ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 8, Issue 5, 2015

ISSN - 0974-2441

Research Article

PHYTOCHEMICAL ANALYSIS AND *IN VITRO* ANTIOXIDANT ACTIVITY OF A WILD EDIBLE MUSHROOM *ENTOLOMA LIVIDOALBUM*

ADHIRAJ DASGUPTA[1], MANJULA RAI[2], KRISHNENDU ACHARYA[1]*

[2]Department of Botany, St. Joseph's College, Darjeeling - 734 104, West Bengal, India. Email: krish_paper@yahoo.com

Received: 01 June 2015, Revised and Accepted: 14 July 2015

ABSTRACT

Objective: In the course of inventorying and bioprospecting wild edible mushrooms of West Bengal, the ethanolic fraction of *Entoloma lividoalbum* was tested for its potential as an antioxidant *in vitro* and estimate the amounts of putative bioactive compounds present in it.

Methods: The fraction's antioxidant potential was tested by employing various *in vitro* assay systems, namely, total antioxidant capacity, chelating effect on ferrous ions, reducing the effect of ferric iron and 1,1-diphenyl-1-picrylhydrazyl radical scavenging assays. Estimation of bioactive components was carried out following previously established methods.

Results: It was found to be a great reducer of ferric iron, as well as an effective ferrous iron chelator and free radical scavenger. In an attempt to quantify the bioactive components, the fraction was found to be comprised of mention worthy amounts of phenols, β-carotene, lycopene and flavonoids.

Conclusion: This fraction can be used to treat oxidative stress related ailments.

Keywords: Antioxidant, 1,1-diphenyl-1-picrylhydrazyl, Reducing power, Scavenging activity.

INTRODUCTION

The world of fungi is large and diverse. They are not only one of the most primitive life forms; they intrigue mycologists with their diversity and other biologists with their unique richness of biologically active constituents. Mushrooms are just a small part of the world of fungi, but they are presently one of the most extensively studied organisms of the present scientific era.

Mushrooms have been accepted in the normal diet of people worldwide, due to their delicious earthy flavor and texture. Even though cultivated mushrooms are abundantly available in the markets, wild mushrooms are as such considered as delicacies [1]. Owing to its geo-climatic exclusiveness, a large variety of mushrooms are found in the state of West Bengal, many of which are eaten by the local natives [2] Apart from that, rigorous research has established mushrooms to be of potent therapeutic value. Mushrooms are rich in natural antioxidants [3-6], they also accumulate a variety of secondary metabolites including phenolic compounds, polyketides, terpenes, and steroids. Some of these compounds have tremendous importance to humankind displaying a broad range of useful antibacterial, antiviral, and pharmaceutical activities as well as less toxic effects [7]. Studies have shown that mushrooms have hepatoprotective [8-10], anticancer [11-13], leishmanicidal [14], anti-microbial [15,16], anti-diabetic [17,18], and immunomodulatory [19-23] activities.

Over the years, we have been collecting, inventorying mushrooms from various corners of West Bengal and assessing their biological potentiality. In the course, we report the antioxidant efficacy of the ethanolic extract of a wild edible mushroom, *Entoloma lividoalbum*, a local delicacy of Darjeeling and its suburbs.

MATERIALS AND METHODS

Chemicals

L-ascorbic acid, quercetin, gallic acid, ethylenediaminetetraacetic acid (EDTA), potassium ferricyanide, ferrous chloride, ferric chloride, ferrozine, Folin–Ciocalteu reagent, 1,1-diphenyl 1-2-picrylhydrazyl (DPPH), trichlroacetic acid, sodium sulfate, and ammonium molybdate were purchased from Sigma chemicals Co. (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

Materials

The mushroom *E. lividoalbum* was purchased from the local markets of Darjeeling, West Bengal, India. The fruit bodies were thoroughly cleaned and then dried. The dried mushroom was extracted as described [24]. 25 g of the dried mushroom was extracted with 100 ml ethanol overnight at room temperature and was filtered using Whatman No. 2 filter paper. The residue was then extracted with an additional portion of ethanol under the same conditions. The ethanolic extract was evaporated using a rotary evaporator at 50°C for dryness. The dried extract was resolubilized in ethanol to obtain various concentrations of ethanolic fraction of *E. lividoalbum* (EfraEliv).

