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Studies On Antibacterial And Antioxidant Activities Of *Agaricus Bisporus* (J.E. Lange) Imbach

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Abstract

Agaricus bisporus (J. E. Lange) Imbach is one of the most cultivated edible mushrooms in India. In the present study, antibacterial activities of acetone and methanol extract of *A. bisporus* were evaluated against *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli* through the disc diffusion method. Antioxidant potential was evaluated through reducing power, DPPH scavenging activity, total phenol contents (TPC), superoxide dismutase activity (SOD), ascorbate peroxidase activity (APX) and non-protein thiol contents (NPT).The zones of inhibition (ZOI) showed by acetone extract were7.73 mm to 9.44 mm and methanol extract was6.46 mm to 6.61mm. The reducing powers of acetone and methanol extracts were 0.196 and 0.272 respectively, the IC50 values of DPPH scavenging activities of acetone and methanol extracts were 1132.79 µg/ml and 381.03 µg/ml respectively and TPC of acetone and methanol extracts were 1.815 µg GAE/mg DW and2.775 µg/mg DW respectively observed. The SOD activity of 10.30 ± 0.33 U/mg protein, the APX activity of 2.58 ± 0.26 µ moles/mg protein min-1, and NPT contents 1.413 ± 0.040 µ moles/g DW were observed in *A. bisporus*. The results of this study emphasize further isolation and characterization of natural antibacterial substances and antioxidants and their use in the medicine and food industries.

Keywords: *Agaricus bisporus*, antibacterial activities, reducing power, DPPH, phenols

Introduction

Agaricus bisporus (J. E. Lange) Imbach (Button mushroom) is one of the most cultivated edible macrofungi belonging to family Agaricaceae. It is well known for its edibility, taste, aroma nutritional and medicinal values (Zhang *et al*., 2017).*A. bisporus* was first cultivated in France in 1650 AD and Tournefort a French botanist was the first who described the primitive method of cultivation to grow the button mushroom in 1707 (Chang and Miles, 2004). The first formal cultivation of Button mushrooms was attempted in India by ICAR in 1961 at Solan, Himachal Pradesh (Vaidya, 2001). In India, button mushroom ranks first in production and consumption with share of 73% of the total production of mushrooms (Sharma *et al*., 2017). *A. bisporus* have great nutritional value since it is quite rich in protein, dietary fiber, minerals, vitamins and poor in fat. (Stamets, 2005; Reis *et al*., 2012b; Ahlawat *et al*. 2016). The dietetic and medicinal properties of *A. bisporus* are due to its wealthy make-up of metabolites and biologically-active components. Mushrooms are also considered functional or health food because of their positive effects on body functions besides high nutritional values. Button mushroom is considered health food with high contents of essential amino acids, dietary fiber, polyphenols, sterols, ergothioneine, vitamins, minerals and polysaccharides (Dubost *et al*., 2007; Elmastas *et al*., 2007; Tian et al., 2012). It has

been also demonstrated that *A. bisporus* possess biological properties like antitumor, anti-aromatase, antimicrobial, immunomodulatory, anti-inflammatory and antioxidant activities (Chen *et al*., 2006; Jeong *et al*., 2010; Kozarski *et al*., 2011; Moro *et al*., 2012).

The unwise and extensive use of synthetic antimicrobial substances for the cure of infectious diseases caused multiple drug resistance in pathogenic microorganisms. The development of multi drug resistance in microbes forced scientists to search for accessible, natural and novel antimicrobial substances from plant resources. The plant-derived drugs are getting more popularity among people due to the widespread belief that "green medicine" is safe, more reliable than costly synthetic drugs and has no adverse side effects (Nair and Chanda, 2007). Secondary metabolites from mushrooms have been shown antimicrobial activity against a wide range of pathogenic bacteria (Mishra and Tiwari *et al*., 2011; Dimitrijević *et al*., 2017). The antimicrobial potential of *A. bisporus* earlier was studied by many workers. (Jagadish *et al*., 2009; Akyüz*et al*., 2010; Priya and Srinivasan, 2013; Padmavathy *et al*., 2014; Sharma *et al*., 2015, Kumar *et al*., 2022).

