. BHT was used as positive control.

Chelating Ability of Ferrous Ions

Chelating ability was determined according to the method of Dinis et al. [30]. Reaction mixture (4 ml) contained different concentration of POCP (100 – 400 µg/ml) mixed with 3.7 ml of water and 0.1 ml of 2 mM ferrous chloride. The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine. After 10 min incubation at room temperature, the absorbance was determined at 562 nm against a blank. EDTA was used as positive control. The percentage of inhibition of ferrozine- Fe^{2+} complex formation is given by this formula:

% inhibition = $\{(A_0-A_1)/ A_0\} \times 100.$

Where A_0 was the absorbance of the control and A_1 the absorbance in the presence of mushroom

Determination of NOS Activity

NO was determined according to Jia et al. [31]. Typically, NO content was estimated by conversion of oxyhaemoglobin to methaemoglobin. The reaction mixture (2.5 ml) containing RBC (10^8 cells) was incubated with L-arginine (10 µM), haemoglobin (30 µM) and POCP for different time periods at 37°C. After each incubation period, a portion of reaction mixture was centrifuged at 8,000 g for 5 min at 37°C and NO content of the supernatant was compared with an appropriate control set.

Statistical Analysis

Results were subjected to statistical analysis using Student's t test. Values are mean ± SD of 3 replications.

RESULTS AND DISCUSSION

Physico-Chemical Characterization

Estimation of crude polysaccharide from dried fruit bodies gave the yield of 5.24%, which appeared to be white powder and highly soluble in water. The hot water extraction yield of crude polysaccharides from *Lentinus polychrous* showed a similar yield from dried fruit bodies [32] which were clearly higher than *Ganoderma tsugae*, 1.7% [33] and *Russula virescens*, 1.94% [34]. The chemical compositions of POCP were summarized in table-1. Total carbohydrate content of POCP was 62.67% which was more than that of *Ganoderma lucidum*, 56.6% [35]. Protein content of POCP when compared with few other samples was seen to be in the following increasing order POCP (15.75%) > *Agaricus brasiliensis* (7.3%) > *Phellinus linteus* (5.7%) > *Ganoderma lucidum* (2.6%) > *Agaricus bisporus* (0.9%) [36]. Total glucan content of POCP (43.9%) was also in a superior position than *Ganoderma applanatum* (22.4%), *Ganoderma lucidum* (36.3%), *Lentinus edodes* (42.6%), *Agaricus brasiliensis* (40.1%), *Ganoderma lucidum* (20.8%), *and Phellinus linteus* (24.5%) [35,36]. The α glucan and β glucan content of POCP were 3.9% and 40% respectively. Whereas α glucan content of *Lentinus edodes* (1.4%), and *Trametes versicolor* (2.9%) and the β glucan of *Ganoderma applanatum* (16%) was lower than POCP [35]. Very negligible amount of phenolic was detected from this crude polysaccharide though Kozarski et al. [35] reported that *Ganoderma applanatum, Ganoderma lucidum, Lentinus edodes, Trametes versicolor* had much higher amount of it.

Table 1: Contents of total polysaccharides, protein, phenolics, total glucan, α glucan and β glucan of crude polysaccharide.

Total Polysaccharide (gm/100gm)	Total Protein (gm/100gm)	Total Phenol (gm/100gm)	Glucan Content (gm/100gm)		
			Total Glucan	α Glucan	ß Glucan
62.67 ± 7.67	15.75 ± 0.75	4.5 ± 0.3	43.9 ± 1.2	3.9 ± 0.7	40 ± 1.5

Superoxide Radical Scavenging Activity

Superoxide radical is known to be very harmful to cellular components and plays a major role in the formation of other reactive oxygen species such as hydroxyl radical, hydrogen peroxide and singlet oxygen in living system. Regarding data presented in Figure