Methods

Determination of total phenols

Total phenols in the extract were measured using Folin–Ciocalteu reagent [25]. The volume of 1 ml of ethanolic extract (100 mg/ml) was mixed with 1 ml Folin–Ciocalteu reagent and incubated for 3 minutes at room temperature. After incubation, 1 ml of 35 % saturated Na_2CO_3 solution was added in the reaction mixture, volume adjusted to 10 ml with distilled water and incubated in the dark for 90 minutes, after which the absorbance was monitored at 725 nm with a spectrophotometer. Gallic acid was used as a standard. Total phenol content of the sample was expressed as mg of gallic acid equivalents per gram of extract.

Determination of total flavonoid content

Flavonoid concentration was determined by the method as described [26]. Mushroom extract (100 mg/ml) was diluted with 4.3 ml of 80% aqueous methanol and 0.1 ml of 10% aluminum nitrates and 0.1 ml of 1 M aqueous potassium acetate was added to it. After 40 minutes at room temperature, the absorbance was determined spectrophotometrically at 415 nm. Total flavonoid concentration was calculated using quercetin as standard.

Determination of total ß-carotene and lycopene content

ß-carotene and lycopene were determined by the processes as suggested [27]. In brief, 100 µl of mushroom extract (10 mg/ml) was vigorously shaken with 10 ml of acetone-hexane mixture (4:6) for 1 minute and absorbance of the mixture was measured at 453, 505 and 663 nm. ß-carotene and lycopene contents were calculated according to the following equations:

$$\text{Lycopene (mg/100 mg)} = -0.0458A_{663} + 0.372A_{505} - 0.0806A_{453.}$$

$$\text{ß-carotene (mg/100 mg)} = 0.216A_{663} - 0.304A_{505} + 0.452A_{453}$$

Total antioxidant capacity

The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH [28]. The tubes containing extract (1 mg/ml) and reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95°C for 90 minutes. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm against a blank. The antioxidant capacity was expressed as ascorbic acid equivalent (AAE).

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

Different concentrations of EfraEliv (0.5-2 mg) was added to 0.004% methanolic solution of DPPH [29]. The mixture was shaken vigorously and left to stand for 30 minutes in the dark. Absorbance was measured at 517 nm against a blank. EC_{50} value is the effective concentration of extract that scavenged DPPH radicals by 50%, and it was obtained by interpolation from linear regression analysis.

Chelating effect on ferrous ions

The ability of the extract of EfraEliv to chelate ferrous ions was estimated by the method of Dinis *et al*., 1995 [30]. Briefly, the extract was added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml), and the mixture was then shaken vigorously and left to stand at room temperature for 10 minutes. The absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine - Fe^{2+} complex formation was calculated as $[(A_0-A_1)/A_0]\times100$, where A_0 was the absorbance of the control, and A_1 of the mixture containing the extract or the absorbance of a standard solution.

Reducing power

Reducing the power of EfraEliv was determined following the method of Oyaizu [31]. Variable concentrations of EfraEliv were added to 2.5 ml 0.2 M phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes. 2.5 ml of 10% trichloroacetic acid was added to the mixture and was centrifuged at 12,000 rpm for 10 minutes. The volume of 2.5 ml of the supernatant was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride, and absorbance was measured at 700 nm. An increase in absorbance of the reaction mixture was taken to mean an increase in reducing the power of the sample.

RESULTS

The percent yield, total phenol, flavonoids, beta-carotene, and lycopene content in the EfraEliv are shown in Table 1. Data show that phenols, *β*-carotene, and lycopene were the major antioxidant components whereas, flavonoids were found in lower amounts.

Total antioxidant capacity

Total antioxidant capacity of EfraEliv was indicated by the formation of green phosphomolybdenum complex within the reaction mixture. The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidants and the formation of green phosphate/Mo (V) complex with the maximal absorption at 695 nm. Total antioxidant activity of the extract was estimated, using ascorbic acid as standard. Analyzing the data, it was found that 1 mg of extract is as functional as approximately 53.9±1.3 µg of ascorbic acid, expressed as 53.9 µg AAE.

DPPH assay

DPPH is a stable free radical that has a characteristic absorption at 517 nm. On treatment with an increasing concentration of the EfraEliv, a marked decrease in absorption was observed, indicating a potent DPPH scavenging ability of the extract (Fig. 1). EC_{50} of DPPH radical scavenging activity was 2.13±0.02 mg/ml.