Free radicals like reactive oxygen species (ROS) are synthesized during natural metabolism of body cells and most of these free radicals are neutralized by the cellular antioxidant defence system. Oxidative stress caused by an excess of reactive oxygen species (ROS) resulted in multiple disorders *i.e*., metabolic disease, heart disease, severe neural disorders such as Alzheimer's and Parkinson's, premature ageing and cancers (Kozarski *et al*., 2015). Living organisms have two types of antioxidant systems, endogenous and exogenous. The endogenous antioxidant system includes enzymatic antioxidant (superoxide dismutase, ascorbate peroxidase, glutathione peroxidase, and catalase) and nonenzymatic (linolenic acid, polyamides, albumin, bilirubin, ceruloplasmin, non-protein thiol, transferrin, uric acid and coenzyme Q10). The exogenous antioxidants taken externally include vitamins, carotenoids, xanthophylls, polyphenols and minerals like Zn, Cu, Mn, Fe, and Se (Duarte and Lunec, 2005; Caverzan *et al.,* 2012; Kozarski*et al.,* 2015; Kuciel-Lewandowska *et al.,* 2020). Mushrooms are used to enhance the antioxidant defence system of body due to their bioactive compounds. *A. bisporus* has many bioactive compounds such as polysaccharides, fatty acids, ergosterol, phosphoric triamide, selenium and vaccenic acid, glycoproteins, sterols, vaccenic acid, agaritine, alcohols and triterpenoids (Wu *et al*., 2007; Ey *et al*., 2007; Borchers *et al*., 2008; Mohamed, 2012). The antioxidant potential of *A. bisporus* was studied through total phenolic contents, DPPH free radical scavenging activity, reducing power evaluation in different parts of the world (Elmastas *et al*., 2007; Ramirez-Anguiano *et al*., 2007; Savoie *et al*., 2008; Tsai *et al*., 2008; Barros *et al*., 2008; Jagadish *et al*., 2009; Palacios *et al*., 2011; Reis *et al*., 2012a; Liu *et al*., 2013; Muna *et al*., 2015).

The antimicrobial evaluation and antioxidant potential of wild and cultivated mushrooms were studied thoroughly in different parts of the world, but a few studies were conducted on cultivated mushrooms in India. So, the present study was undertaken with the objectives to evaluate the antibacterial and antioxidant activities of *A. bisporus* through organic solvent methanol and acetone.

Materials and Methods

The fruiting bodies of *Agaricus bisporus* (NBS-5) were obtained from the Directorate of Mushroom Research, Solan, Himachal Pradesh, India in 2020 (Fig 1).

Preparation of mushroom extracts

The fruiting bodies were dried at 40-45°C in a mushroom dryer under aseptic conditions. 10 g powdered samples of dried fruiting bodies were soaked separately in 100 ml methanol and acetone in an Erlenmeyer flask. Flasks were covered with aluminium foil and kept at 29°C for seven days for extraction. The samples were filtered through Whatman filter paper no. 1 and filtrates were

evaporated at room temperature. The extracts were collected and stored at a temperature below 4°C in a refrigerator. A stock solution of 50 mg/ml of extracts was prepared by dissolving them in dimethyl sulphoxide (DMSO).

Preparation of microbial inoculums

Human pathogenic bacterial species *Bacillus cereus* (MTCC-430), *Staphylococcus aureus* (MTCC-96), *Salmonella typhi* (MTCC-98), and *Escherichia coli* (MTCC-1687) were obtained from the Department of Microbiology and Biotechnology, H.P. University, Summer Hill Shimla. The bacterial cultures were raised in nutrient broth media (Beef extract 1 g, Peptone 5 g, Sodium Chloride 1 g, Yeast extract 2 g, distilled Water 1000 ml). The culture tubes filled with media were sterilized in an autoclave, cooled and inoculated with the pure culture of bacterial species. The culture tubes were incubated in a BOD incubator at 37°C for 24 hrs compared against 0.4-0.5 McFarland turbidity standard tubes and were further used for the antibacterial assay.