1, the scavenging activity of POCP increased with increasing concentration up to a certain level and reached a plateau. The EC_{50} value was found to be 390 and 30 µg/ml for POCP and BHA, respectively. Recently, a pure heteroglycan was isolated from *P. ostreatus* showed EC_{50} value of 553 µg/ml [22]. He et al. stated that the crude polysaccharide from *Agaricus bisporus* had EC_{50} value of 1.17 mg/ml [37]. The results suggested that the crude

polysaccharide exhibited scavenging effect on superoxide anion radical generation that could help prevent or ameliorate oxidative damage.
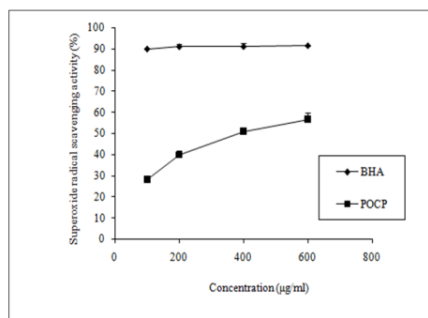
Figure 1: Superoxide radical scavenging activity of POCP. Results are the mean ± SD of three separate experiments, each in triplicate.

Hydroxyl Radical Scavenging Activity

Hydroxyl radicals are the major active oxygen species capable of modifying almost every molecule in the living cells. This radical has the capacity to cause strand damages in DNA leading to carcinogenesis, mutagenesis, and cytotoxicity. Furthermore, this radical is also capable of stealing hydrogen atoms from unsaturated fatty acids leading to quick initiation of lipid peroxidation process [38, 39]. Ferric – EDTA was incubated with H_2O_2 and ascorbic acid at pH 7.4. Hydroxyl radicals were formed in free solution and were detected by their ability to degrade 2-deoxy-2-ribose into fragments that formed a pink chromogen upon heating with TBA at low pH [40]. Data presented in Figure 2 showed that the crude polysaccharide POCP was a potent scavenger of hydroxyl radicals which increased in a concentration dependent manner. The EC_{50} value of the POCP was 665 µg/ ml. EC_{50} value of *Agaricus bisporus* and *Lentinus edodes* was seen to be 1.05 mg/ml and 1.90 mg/ml respectively [37]. Jayakumar et al. [41] found that the EC_{50} value of the ethanolic extract of *P. ostreatus* was 8 mg/ml. Patra and his co-workers [23], reported that EC_{50} value of a pure water soluble heteroglycan of *P. ostreatus* was 943 µg/ml, which was 1.5 fold higher than the present observation.
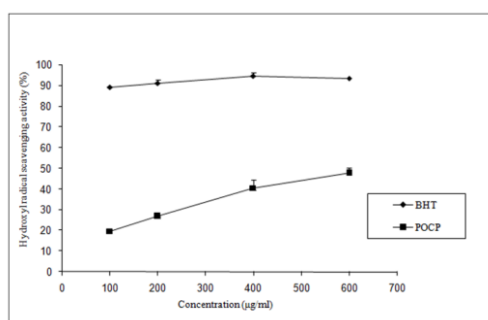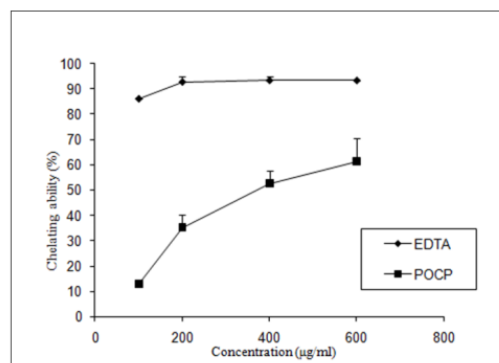

Figure 2: Hydroxyl Radical scavenging activity of POCP. Results are the mean ± SD of three separate experiments, each in triplicate.