Chelating effect on ferrous ions

Like many transition metals, ferrous ions in a biological system could catalyze Heber-Weiss and Fenton-type reactions leading to the formation of hydroxyl radicals. Antioxidants chelate these transition metal ions resulting in the suppression of hydroxyl radical generation and inhibition of peroxydation process of biomolecules. The range and the mean of Fe++ chelating capacity is directly related with antioxidant potentiality. At 125-500 µg/ml the chelating effects of the EfraEliv was between 19.51% and 56.63% (Fig. 2). At the same concentration range, the chelating effects of the known metal chelator EDTA, was between 83% and 85%. In the present study, EfraEliv was determined to have potential ferrous iron chelating ability, with the calculated EC_{50} being 4.4±0.37 mg/ml.

Reducing power

The presence of reducers among antioxidants causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. In the reducing power assay the presence of antioxidants in the EfraEliv would effect in the reduction of Fe^{3+} to Fe^{2+} by the donation of an electron. The increasing absorbance at 700 nm by measuring the formation of Perl's Prussian Blue indicates an increase in reductive ability. A steady increase in reducing power was observed (Fig. 3). Results showed that EC_{50} for the reducing power of EfraEliv was of 0.487±0.03 mg/ml.

DISCUSSION

Phenolic compounds are known to be powerful chain-breaking

Table 1: Yield percentage and antioxidant components of EfraEliv

Yield %	Flavonoids (µg/mg)	Total phenols (µg/mg)	*β*-carotene (µg/mg)	Lycopene (µg/mg)
3.8±0.6	0.97±0.37	4.733±0.45	2.202±0.14	1.675±0.098

Values are the mean±standard deviation of three separate experiments, each in triplicate. Total phenols are expressed in GAE and flavonoids as QE. GAE: Gallic acid equivalent, QE: Quercetin equivalent, EfraEliv: Ethanolic extract of *Entoloma lividoalbum*

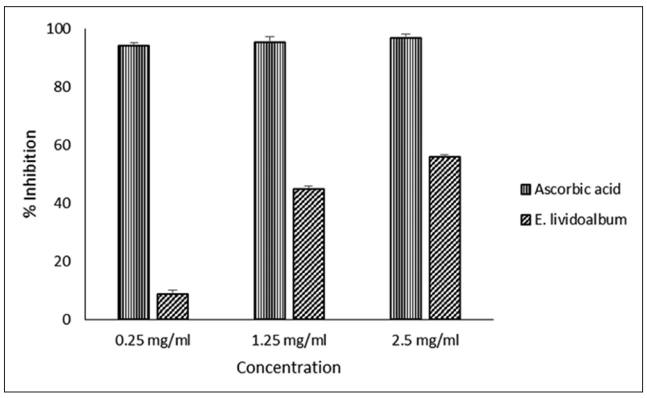

Fig. 1: 1,1-diphenyl 1-2-picrylhydrazyl radical scavenging activity of ethanolic extract of *Entoloma lividoalbum* compared with that of the standard, ascorbic acid. Values are the mean ± standard deviation of three separate experiments, each in triplicate

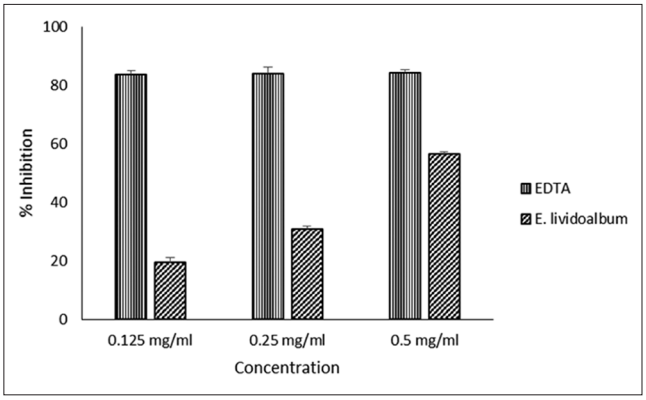

Fig. 2. Chelating effects of ethanolic extract of *Entoloma lividoalbum* on ferrous ions compared with that of ethylenediaminetetraacetic acid, used as standard. Values are the mean ± standard deviation of three separate experiments, each in triplicate