Antibacterial Assay

The antibacterial tests were carried out through the disc diffusion method (Collins and Lyne, 1987) using Mueller Hinton Agar. The nutrient medium was poured into petriplates under a laminar flow hood and was kept overnight at room temperature to check for any microbial contamination to appear. A 100µl of bacterial suspension culture was spread on the surface of each plate. The discs (6 mm) impregnated with 20 µl of mushroom extract (1 mg) were then placed on the inoculated plates. These plates were incubated at 37°C for 24 hours and zones of growth inhibition were measured.

Antioxidant Potential

1) Reducing power

The reducing power of methanol and acetone extract from mushrooms was determined by a method described by Oyaizu (1986). Different concentrations of 100, 200, 300, 400 and 500 µg/ml of each extract were made. Each concentration (0.5 ml) was mixed with 0.5 ml of a 0.2 M phosphate buffer (pH 6.6) and 0.5 ml of 1% potassium ferricyanide [K3Fe (CN) $_6$]. The mixture was incubated at 50°C for 20 min, followed by the addition of 0.5 ml of 10% trichloroacetic acid (TCA) and then centrifuged for10 min at 3000 rpm. The upper layer of supernatant (0.5 ml) was taken and mixed with 0.5 ml of distilled water and 0.1 ml of 0.1% $FeCl_3$. The mixture was incubated at room temperature for 10 min, and the absorbance was measured at 700 nm against a blank. The presence of reductants (antioxidants) in the tested sample resulted in reducing Fe^{3+}/Ferricyanide complex to ferrous form (Fe^{2+}). The Fe^{2+} was monitored by measuring the formation of Perl‟s Prussian blue at 700nm (Chung *et al*., 2002). A higher absorbance indicates a higher reducing power.

2) Scavenging activity of DPPH

DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging activity of mushroom extracts was estimated by the method given by Barros *et al*. (2008) with few modifications. Concentrations of 100, 200, 300, 400 and 500 µg/ml of methanol and acetone extracts of mushroom (1 ml) were taken in the test tube. To each test tube, 1 ml of (0.1mM) DPPHwas added and the mixture shaken vigorously and kept for 30 min in dark at 25°C. The reduction of the DPPH radical was determined by measuring the bleaching of the purple-coloured methanol solution of DPPH at 517 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration through the following formula: %RSA $= \frac{A\ Control - A\ Sample}{A\ Control} \times 100$

Where A control is the absorbance of the DPPH solution and A sample is the absorbance of the reaction mixture when the sample extract of a particular concentration has been added. The IC_{50}value was calculated from the plotted graph of scavenging percentage against extract concentration by the followingformula: $IC_{50} = \frac{50 - Y\ intercept}{slope}$

3) Total Phenolic Contents (TPC)

Total phenolic contents were determined by using the Folin-Ciocalteu phenol reagent method described by Goldstein and Swain (2001) with some alterations. 100 mg of each mushroom powder was homogenized in 2ml of 0.3N HCl in methanol, 0.3N HCl in water and 0.3N HCl in acetone separately and centrifuged at 6000 rpm for 10 minutes. Each supernatant was evaporated and the residue was dissolved in 2 ml distilled water in the case of methanol and aqueous extracts and acetone in acetone extract. The resulting solution was added with 0.5 ml of Folin and Ciocalteu‟s phenol reagent and shaken vigorously to mix thoroughly. After three minutes 1 ml of saturated sodium carbonate (35%) solution was added to the mixture. The reaction mixture was kept in dark for 90 min, after which the absorbance was read at 725 nm against blank. The content of the total phenolic compound was calculated on the basis of the calibration curve of gallic acid. The concentration of phenolic compound was expressed as mg of gallic acid equivalents (GAEs)/g of DW of tissue.