Chelating Ability of Ferrous Ion

Like many transition metals Fe^{2+} can trigger process of free radical reaction such as hydroxyl radical formation. As the ferrous ions are effective prooxidants in food system, so ferrous ion chelating abilities to some extent would be beneficial [12]. The range and the mean of Fe^{2+} chelating capacity showed a marked capacity for iron binding ability. At 100 – 400 µg/ml, the chelating ability of this polysaccharide was between 12.85% and 52.55% (Figure 3). At the same concentration range, the chelating effect of the synthetic metal chelator, EDTA, was between 68.62% and 82.35%. The pure polysaccharide of *P. ostreatus* demonstrated iron binding ability of 54.82% at a concentration of 1 mg/ ml [23] whereas ethanolic

extract of *P. ostreatus* was seen to have an ability of 50% chelation at 6 mg/ml [41].

Figure 3: Ferrous ion chelating ability of POCP. Results are the mean ± SD of three separate experiments, each in triplicate.

Determination of NOS Activity

NO is recognized to be an inter and intra- cellular mediator of several cell functions. It acts as a signal molecule in immune, nervous and vascular systems [42]. POCP showed more than 2 fold increase in NOS activity when compared with the control. Our findings suggested that the crude polysaccharide is a potent activator of NOS. Similar observations were made earlier where hot water extract of *Astreus hygrometricus* [43], *Ramaria aurea* [20], *Volvariella volvacea* [21], *Meripilus gigantium* [22] were capable of inducing NOS in *in vitro* model system.
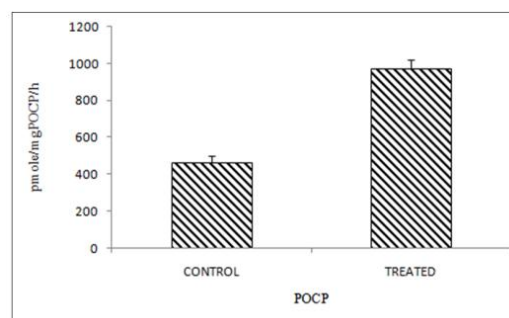
Figure 4: NOS Activation property of POCP. Results are the mean ± SD of three separate experiments, each in triplicate.

CONCLUSION

The data recorded in the above experiments showed that the crude polysaccharide of *P. ostreatus* had a good antioxidant property and NOS activation power, which might be good sources for the development of antioxidant food additives. Carbohydrate component mostly β-glucan seemed to be responsible for the antioxidant activity as the extract contained little amount of phenolic substances and protein. So, the crude polysaccharide of *P. ostreatus* could be used as a possible food supplement or in pharmaceutical industry.

REFERENCE

1. Halliwell B. Establishing the significance and optimal intake of dietary antioxidants: the biomarker concept. Nutr Rev 1999; 57: 104-13.
2. Rai M, Biswas G, Mandal SC, Acharya K. Free radicals and human diseases. In: Mandal SC, editor. Herbal Drugs: A modern approach to understand them better. Kolkata, India: New Central Book Agency (P) Ltd; 2011. p. 479-96
3. Wijk RV, Wijk EPV, Wiegant FA, Ives J. Free radicals and low level photon emission in human pathogenesis: State of the art. Indian J Exp Biol 2008; 46: 273-309.
4. Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, et al.The comet assay with 8 mouse organs: Results

with 39 currently used food additives. Mutat Res-Gen Tox En 2002; 519: 103-09.

5. Achike FI, Kwan CY. Nitric oxide, human diseases and the herbal products that affect the nitric oxide signalling pathway. Clin Exp Pharmacol Physiol 2003; 30: 605-15.

6. Ignaro LJ. Nitric Oxide Biology and Pathobiology. San Diego, CA: Academic Press; 2000.

7. Sinha AK, Acharya K, Bhattacharya S, Patra SC, Guha M, Ray U et al. Neutralization of "antineoplastin" of insulin activated nitric oxide synthase antibody and its effects in cancers. J Cancer Res Clin Oncology 2002; 128: 659-68.