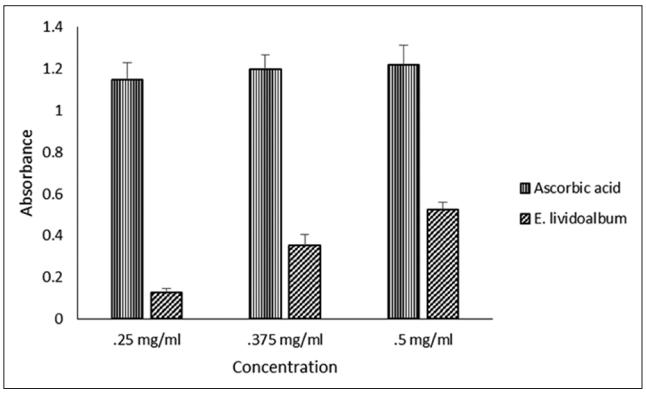

Fig. 3. Reducing the power of ethanolic extract of *Entoloma lividoalbum* with respect to that of ascorbic acid used as standard. Values are the mean ± standard deviation of three separate experiments, each in triplicate

antioxidants and they possess scavenging ability due to their hydroxyl groups. The phenolic compounds contribute directly to the anti-oxidative action. In the present study, the total phenolic content of EfraEliv (4.733 ± 0.45 µg/mg) was found to be lower than that of the ethanolic extract of *Pleurotus ostreatus*, which was reported to be 5.49 µg/mg [32], *Pleurotus squarrosulus*, which was 18.1 µg/mg [33] and *Pleurotus citrinopileatus*, that being 8.62 µg/mg [34].

Many other naturally occurring antioxidant components, including β-carotene, lycopene and flavonoids are known to possess strong anti-oxidative characteristics [35]. In this study β-carotene and lycopene were found in considerable amounts, i.e. 2.202 µg/mg and 1.675 µg/mg respectively, which are higher than that of the methanolic extract of *P. squarrosulus*, which were 570 ng and 225 ng per mg respectively [33] and *P. ostreatus* [32]. β-carotene was not detected in the ethanolic extract of *P. citrinopileatus* [34]. The estimated flavonoid content of EfraEliv is 0.97 µg/mg which is lower than *P. squarrosulus*, where it was reported to be 3.07 µg/mg [33], but the higher than *P. citrinopileatus*, 71.2 ng/mg [34]. Total phenols, β-carotene, and lycopene were the major naturally occurring antioxidant components estimated in this study. The higher amounts of these components in this extract might explain its effectiveness in antioxidant properties.

Total antioxidant capacities of EfraEliv were analyzed by the phosphomolybdenum method. A high absorbance value of the sample indicates high antioxidant activity. The total antioxidant activity of EfraEliv was found to be equivalent to the activity of 53.9±1.3 µg of ascorbic acid. The total antioxidant capacity of EfraEliv may be attributed to their chemical composition and phenolic acid content. A recent study [36] showed that some bioactive compounds from citrus fruits had strong total antioxidant activity, which was probably due to the presence of flavonoids and carotenoids.

DPPH is a stable free radical and possesses 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 chelation. The EC_{50} value of EfraEliv was lower than the ethanolic extract of *Calocybe gambosa*, *Armillaria mellea*, *Clitocybe odora*, and *Coprinus comatus* [3]. In our previous studies, we have found the ethanolic extracts of *Pleurotus flabellatus* [24] and *Russula albonigra* [37] to be lower than EfraEliv. In a related study, the edible mushroom *Volvariella volvaceae* showed 57.8% DPPH scavenging at a concentration of 9 mg/ml [38]. Thus, it can be said that the ethanolic extract of *P. flabellatus* has significant DPPH radical scavenging ability.

Iron can stimulate lipid peroxidation by the Fenton reaction and accelerate peroxidation by decomposing lipid peroxide into peroxyl and alcoxyl radical that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation. Iron toxicity is associated with an increased risk of free radical damage and cancer. The ferrous ion chelating ability of EfraEliv was effective and the EC_{50} value was found to be 4.4 mg/ml. Previous investigators have shown that the EC_{50} value of the ethanolic extract for *Hypsizigus marmoreus* [39] were more than 3 mg/ml which is much higher than that of EfraEliv. In our earlier investigations, the EC_{50} value of the ethanolic extract of *Tricholoma giganteum* was very close to 1 mg/ml [40]. Hence, the studied mushroom extract shows higher interference with the formation of ferrous and ferrozine complex and can be considered as a good chelator of ferrous ions.