4) Superoxide dismutase activity (SOD)

The superoxide dismutase activity (SOD) was assayed by the method of Beauchamp and Fridovich (1971). Mushroom tissue (100 mg) was homogenized with extraction buffer (2.8 ml phosphate buffer 50 mM, pH 7.8 and 0.2 ml EDTA 0.1mM). Homogenate was centrifuged at 13,000 rpm for 25 min (4°C). Supernatant used as enzyme extract. For each test sample a corresponding control is maintained and a common standard and blank are kept for each set analyzed. Test (T) reaction mixture included 1.5 ml methionine, 0.3 ml riboflavin, 0.1ml NBT and 0.1 ml enzyme extract. Control (C) reaction mixture included 1.5 ml methionine, 0.3 ml riboflavin and 0.1ml phosphate buffer (pH 7.8), 0.1 ml enzyme extract. Standard (S) reaction mixture included 1.5 ml methionine, 0.3 ml riboflavin, 0.1 ml NBT, and 0.1 ml phosphate buffer. Blank (B) reaction mixture included 1.5 ml methionine, 0.3 ml riboflavin and 0.2 ml phosphate buffer (pH 7.8) The test tubes labelled as T, S, C and B are subjected to illumination in 15W fluorescent lampfor 10 minutes in an illumination chamber lined with aluminium foil. Following illumination, immediately the absorbance of all thereaction mixtures was read at 560 nm.Units of enzyme present in the sample are calculated using the following formula and expressed as U/mg protein.

Decrease in OD= (S-B)-(T-C)

SOD activity $=\frac{Decrease\ in\ OD \times 2}{S-B}$

SOD activity/mg of protein $=\frac{SOD\ activity \times Dilution\ factor}{Total\ protein\ (mg/\mu l)}$

5) Ascorbate peroxidase activity (APX)

The ascorbate peroxidase (APX) activity was estimated according to Nakano and Asada (1981) with some modifications. 100 mg of mushroom tissue was homogenized with 1ml of HEPES- NaOH buffer (100 mM, pH 7.6) and containing 5 mM ascorbate. The homogenate was centrifuged at 10,000 rpm for 5 min. at 4°C. The supernatant used as an enzyme extract. The reaction mixture contained 1ml (50 mM, pH 7.6) HEPES- NaOH buffer, 50 µl (5 mM) ascorbate, 50 µl enzyme extract and 100 µl (4 mM) H_2O_2. Following the addition of substrates, a change in absorbance was recorded at 290 nm for up to 3 minutes. The enzyme activity was determined using the extinction coefficient 2.8 $mM^{-1}cm^{-1}$ for ascorbate. Enzyme activity was calculated according to the following formulas.

Enzyme activity (units/ml enzyme) $=\frac{\Delta Abs \times Total\ assay\ volume \times Dilution\ factor}{\Delta t \times Extinction\ coefficient \times 1 \times Enzyme\ sample\ volume}$

Where: ΔAbs is the change in absorbance, Δt is time in minutes and l is the path length (1 cm).

Units/mg protein $=\frac{Units/ml\ enzyme}{mg\ protein\ /ml\ enzyme\ sample}$

6) Non-Protein Thiols (NPTs)

The non-protein thiol (NPT) contents were estimated as per the method given by Noctor and Foyer (1998). 100 mg of powdered mushroom sample was homogenized with 3 ml of extraction buffer (sodium phosphate buffer 0.1 M, pH 7.8 and EDTA 1 mM). The homogenate was centrifuged at 10,000 rpm for 10 minutes andthe supernatant was served as enzyme extract. The reaction mixture contained 2.5 ml buffers (sodium phosphate buffer 0.1M, pH 7.8 and EDTA), 50 µlEllman‟s reagent (Dithiobisnitrobenzoic acid DNTB) 4 mg/ml and 250 µl enzyme extract. The absorbance was observed at 412 nm and NPTs were quantified from the standard calibration curve of reduced glutathione (GSH). The NPTs were determined using the molar extinction coefficient 13600 mM-1cm-1 for DNTB.

Results and Discussion

Antibacterial activity of *Agaricus bisporus* against four human pathogenic bacteria

Acetone extract of *A. bisporus* showed higher antibacterial activity than methanol extract. The zones of inhibition (ZOI) showed by acetone extract (1 mg) were 9.44 ± 0.1 mm against *Escherichia coli,* 9.14 ± 0.01 mm against *Salmonella typhi*, 7.78 ± 0.05 mm against *Bacillus cereus* and 7.73 ± 0.07 mm against *Staphylococcus aureus*. The methanol extract (1 mg) showed ZOI of 6.61 ± 0.06 mm, 6.57 ± 0.03 mm, 6.50 ± 0.12 mm and 6.46 ± 0.07 mm against *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* respectively (Table 1).

