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Original Article

ANTIOXIDATIVE AND FREE RADICAL SCAVENGING ACTIVITY OF ETHANOLIC EXTRACT OF *LENTINULA EDODES*

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ABSTRACT

Objective: Aim of this work was to evaluate the antioxidant properties of the edible mushroom species, *Lentinula edodes*.

Methods: Presence of potential antioxidant compounds, such as, ascorbic acid, β carotene, lycopene, phenolics and flavonoids were tested. To understand the antioxidant potentiality like scavenging of superoxide radical, chelating of ferrous ion, scavenging capacity of DPPH radicals and reducing power of the extract were investigated.

Results: The extraction yield achieved from *L. edodes* extraction was 2.4%. Total phenolic content was 25.5 mg GAE/g and total flavonoids content was 2 mg CE/g. The EC_{50} value for superoxide radical scavenging activity was found to be at a concentration of 0.64 mg/ml.

Conclusion: The results obtained reveal that *L. edodes* can be a potential source of natural antioxidant which may be used to treat various oxidative stress related diseases.

Keywords: Ascorbic acid, edible mushroom, *Lentinula edodes,* flavonoids, phenols, reactive oxygen species

INTRODUCTION

Different biochemical reactions in our body generate free radicals and these are capable of damaging crucial biomolecules. Over-production of reactive oxygen species results in increase of oxidative stress and if they are not effectively neutralized by oxidative stress-defence system, they lead to the development of diseased conditions in our body [1]. Every organism is equipped with defence system against oxidative damage but they often fall short, thus making the search for external antioxidants increasingly important. Antioxidants can protect human body from damages caused by reactive oxygen species. Although synthetic antioxidants are available in the market, their use is being restricted due to reported carcinogenicity and hepatotoxicity. Thus, in recent times anti-oxidants from natural sources are being sought extensively [1].

Mushrooms have been a part of human diet, for time immemorial and are well known for its nutritional and medicinal values. Recently, mushrooms have become attractive as functional food and as a source of physiologically beneficial medicine, while being devoid of unstable side effects [2]. Mushrooms are rich in different biologically active compounds like phenolics, tocopherol, lycopene, β-carotene etc. [1]. Recent investigations of different wild edible mushrooms from India have shown their potentiality for treatment of cancer [3-5], diabetes [6], ulcer [7], hepatic [8-11], cardio-vascular diseases [12], microbial pathogens [13-16], immunomodulation [17-19] etc. Here, an attempt has been made to evaluate the free radical scavenging activity of ethanolic extract of *Lentinula edodes* using various *in vitro* assay models.

MATERIAL AND METHODS

Preparation of extract

Ethanolic extract of the sample was prepared following Dasgupta et al. 2013 [20]. The sample was dried, powdered and extracted with ethanol at 25ºC for 2 days. After filtration, the residue was then re-extracted with ethanol, as described above. Precipitate was discarded by centrifugation and the supernatant was concentrated under reduced pressure in a rotary evaporator. Then, this ethanolic extract of *Lentinula edodes* was stored at 4ºC until further analysis. The percentage yield extracts were calculated based on dry weight as:

Yield (%) = $(W_1 \times 100)/ W_2$

Where W_1 = weight of extract after solvent evaporation; W_2 = Weight of the minced mushroom.

Determination of total phenolic content

The method for determination of phenol was performed following by Singleton and Rossi, 1965 [21]. To the sample extract (100 µl), 1 ml of 1N Folin–Ciocalteu reagent was added. After 3 min, a saturated sodium carbonate solution (approximately 35 g/100 ml, 1 ml) was added to it. The absorbance of the reaction mixture was measured at 725 nm after incubation for 1 hr 30 min at room temperature. Gallic acid was used as a standard, and the results were expressed as milligram gallic acid equivalent (mg GAE)/g of extract.

Determination of flavonoids

The flavonoid content was estimated following the method of Park et al. 1997 [22] 100µl of the sample extract was added to 80% ethanol containing 0.1ml of 10% aluminium nitrate and 0.1 ml of 1M potassium acetate. The mixture was incubated at room temperature for 40 min and its absorbance was measured at 415 nm. Quercetin was used as standard.

Determination of β-carotene and lycopene

β-carotene and lycopene determination was done by Nagata and Yamashita, 1992 [23]. 100µl of the sample extract was vigorously shaken with 10 ml of an acetone–hexane mixture (4:6) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505, and 663 nm. β-carotene and lycopene content were calculated according to the following equations:

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

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

The results were expressed as mg of carotenoid/g of extract.