Reducing the power of a compound serves as a significant indication of its potential antioxidant activity. The presence of reducers (i.e. antioxidants) causes the reduction of Fe^{3+}/ferrocyanide complex to ferrous form. The yellow color of the test solution changes to various shades of green and blue, which depends on the reducing power of each compound. EfraEliv was found to be a potent reducing agent, with an EC_{50} value of 0.48 mg/ml. Compared with the reducing powers of previously studied edible mushrooms from previously reported studies, the EfraEliv was an excellent reducer of ferric ions. The reducing power of ethanolic extracts of different edible mushrooms in descending order are *E. lividoalbum* > *H. marmoreus* [40] > *C. gambosa* [41] > *A. mellea* [41] > *C. odora* [3] > *T. giganteum* [40] > *C. comatus* [3] > *R. albonigra* [37] > *P. flabellatus* [24]. Apparently the ethanolic extract of *P. flabellatus* is an excellent reducing agent.

CONCLUSION

EfraEliv was found to possess effective antioxidant properties, being an excellent reducer of ferric ions and considerable chelator and free radical scavenger. With high amounts of natural phenolics, β-carotene and lycopene, this fraction can highly be recommended for use as dietary supplements and in the pharmaceutical industries.

REFERENCES

1. Kalac P. Chemical composition and nutritional value of European species of wild growing mushrooms: A review. Food Chem 2009;113:9-16.
2. Pradhan P, Dutta AK, Roy A, Basu SK, Acharya K. Inventory and spatial ecology of macrofungi in the *Shorea robusta* forest ecosystem of lateritic region of West Bengal. Biodiversity 2012;13(2):88-99.
3. Khatua S, Paul S, Acharya K. Mushroom as the potential source of new generation of antioxidant: A review. Res J Pharm Tech 2013;6(5):496-505.
4. Acharya K, Chatterjee S, Ghosh S. Comparative evaluation on the free radical scavenging activity of eleven Indian cultivated strains of *Pleurotus ostreatus*. Pharmacol Online 2011;1:440-50.
5. Chatterjee S, Saha GK, Acharya K. Antioxidant activities of extracts