Table-1

Extract 1mg/20 µl	Zone of Inhibition (mm)			
	B. Cereus	*S. aureus*	*S. typhi*	*E. coli*
Acetone	7.78 ± 0.09	7.80 ± 0.13	9.14 ± 0.17	9.44 ± 0.01
Methanol	6.61 ± 0.11	6.46 ± 0.12	6.50 ± 0.20	6.57 ± 0.05

Similar antibacterial activity of the methanol extract of *A. bisporus* was reported by earlier workers which were comparable to the current results. Jagadish *et al*. (2009) documented the antibacterial activity of ethanol extract of *A. bisporus* (20 mg) against *Staphylococcus aureus* (18-22 mm) and *Bacillus subtilis* (12 mm) but no growth inhibition against *E. coli* was recorded. Akyüz *et al*. (2010) reported the antibacterial activity of methanol extract of *A. bisporus* (100µg) against *Staphylococcus aureus* (8.5±0.7 mm) and *Escherichia coli* (7.5±0.7 mm), by disc diffusion method. However, Priya and Srinivasan (2013) did not detect any zone of growth inhibition against *Bacillus* sp., *Staphylococcus aureus* and *Escherichia coli* by disc diffusion method and stated that these bacterial species were resistant to *A. bisporus*. Padmavathy *et al*. (2014) reported a 15 mm zone of growth inhibition in petroleum ether extract of *A. bisporus* against *Staphylococcus aureus*. The antibacterial activity of acetone and methanol extracts of fruiting bodies of *A. bisporus* from DMR Solan also reported by Sharma *et al*. (2015), which was comparable with the present study.

Antioxidant Potential

1) Reducing power

The reducing activity was estimated through the reduction of Fe3+/Ferricyanide complex to ferrous form (Fe2+). The colour of the reaction mixture changes to various shades of green and blue. A higher absorbance indicates a higher reducing power. The reducing power of mushroom extracts increased with an increase in concentration **(Fig 2)**. The reducing powers of *A. bisporus* in acetone extract were 0.027 ± 0.002, 0.079 ± 0.003, 0.126 ± 0.002, 0.167 ± 0.003 and 0.196 ± 0.004 at 100, 200, 300, 400 and 500 µg/ml respectively. The reducing powers of *A. bisporus* in methanol extract were 0.055 ± 0.0, 0.121 ± 0.003, 0.182 ± 0.002, 0.221 ± 0.003 and 0.272 ± 0.002 at 100, 200, 300, 400 and 500 µg/ml respectively. In the earlier studies ethanolic extract of *A. bisporus* at 5 mg/ml was reported to have a reducing power of 0.34 (Lo, 2005) and 0.52–0.70 (Tsai *et al*., 2008). Muna *et*

al. (2015) reported a much higher reducing power of *A. bisporus* in hot water extract and ethanol extract than in the present study.

2) Scavenging activity of DPPH

The free radical scavenging activity (RSA) of methanol and acetone extracts of *A. bisporus* was tested against DPPH. The hydrogen atom or electron donation abilities of the corresponding extracts were estimated from the bleaching of the purple-coloured methanol solution of DPPH. The decrease in absorbance was taken as the measure of the amount of radical scavenging. The results revealed that there was an increase in RSA with increasing concentration of extracts **(Fig 3)**. The DPPH radical scavenging activity (RSA) of *A. bisporus* in acetone extract was 5.556 ± 0.117, 9.978 ± 0.176, 13.808 ± 0.384, 20.334 ± 0.159 and 22.168 ± 0.925 % at 100, 200, 300, 400 and 500 µg/ml respectively. The IC_{50} values of RSA in acetone extract was1132.79 µg/ml. The RSA of *A. bisporus* in methanol extract was 19.017 ± 0.286, 28.900 ± 0.380, 42.895 ± 0.388, 53.259 ± 0.265 and 62.073 ± 0.341 % at 100, 200, 300, 400 and 500 µg/ml respectively. The IC_{50} value of methanol extract of A. *bisporus* was measured 381.03 µg/ml.