Determination of ascorbic acid content

Ascorbic acid content was determined by a method followed by Rekha et al. 2012 [24], with few relevant modifications. Standard ascorbic acid (100 µg/ml) was taken in a conical flask and made up to 10 ml by 0.6% oxalic acid. It was titrated with 2, 6-dichlorophenol indophenol dye. The amount of dye consumed (V_1 ml) was equivalent to the amount of ascorbic acid. Similarly the sample (w µg/ml) was titrated with the dye (V_2 ml). The amount of ascorbic acid was calculated using the formula,

Ascorbic acid (µg/mg) = [{(10 µg /V_1ml) × V_2ml} × w µg] × 1000

DPPH radical scavenging assay

The model of scavenging DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. Effect of antioxidants on DPPH scavenging was thought to result from their hydrogen donating ability. Upon reduction, solution of DPPH fades from purple to yellow. Thus, a lower absorbance at 517 nm indicates a higher radical scavenging activity of extract. The DPPH radical scavenging ability of the sample extract was measured according to Shimada et al. 1992 [25]. 2ml of reaction mixture was prepared using different concentrations of sample (1-2.5 mg/ml) and methanol solution of DPPH (0.004%) (w/v). The absorbance was read against methanol as blank at 517 nm after 30 min incubation at room temperature in dark. The degree of scavenging was calculated by the following equation

Scavenging effect (%) = {(α_0-α_1)/α_0} × 100

Where α_0 and α_1 were the absorbance of control and in presence of sample.

Chelating ability

Ferrozine can react with Fe^{2+} to form violet complex. When there is other chelating agent, the ferrozine-Fe^{2+} formation is disrupted with decrease in colour of the complex. Therefore, measurement of absorption of the reaction mixture at 562 nm could be used to estimate the metal chelating activity of an antioxidant. To measure the ferrous ion chelating ability of the sample the method proposed by Dinis et al. 1994 [26] was followed with little modification. The sample was mixed with 2mM $FeCl_2$ (0.5 ml) to which 5mM ferrozine was added. The mixture was shaken vigorously and left to stand at room temperature for 10 min. The absorbance was measured at 562 nm. The decrease in absorbance indicated an increase in the ferrous ion chelating ability of the sample. The following formula was used for determination of the percentage inhibition of ferrozoin Fe^{+2}, complex formation

Scavenging effect (%) = {(α_0-α_1)/α_0} × 100

Where α_0 and α_1 were the absorbance of control and in presence of sample.

Reducing power

Reducing power of the sample was determined following Oyaizu 1986 [27]. Varied concentration of the sample (0.5-2 mg/ml) were added to 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. 2.5 ml of 10% trichloro acetic acid was added to the mixture after an incubation of 20 min. It was then centrifuged for 10 min at 12000 rpm. 2.5 ml of the supernatant was mixed with distilled water and 0.5 ml of 0.1% ferric chloride. Its absorbance was interpreted as an increase in reducing power of the sample. Antioxidants have ability to donate electrons and cause conversion of (Fe^{+3}) in ferric chloride to ferrous (Fe^{+2}). Resulting Perl's Prussian blue is measured at 700 nm and higher absorbance indicates higher reducing power.

Superoxide radical scavenging activity

The riboflavin – light – nitroblue tetrazolium (NBT) system as suggested by Martinez et al. 2001 [28] was used to study the superoxide radical scavenging activity of the ethanolic extract of *L. edodes*. This method was followed with minor modification. 3 ml of reaction mixture was prepared containing 50 mM sodium phosphate buffer (pH 7.8) 13 mM methionine and ethanolic extracts of various concentrations, 75 µM NBT, 100 µM EDTA and 2 µM riboflavin. One set of the reaction mixture was kept exposed to light for 10 min to activate the NBT and absorbance of each mixture was measured at 560 nm against identical mixtures from another set kept in the dark for same duration. BHA (Butylated hydroxylanisole) was used as standard. The degree of scavenging was calculated as follows

Scavenging effect (%) = {(α_0-α_1)/α_0} × 100

Where α_0 and α_1 were the absorbance of control and in presence of sample.