8. Malinski T. Understanding nitric oxide physiology in the heart: a nano-medical approach. Am J Cardiol 2005; 96:13-24.

9. Sadler M. Nutritional properties of edible fungi. Nutr Bull 2003; 28: 305-08.

10. Acharya K. Medicinal properties of mushroom. In: Acharya SN Thomas JE, editors. Advances in Medicinal Plant Research. Kerala, India: Research Signpost; 2007. p. 215-36.

11. Biswas G, Rana S, Sarkar S, Acharya K. Cardioprotective activity of ethanolic extract of *Astraeus hygrometricus* (Pers.) Morg. Pharmacologyonline 2011; 2: 808-17.

12. Chatterjee S, Dey A, Dutta R, Dey S, Acharya K. Hepatoprotective Effect of the Ethanolic Extract of *Calocybe indica* on Mice with CCl$_4$ Hepatic Intoxication. Int J PharmTech Res 2011**;** 3: 2162-68.

13. Biswas G, Chatterjee S, Acharya K. Chemopreventive activity of the ethanolic extract of *Astraeus hygrometricus* (pers.) morg. on Ehrlich's Ascites Carcinoma cells. Dig J Nanomater Bios 2012; 7: 185-91.

14. Acharya K, Chatterjee S, Biswas G, Chatterjee A, Saha GK. Hepatoprotective effect of a wild edible mushroom on carbon tetrachloride-induced hepatotoxicity in mice. Int J Pharm Pharm Sci 2012; 4: 285-88.

15. Lai TK, Biswas G, Chatterjee S, Dutta A, Pal C, Banerji J, Bhuvanesh N, Reibenspies JH, Acharya K. Leishmanicidal and anticandidal activity of constituents of Indian edible mushroom *Astraeus hygrometricus*. Chem Biodivers 2012; 9: 1517-24.

16. Chatterjee S, Biswas G, Chandra S, Saha GK, Acharya K**.** Apoptogenic effects of *Tricholoma giganteum* on Ehrlich's ascites carcinoma cell. Bioprocess Biosyst Eng 2013; 36**:** 101-7.

17. Biswas G, Acharya K. Hypoglycemic activity of ethanolic extract of *Astraeus hygrometricus* (Pers.) Morg. in alloxan-induced diabetic mice. Int J Pharm Pharm Sci 2013; 5: 391-94.

18. Chatterjee S, Saha GK, Acharya K. Antioxidant activities of extracts obtained by different fractionation from *Tricholoma giganteum* basidiocarps. Pharmacologyonline 2011; 3: 88-97.

19. Acharya K, Chatterjee S, Ghosh S. Comparative evaluation on the free radical scavenging activity of eleven Indian cultivated strains of *Pleurotus ostreatus*. Pharmacologyonline 2011; 1: 440-50.

20. Rai M, Acharya K. Proximate composition, free radical scavenging and NOS activation properties of *Ramaria aurea*. Res J Pharm Technol 2012; 5: 1421-27.

21. Rai M, Acharya K. Evaluation of antioxidant and nitric oxide synthase activation properties of *Volvariella volvacea*. Int J Pharm Pharm Sci. 2012; 4: 460-63.

22. Acharya K, Rai M. Proximate composition, free radical scavenging and NOS activation properties of a wild edible mushroom. Int J Pharm Pharm Sci 2013; 4: 67-72.

23. Patra S, Patra P, Maity KK, Mandal S, Bhunia SK, Dey B et al. A heteroglycan from the mycelia of *Pleurotus ostreatus*: Structure determination and study of antioxidant properties. Carbohydr Res 2013; 368: 16-21.

24. Samanta S, Maity K, Nandi AK, Sen IK, Devi KSP, Mukherjee S et al. A glucan from an ectomycorrhizal edible mushroom *Tricholoma crassum* (Berk.) Sacc.: isolation, characterization and biological studies. Carbohydr Res 2013; 367: 33-40.

25. Dubois M, Gilles A, Hamitton JK, Reders PA, Smith F. Colorimetric method for determination of sugars related substances. Anal Chem 1956; 28: 350-56.