The results of IC_{50} values reported previously in methanol extract of *A. bisporus* were 3.13 mg/ml from Portugal (Reis *et al*., 2012a), 9.61 mg/ml (Barros *et al*., 2008), ~18 µg/ml from Turkey (Elmastas *et al*., 2007). The IC_{50} values of ethanol extract of *A. bisporus* was reported 0.38 mg/ml from China (Liu *et al*., 2013), 0.52 mg/ml from Spain (Ramirez-Anguiano *et al*., 2007) and 1.77 mg/ml) from France (Savoie *et al*., 2008). Jagadish *et al*. (2009) reported 65.76% DPPH free radical scavenging of ethanol extract of *A. bisporus* at 600 µg/ml concentrations. The results indicated that extracts of *A. bisporus* had strong DPPH radical scavenging activity.

3) Total Phenolic Contents (TPC)

The phenolic contents were estimated by extracting the mushrooms separately in acetone, aqueous and methanol. The results were expressed in µg GAE/mg dry weight (DW) of mushroom tissue. The total phenol contents of A. *bisporus* in acetone, aqueous and methanol solvent were found to be 1.815 ± 0.05 µg GAE/mg DW, 2.483±0.034µg/mg DW 2.775 ± 0.02 µg/mg DW respectively. The phenol content *A. bisporus* in methanol extract was comparable with Palacios *et al*. (2011) but less than the reports of Ramirez-Anguiano *et al*., 2007; Dubost *et al*., 2007). Liu *et al*. (2013) reported 6.18 mg GAE/g DW phenol in the aqueous extract which was more than in the present study.

4) Superoxide dismutase (SOD) activity

Superoxide dismutases (SODs) are the group of enzymes which catalyze the conversion of superoxide anions into H_2O2 and O_2. Thepresent study reported the superoxide dismutase activity as10.30 ± 0.33 U/mg protein in A. *bisporus.* Turfan *et al*. (2020) measured the SOD activity of 15 wild and cultivated mushroom species from Turkey and found the SOD activity in the range of 19.12 ±0.06 - 58.23±0.12 U /mg protein. Cheng *et al*. (2012) studied the Superoxide dismutase activity of seventeen edible mushrooms from China and found that *A. bisporus*, *V. volvacea*, *P. ostreatus* had high and *F velutipes* had low SOD activity. Dama *et al*. (2010) found that the SOD activity increases during low temperature storage. The value of SOD activity at 5°C storage for 0 hours was 1.69 U/mg protein in *A. bisporus*. Ramkumar *et al*. (2012) evaluated SOD activity of *V. volvacea* (23.92±0.07-29.21±0.04 U/ mg protein). Khatuna *et al*. (2015) reported SOD activity of 347.5±0.06, 260.4±0.08 and 203.7±0.01 units/g DW in *P. florida*, *P. pulmonarius* and *P. citrinopileatus* respectively.

5) Ascorbate peroxidase (APX) activity

The ascorbate peroxidase (APX) is a member of the group of heme-containing peroxidases that are found in higher plants, green, red algae and in members of protista. The APX activity was of A. *bisporus* measured 2.58 ± 0.26 µ moles/mg protein min^{-1}. Turfan *et al*. (2020) measured the APX activity of 15 wild and cultivated mushroom species from Turkey and found the APX activity in the range of 0.201 - 2.118 U mg-1 protein min-1 which was in agreement with the present study. Sahu *et al*. (2018) recorded APX activity in White Button Mushrooms (10.07 ± 2.17 m moles min^{-1} g^{-1} FW) and Oyster mushrooms (9.09 ± 2.11 m moles min^{-1} g^{-1} FW). Sagar and Thakur (2018) reported

ascorbate peroxidase activity of *Lactarius deliciosus* (4.64 µ moles/mg protein min-1) and *L. salmonicolor* (0.76 µ moles/mg protein min-1) from Indian Himalayas.