Determination of total antioxidant capacity (TAC)

The TAC was determined on the basis of reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of green phosphate/Mo (V) complex at acidic pH. Total antioxidant capacity of the sample was investigated and compared against ascorbic acid. The TAC of the sample was determined by the assay prescribed by Preito et al. 1999 [29] with modifications. A reaction mixture was prepared consisting of 0.3 ml of reagent solution (0.6 M H_2SO_4, 28 mM Na_2SO_4, 4mM NH_4Mo). Absorbance was measured at 695 nm after heating tubes at 95°C for 90 min. Ascorbic acid was used to draw a standard curve and TAC was expressed as the equivalent of ascorbic acid.

Statistical analysis

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

RESULTS AND DISCUSSION

In the present study, ethanol fraction from fruiting bodies of *L. edodes* was successfully isolated and physicochemical parameters were documented. Measurement of antioxidant properties of *Lentinula edodes* showed relatively high antioxidant activity.

Extractive value and bioactive components of ethanolic fraction of *L. edodes* were recorded.

The extract was brown in colour, sticky in nature and had extractive value of 10 ± 0.55%.

Phytochemicals assay

Naturally occurring antioxidant components including phenols, flavonoids, β-carotene, lycopene and ascorbic acid were found in ethanolic extract of *L. edodes*. Total phenol being the major naturally occurring antioxidant components was found as 25.5 µg gallic acid equivalent/mg of extract. Ethanolic extract of *L. edodes* also contained flavonoid as 2 ± 0.025 µg quercetin equivalent/mg of extract. Very negligible amount of β-carotene and lycopene were found such as 0.015 ± 0.002 µg/mg and 0.01 ± 0.002 µg/mg of the extract respectively. Ascorbic acid was also found (1.9 ± 0.06 µg/mg of extract) in small quantities.

Phenolic compounds possess scavenging ability due to their hydroxyl groups and are known to be powerful antioxidant. In the present study, the total phenolic content of *L. edodes* was found higher than *Pleurotus ostreatus* [30] and *Pleurotus citrinopileatus* [31] which were reported to be 5.49 µg/mg and 8.62 µg/mg respectively and similar to that of *Pleurotus squarrosulus* 18.1 µg/mg [32]. The phenolic content was also found to be comparatively higher than that of *Russula albonigra* (9.53 µg/mg) as reported [33].

DPPH radical scavenging assay

The model of scavenging DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. Effect of antioxidants on DPPH scavenging was thought to result from their hydrogen donating ability.

DPPH is a stable organic nitrogen radical and accepts an electron or hydrogen to become stable diamagnetic radical. Upon reduction, solution of DPPH fades from purple to yellow. Thus, a lower absorbance at 517 nm indicates a higher radical scavenging activity of extract. As shown in figure 1, ethanolic extract of *L. edodes*

exhibited the radical scavenging activity at the rate of 21.86%, 57.11%, 72.72% at 0.1, 0.3 and 0.5 mg/ml concentrations respectively (EC_{50} 0.283 ± 0.007 mg/ml). This activity was much better than *Amanita vaginata* which had EC_{50} at a concentration of 1.48 mg/ml [34].

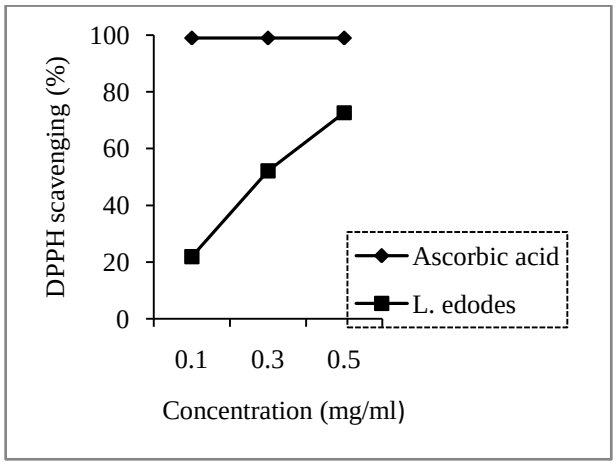

Fig.1: DPPH scavenging activity of ethanolic extract of *L. edodes*.

(Values are the mean ± standard deviation of three separate experiments, each in triplicate)

Chelating ability of ferrous ions

Transition metals e.g. Fe^{2+}, Cu^{+}, Pb^{2+}, Co^{2+} may serve as catalysts for the initial formation of radicals. Chelating agents has ability to stabilize transition metals in living systems and inhibit generation of free radicals. Development of potential chelating agents from natural mushrooms, thus provides an effective way to protect human beings from free radical damage.