26. Bradford MM. A refined sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. Anal Biochem 1976; 72: 248-55.

27. Ziestin N, Ben-Zaker R. Peroxidase activity presence of phenolic substance in peduncles of rose flowers. Plant Physiol Biochem 1993; 31: 333-39.

28. Martinez AC, Marcelo EL, Marco AO, Moacyr M. Differential responses of superoxide dismutase in freezing resistant *Solanum curtibolum* and freezing sensitive *Solanum tuberosum* subjected to oxidative and water stress. Plant Sci 2001; 160: 505-15.

29. Halliwell B, Gutteridge JMC, Arumo OI. The deoxyribose method: A simple test tube assay for determination of rate constants for reactions of hydroxyl radicals. Anal Biochem 1987; 165: 215-19.

30. Dinis TCP, Mudaira VMC, Alnicida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-amino salicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys 1994; 315: 161-69.

31. Jia L, Bonaventua C, Bonaventura J, Stamler SJ. S-nitrosohemoglobin: A dynamic activity of blood involved in vascular control. Nature 1996; 380: 221-26.

32. Thetsrimuang C, Khammuang S, Chiablaem K, Srisomsap C, Sarnthima R. Antioxidant properties and cytotoxic properties of crude polysaccharides from *Lentinus polychrous* . Food Chem 2011; 128: 634-39.

33. Tseng YH, Yang JH, Mau JL. Antioxidant properties of polysaccharides from *Ganoderma tsugae*. Food Chem 2008; 107: 732-38.

34. Sun ZW, Zhang LX, Zhang B, Niu TG. Structural characterisation and antioxidant properties of polysaccharides from the fruit bodies of *Russula virescens.* Food Chem 2010; 118: 675-80.

35. D,Griensven LJLDV et al. Antioxidative activities and chemical characterization of polysaccharide extracts from the widely used mushrooms *Ganoderma applanatum, Ganoderma lucidum, Lentinus edodes* and *Trametes versicolor* J Food Comp Anal 2012; 26: 144-53.

36. Kozarski M, Klaus A, Nikšić M, Jakovljevic D, Helsper JPFG, Griensven LJLDV. Antioxidative and immunomodulating activities of polysaccharide extracts of the medicinal mushrooms *Agaricus bisporus, Agaricus brasiliensis, Ganoderma lucidum* and *Phellinus linteus*. Food Chem 2011; 129: 1667-75.

37. He JZ, Ru QM, Dong DD, Sun PL. chemical characteristics and antioxidant properties of crude water soluble polysaccharides from four common edible mushrooms. Molecules 2012; 17: 4373-87.

38. Kappus H. Lipid peroxidation-mechanism and biological relevance. In: Aruoma OI, Halliwell B editors. Free Radicals and Food Additives. London, UK: Taylor and Francis Press; 1991. p. 59-75.

39. Aruoma OI, Grootveld M, Halliwell B. The role of iron in ascorbate- dependent deoxyribose degradation. Evidence consistent with a site-specific hydroxyl radical generation caused by iron ions bound to the deoxyribose molecule. J Inorg Biochem 1987; 29: 289-99.

40. Aruoma OI, Laughton MJ, Halliwell B. Carnosine, homocarnosine and anserine: could they act as antioxidants *in vivo*? Biochem J 1989; 264: 863-69.

41. Jayakumar T, Thomas PA, Sheu JR, Geraldine P. *In-vitro and in-vivo* antioxidant effects of the oyster mushroom *Pleurotus ostreatus*. Food Res Int 2011; 44: 851-61.

42. Schmidt HHHW, Lohmann SM, Walter U. The nitric oxide and cGMP signal transduction system - regulation and mechanism of action. Biochem Biophys Acta 1993; 1178: 153-75.

43. Biswas G, Chatterjee S, Sankar S, Acharya K. Evaluation of antioxidant and nitric oxide synthase activation properties of *Astraeus hygrometricus* (Pers.) Merg. Intl J Biomed Pharma Sci 2010; 4: 22-6.