6) Non-Protein Thiols (NPTs)

The non-protein thiols (NPTs) were measured by calibrating them with reduced glutathione (GSH) standard curve. Results were presented in µ moles/g DW of mushroom tissues. The present study showed 1.413 ± 0.040 µ moles/g DW NPTs in A. *bisporus*. Kalaras *et al*. (2017) studied thirteen species of mushrooms in the USA and found that the glutathione level ranged from 0.35 µ moles/g DW to 7.84 µ moles/g DW, which was in accordance with the present study. Selvi *et al*. (2007) reported GSH as 0.156 ± 0.025 n moles/g (fresh samples) in *C. indica* and 0.176 ± 0.025 n moles/g (fresh samples) in *P. florida*. Sudha *et al*. (2008) evaluated total glutathione (125.70 to 395.25 µg /g DW) in *V. volvacea* grown on substrates supplemented with different organic and inorganic additives.

Conclusions

In the present investigations, *A. bisporus* displayed considerable antibacterial activity against human pathogens (*B. cereus*, *S. aureus*, S. *typhi* and *E. coli*) and high antioxidant potential. This study will form the basis for further isolation and characterization of natural antibacterial substances and antioxidants and their use in the medicine and food industries for human health.

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Fig 1

A B C

Fig 1: *Agaricus bisporus* (Fruiting bodies)

Fig 2

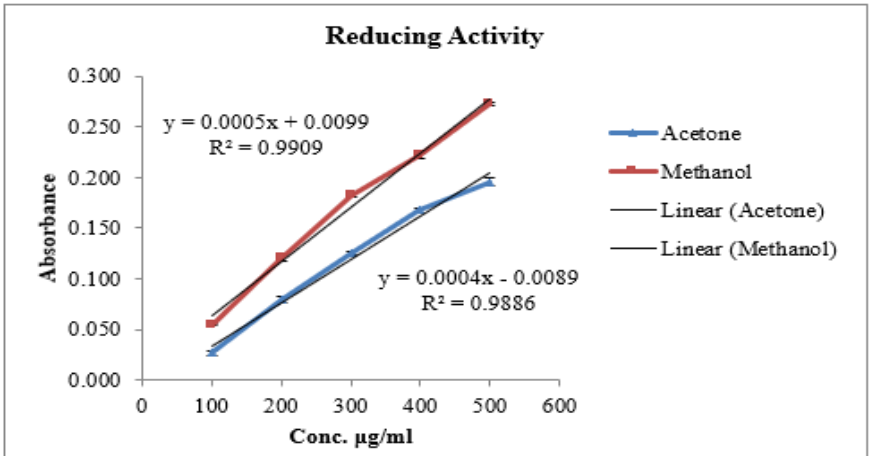

Fig 2: Reducing activity of acetone and methanol extracts of *A. bisporus*

Fig 3

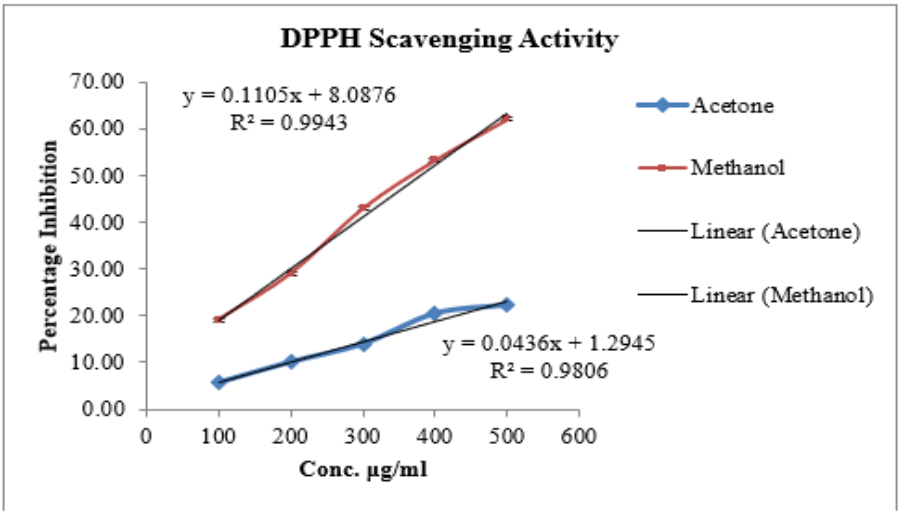

Fig 3: DPPH scavenging activity of acetone and methanol extracts of *A. bisporus*