In ethanol or water solution, ferrozine can react with Fe^{2+} to form violet complex. When there is other chelating agent, the ferrozine-Fe^{2+} formation is disrupted with decrease in colour of the complex. Therefore, measurement of absorption value of reaction mixture at 562 nm could be used to estimate the metal chelating activity of antioxidant. Figure 2 reveals that the ethanolic extract of *L. edodes* demonstrated a marked capacity for iron binding ability which showed a dose dependent response. 50% chelation was found at a concentration of 0.85 ± 0.05 mg/ml. EDTA exhibited excellent chelating ability of about 97% at these concentrations. In previous studies, it was showed that the EC_{50} value of the ethanolic extract for *Russula delica* [35] was more than 3 mg/ml which is much higher than that of the sample of our study. So, *L. edodes* could be considered as a good metal ion chelating agent.

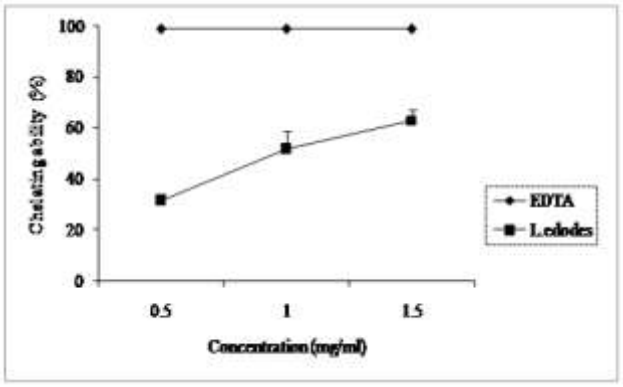

Fig 2 Chelating activity of ethanolic extract of *L. edodes*.

(Values are the mean ± standard deviation of three separate experiments, each in triplicate)

Determination of reducing power

The ability of extract to donate electrons can be evaluated using the reducing power assay. Ferrous ion has the capacity to reduce oxygen to superoxide radical and generation of hydroxyl radical from hydrogen peroxide. In presence of the extract reductive ability of Fe^{3+} to Fe^{2+} transformations was investigated. The reducing capacity of analysed mushroom extract may serve as the indicator of its potential antioxidant activity.

In reducing power assay, yellow colour of the test solution changes to various shades of green to blue as the reducing power of sample increases. Antioxidant has ability of donation of electron and causes conversion of the oxidation form of iron (Fe^{+3}) in ferric chloride to ferrous (Fe^{+2}). Resulting Perl`s Prussian blue is measured at 700 nm and higher absorbance indicates higher reducing power. Figure 3 reveals the reducing power of ethanolic extract of *L. edodes*, as a function of their concentration. EC_{50} value was at the concentration of 0.45 ± 0.2 mg/ml which was much less than that of *Amanita vaginata* (0.91 mg/ml) [34].

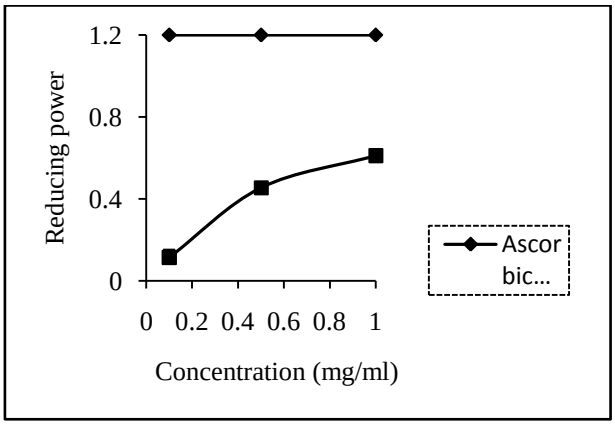

Fig.3: Reducing power of ethanolic extract of *L edodes*.

(Values are the mean ± standard deviation of three separate experiments, each in triplicate)

Superoxide radical scavenging assay

The method used by Martinez et al. [19] is based on generation of superoxide radical by auto-oxidation of riboflavin which in turn reduces yellow dye NBT to produce blue formazon in presence of light. Decrease in absorbance at 560 nm with antioxidants designates the consumption of superoxide anion. In the present study, ethanolic extract of *L. edodes* was found to be a notable scavenger of superoxide radicals (Fig.4) (EC_{50} 0.283 ± 0.007 mg/ml) and showed comparable scavenging activity with BHA (EC_{50} 0.34 ± 0.01 mg/ml). The action may depend on hydrogen atom donation by its components leading to the formation of secondary stabilized radical species.