obtained by different fractionation from *Tricholoma giganteum* basidiocarps. Pharmacol Online 2011;3:88-97.
6. 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.
7. Asatiani MD, Elisashvili V, Songulashvili G, Reznick AZ, Wasser SP. Higher basidiomycetes mushrooms as a source of antioxidants. Progress in Mycology. Netherlands: Springer; 2010. p. 311-26.
8. Chatterjee S, Datta R, Dey A, Pradhan P, Acharya K. *In vivo* hepatoprotective activity of ethanolic extract of *Russula albonigra* against carbon tetrachloride-induced hepatotoxicity in mice. Res J Pharm Tech 2012;5(8):1034-8.
9. Chatterjee S, Dey A, Datta R, Dey S, Acharya K. Hepatoprotective effect of the ethanolic extract of *calocybe indica* on mice with ccl_4 hepatic intoxication. Int J Pharm Tech Res 2011;3:2162-8.
10. Biswas G, Sarkar S, Acharya K. Hepatoprotective activity of the ethanolic extract of Astraeus hygrometricus (Pers.) Morg. Dig J Nanomater Biostruct 2011;6:637-41.
11. Biswas G, Chatterjee S, Acharya K. Chemopreventive activity of the ethanolic extract of *Astraeus hygrometricus* (Pers.) Morg. On Ehrlich's ascites carcinoma cell. Dig J Nanomater Biostruct 2012;7:185-91.
12. 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(1):101-7.
13. Chatterjee S, Biswas G, Chandra S, Saha GK, Acharya K. Chemopreventive effect of *Tricholoma giganteum* against benzo[a] pyrene-induced forestomach cancer in Swiss albino mice. Int J Pharm Sci Rev Res 2014;26(2):189-96.
14. Mallick S, Dutta A, Dey S, Ghosh J, Mukherjee D, Sultana SS, *et al.* Selective inhibition of *Leishmania donovani* by active extracts of wild mushrooms used by the tribal population of India: An *in vitro* exploration for new leads against parasitic protozoans. Exp Parasitol 2014;138:9-17.
15. Giri S, Biswas G, Pradhan P, Mandal SC, Acharya K. Antimicrobial activities of basidiocarps of wild edible mushrooms of West Bengal, India. Int J Pharm Tech Res 2012;4(4):1554-60.
16. Rai M, Sen S, Acharya K. Antimicrobial activity of four wild edible mushrooms from Darjeeling Hills, West Bengal, India. Int J Pharm Tech Res 2013;5(3):949-56.
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 Suppl 1:391-4.
18. Chatterjee A, Khatua S, Chatterjee S, Mukherjee S, Mukherjee A, Paloi S, *et al.* Polysaccharide-rich fraction of *Termitomyces* eurhizus accelerate healing of indomethacin induced gastric ulcer in mice. Glycoconj J 2013;30(8):759-68.
19. Patra P, Bhanja SK, Sen IK, Nandi AK, Samanta S, Das D, *et al.* Structural and immunological studies of hetero polysaccharide isolated from the alkaline extract of *Tricholoma* crassum (Berk.) Sacc. Carbohydr Res 2012;362:1-7.
20. Nandi AK, Sen IK, Samanta S, Maity K, Devi KS, Mukherjee S, *et al.* Glucan from hot aqueous extract of an ectomycorrhizal edible mushroom, *Russula albonigra* (Krombh.) Fr.: Structural characterization and study of immunoenhancing properties. Carbohydr Res 2012;363:43-50.
21. Samanta S, Maity K, Nandi AK, Sen IK, Devi KS, 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.
22. Nandi AK, Structural elucidation of an immunoenhancing heteroglycan isolated from *Russula albonigra* (Krombh.) Fr. Carbohydr Polym 2013;94(2):918-26.
23. Nandi AK, Samanta S, Maity S, Sen IK, Khatua S, Devi KS, *et al.* Antioxidant and immunostimulant ß-glucan from edible mushroom *Russula albonigra* (Krombh.) Fr. Carbohydr Polym 2014;99:774-82.
24. Dasgupta A, Rai M, Acharya K. Chemical composition and antioxidant activity of a wild edible mushroom *Pleurotus flabellatus*. Int J Pharm Tech Res 2013;5(4):1655-63.
25. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 1965;16(3):144-58.
26. Adebayo EA, Oloke JK, Ayandele AA, Adegunlola CO. Phytochemical, antioxidant and antimicrobial assay of mushroom metabolite from *Pleurotus pulmonarius* –LAU 09 (JF736658). J Microbiol Biotechnol Res 2012;2(2):366-74.
27. Nagata M, Yamashita I. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. Nippon Shokuhin Kogyo Gakkaishi 1992;39(10):925-8.
28. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem 1999;269(2):337-41.
29. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem 1992;40(6):945-8.
30. Dinis TC, Maderia VM, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys 1994;315(1):161-9.
31. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Jpn J Nutr 1986;44:307-15.
32. Jayakumar T, Thomas PA, Geraldine P. *In-vitro* antioxidant activities of an ethanolicextract of the oyster mushroom, *Pleurotus ostreatus*. Innov Food Sci Emerg Technol 2009;10(2):228-34.
33. Pal J, Ganguly S, Tahsin KS, Acharya K. *In vitro* free radical scavenging activity of wild edible mushroom, *Pleurotus* squarrosulus (Mont.) Singer. Indian J Exp Biol 2010;48(12):1210-8.
34. Lee YL, Huang GW, Liang ZC, Mau JL. Antioxidant properties of three extracts from *Pleurotus citrinopileatus*. LWT Food Sci Technol 2007;40(5):823-33.
35. Sowndhararajan K, Joseph JM, Manian S. Antioxidant and free radical scavenging activities of indian acacias: *Acacia Leucophloea* (Roxb.) Willd., *Acacia Ferruginea* Dc., Acacia Dealbata Link. and *Acacia Pennata* (L.) Willd. Int J Food Prop 2013;16(8):1717-29.
36. Jayaprakasha GK, Girennavar B, Patil BS. Radical scavenging activities of Rio Red grapefruits and Sour orange fruit extracts in different *in vitro* model systems. Bioresour Technol 2008;99:4484-94.
37. Dasgupta A, Ray D, Chatterjee A, Roy A, Acharya K. *In vitro* antioxidative behaviour of ethanolic extract of *Russula albonigra*. J Chem Pharm Res 2014;6(3):1366-72.
38. Cheung LM, Cheung PC, Ooi VE. Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem 2003;81:249-55.
39. Lee YL, Yen MT, Mau JL. Antioxidant properties of various extracts from *Hypsizigus marmoreus*. Food Chem 2007;104:1-9.
40. Chatterjee S, Saha GK, Acharya K. Antioxidant activities of extracts obtained by different fractionation from *Tricholoma giganteum* basidiocarps. Pharmacology 2011;3:88-97.
41. Vaz AJ, Barros L, Martins A, Santos-Buelga C, Vasconcelos HM, Ferreira IC. Chemical composition of wild edible mushrooms and antioxidant properties of their water soluble polysaccharidic and ethanolic fractions. Food Chem 2011;126(2):610-6.