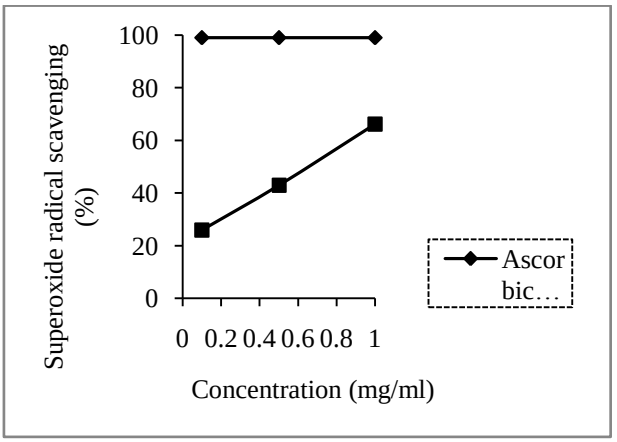

Fig.4: Superoxide radical scavenging activity of ethanolic extract of *L edodes*.

(Values are the mean ± standard deviation of three separate experiments, each in triplicate)

Determination of total antioxidant capacity (TAC)

Phosphomolybdenum method is a good method for evaluation of total antioxidant capacity. The method is based on reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of green phosphate /Mo (V) complex at acidic pH. Total antioxidant capacity of ethanolic extract of *L. edodes* was investigated and compared against ascorbic acid. The extract showed 276 ± 24 µg/mg ascorbic acid equivalent antioxidant capacity.

EC $_{50}$ values

The ethanolic fraction of *L. edodes* showed good antioxidant activity by *in vitro* assays like reducing power, ferrous ion chelating, superoxide radical scavenging activity and DPPH radical scavenging activity along with some amount of total phenol, flavonoids, β carotene, lycopene and ascorbic acid (Fig. 5).

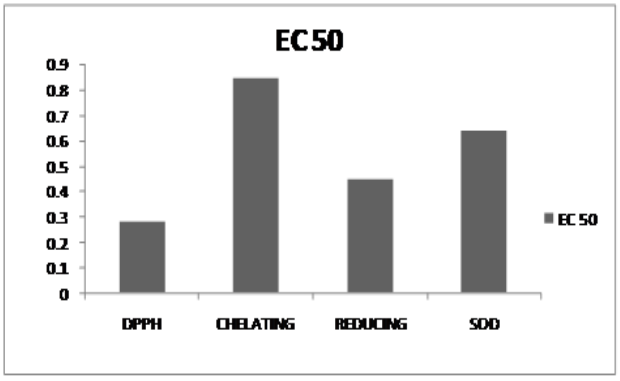

Fig.5: Comparison of the EC$_{50}$ values of free radical scavenging assays of the ethanolic extract of *Lentinula edodes.*

CONCLUSION

The data recorded in the above experiments showed that the ethanolic fraction of *Lentinula edodes* has a good antioxidant property and free radical scavenging power, which might be good sources for the development of antioxidant food additives. Phenolic component mostly polyphenols, flavonoids seemed to be responsible for the antioxidant activity. So, the ethanolic fraction of *Lentinula edodes* could be used as a possible food supplement or in pharmaceutical industry.

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REFERENCES

1. Khatua S, Paul S, Acharya K. Mushroom as the potential source of new generation of antioxidant: a review. Res J Pharm Technol 2013; 6(5): 496-505.
2. Sadler M. Nutritional properties of edible fungi. Nutr Bull 2003; 28: 305-308.
3. 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 Bios 2012; 7: 185-91.
4. 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: 101-107.
5. 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): 189-196.
6. 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: 391-394.
7. Chatterjee A, Khatua S, Chatterjee S, Paloi S, Mukherjee S, Mukherjee A et al. Polysaccharide-rich fraction of *Termitomyces eurhizus* accelerate healing of indomethacin induced gastric ulcer in mice. Glycoconjugate 2013; 30: 759-68.
8. 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: 285-288.
9. 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 Technol 2012; 5(8): 1034-1038.
10. Chatterjee S, Dey A, Datta R, Dey S and Acharya K. Hepatoprotective Effect of the Ethanolic Extract of *Calocybe indica* on Mice with CCl_4 Hepatic Intoxication. Int J PharmTech Res 2011, 3: 2162-2168.
11. Biswas G, Sarkar S and Acharya K. Hepatoprotective activity of the ethanolic extract of *Astraeus hygrometricus* (Pers.) Morg. Dig J Nanomater Bios 2011, 6: 637-641.
12. Biswas G, Rana S, Sarkar S, Acharya K. Cardioprotective activity of ethanolic extract of *Astraeus hygrometricus* (Pers.) Morg. Pharmacologyonline 2011; 2: 808-817.
13. Lai TK, Biswas G, Chatterjee S, Dutta A, Pal C, Banerj J, Bhuvanesh N, Reibenspies JH and Acharya K. Leishmanicidal and anticandidal activity of constituents of Indian edible mushroom *Astraeus hygrometricus*. Chem Biodivers 2012, 9: 1517-1524.
14. Giri S, Biswas G, Pradhan P, Mandal SC, Acharya K. Antimicrobial activities of basidiocarps of wild edible mushrooms of West Bengal, India. Int J PharmTech Res 2012, 4(4): 1554-1560.
15. Rai M, Sen S and Acharya K, Antimicrobial activity of four wild edible mushrooms from Darjeeling Hills, West Bengal, India. Int J PharmTech Res 2013, 5(3): 949-956.
16. Mallick S, Dutta A, Dey S, Ghosh J, Mukherjee D, Sultana SS, Mandal S, Paloi S, Khatua S, Acharya K, Pal C. 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.
17. Patra P, Bhanja SK, Sen IK, Nandi AK, Samanta S, Das D, Devi KSP, Maiti TK, Acharya K, Islam SS. Structural and immunological studies of hetero polysaccharide isolated from the alkaline extract of *Tricholoma crassum* (Berk.) Sacc. Carbohydr Res 2012, 362: 1-7.
18. Nandi AK, Sen IK, Samanta S, Maity K, Devi KSP, Mukherjee S, Maiti TK, Acharya K, Islam SS. 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.
19. Nandi AK, Samanta S, Sen IK, Devi KSP, Maiti TK, Acharya K, Islam SS. Structural elucidation of an immunoenhancing heteroglycan isolated from *Russula albonigra* (Krombh.) Fr., Carbohydr Polym 2013, 94: 918-926.
20. Dasgupta A, Rai M, Acharya K. Chemical composition and antioxidant activity of a wild edible mushroom *Pleurotous Flabellatus*. Int J PharmTech Res 2013; 5: 1655-1663.
21. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. Am J Enol Vitic 1965; 16: 144-158.
22. Park YK, Koo MH, Ikegaki M, Contado JL. Comparison of the flavonoid aglycone contents of *Apis mellifera* propolis from various regions of Brazil. Arq Biol Tecnol 1997; 40: 97-106.
23. Nagata M, Yamashita I. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. J Japan Soc Food Sci Technol 1992; 39(10): 925-928.
24. Rekha C, Poornima G, Manasa M, Abhipsa V, Pavithra DJ, Vijay KHT, Prashith KTR. Ascorbic acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits. Chem Sci Trans 2012; 1(2): 303-310.

25. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soyabean oil in
26. Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivates (acetoaminophen, salycilate, and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophy 1994; 315: 161-169.
27. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Jpn J Nut 1986; 44: 307-15.
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: 505-515.
29. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphor molybdenum complex: specific application to the determination of vitamin E. Anal Biochem 1999; 269: 337-341.
30. 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: 851-861.

cyclodextrin emulsion. J Agric Food Chem 1992; 40: 945-948.

31. Lee YL, Huang GW, Liang ZC, Mau JL. Antioxidant properties of three extracts from *Pleurotus citrinopileatus*. LWT - Food Sci Technol 2007; 40(5): 823-833.
32. 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: 1210-1218.
33. 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): 1366-1372.
34. Paloi S, Acharya K. Evaluation of antioxidative activity and chemical composition of ethanolic extract from *Amanita vaginata* (Bull.) Lam.: an *in vitro* study, Asian J Pharm Clin Res 2014; 7(2): 88-92.
35. Yaltirak T, Aslim B, Ozturk S, Alli H. Antimicrobial and antioxidant activities of *Russula delica* Fr. Food Chem. Toxicol 2009; 47: 2052-2056.