RESEARCH ARTICLE

Antioxidant properties of some common oyster mushrooms grown in North East India

AFROZ MANSURI¹, DILIP KUMAR SARMAH^{2*} and ANANTA SAIKIA³

¹Central Bank of India, Talep Branch, Tinsukia 786 156, Assam, India

²Department of Plant Pathology, ³Department of Horticulture, Assam Agricultural University, Jorhat 785 013, Assam, India

Received: 05 December 2016/ Accepted: 20 February 2017/ Published online: 23 March 2017

© Indian Phytopathological Society 2017

ABSTRACT: Six oyster mushrooms, viz., *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm., *P. sapidus* (Schulz.) Sacc., *P. djamor* (Rumph.) Boedijn, *P. florida* (Mont.) Singer, *P. sajor-caju* (Fr.) Singer and *P. citrinopileatus* Singer were evaluated for their antioxidant properties. The antioxidant activity was more in pink coloured species (the highest in *P. djamor*, followed by *P. ostreatus*, and grey mushroom *P. sajor-caju*) than the white species (*P. florida*, *P. sapidus* and *P. citrinopileatus*), which established the positive effect of fruiting body pigmentation on antioxidant properties. A strong correlation between phenolic content and antioxidant activity was observed for all the species investigated. *P. citrinopileatus* showed higher chelating and radical scavenging effects than *P. florida* though it contained lower amount of phenol than *P. florida*. The investigation revealed the antioxidative potential of the pigmented mushrooms, viz., *P. djamor* and *P. ostreatus*. These species, enriched with beneficial bioactive components, have much potential for inclusion in breeding programmes and should be popularized for cultivation purpose.

Keywords: Antioxidant activity, mushroom, oyster mushroom, pigmented mushroom

Mushrooms are becoming increasingly popular and often find a place in human diets, because of their established nutritional and medicinal properties (Thakur, 2014; Jedinak *et al.*, 2010). Oyster mushroom (*Pleurotus* spp.) is widely cultivated for edible purpose and popular among farmers for its wide adaptability (Gogavekar *et al.*, 2014). Although, about 40 species of oyster mushroom have been recognized and documented scientifically, only 12 species are being cultivated in different parts of India, which perform well in various types of lignocellulosic materials degrading lignin efficiently (Dehariya and Vyas, 2013). Production of oyster mushroom in India is estimated to be 15-20,000 metric tonnes annually (Thakur, 2014). Low cost production technology is available for cultivation of this mushroom. Various species of *Pleurotus* have different temperature requirement for growth and development which makes them ideally suited for cultivation throughout the year in various regions of India.

Besides, being rich sources of nutrients with high food value, mushrooms contain many biologically active compounds of medicinal importance (Khatun *et al.*, 2015). Free radical formation is associated with normal metabolism in our body during consumption of oxygen leading to the generation of a series of oxygen radical (Kozarski *et al.*, 2015), which interact with biological system causing several diseases. Mushrooms are rich in natural antioxidants which increase the anti-oxidative

capacity of plasma and reduce risk of certain diseases like cancer, heart diseases, cirrhosis, atherosclerosis etc. (Kozarski *et al.*, 2015). Consumption of oyster mushroom extract aids in protecting major organs like liver, heart and brain from oxidative damage (Jayakumar *et al.*, 2006). Secondary metabolites, such as phenols and flavonoids from mushrooms, have been regarded as potent free radical scavengers (Visioli *et al.*, 1998). They inhibit xanthine oxidase involved in catalysis of xanthine which may otherwise lead to accumulation of uric acid and cause gout (Zhou *et al.*, 2001).

Forest belts of North Eastern region are very rich in microbial resources including macrofungi (Singh and Chhetri, 2010). Several species of oyster mushrooms have been cultivated in the region mostly for culinary purposes. Considering the limited information available on the oyster mushrooms of North East India, the present work was undertaken to investigate their anti-oxidative potentials.

MATERIALS AND METHODS

Mushroom species

Six mushroom species, viz., *Pleurotus ostreatus* (PO), *P. sapidus* (PS), *P. djamor* (PD), *P. florida* (PF), *P. sajor-caju* (PSC) and *P. citrinopileatus* (PC) were used for the investigation. The species were obtained from the culture collections maintained at the Department of Plant

*Corresponding author: sarmah.dilip@gmail.com

Pathology, Assam Agricultural University, Jorhat 785013, Assam; which were originally sourced from the National Research Centre for Mushroom, Solan (presently Directorate of Mushroom Research).

Sample preparation

Dried mushroom was made into fine powder by using an electric grinder and passing through a 250 mm sieve, packed in plastic bag and stored in refrigerator at 4°C until analysis. Preparation of extract was done as per the method described earlier with some modifications (Elmastaş *et al.*, 2007). About 25 g of dried mushroom sample was weighed and mixed with 60ml of 99% methanol in a 250 ml conical flask and then put on a shaker at 150 rpm for 24 hours at room temperature after sealing the mouth of the flask with DuraSeal™ film (Diversified Biotech, Boston). Whatman No. 42 filter paper was used to obtain the filtrate and the residue was re-extracted under the same condition repeatedly until the extracts became colourless each time adding 60ml methanol to it. The filtrates were combined together and the methanol was removed using an IKA RV10 rotary vacuum evaporator (IKA India Private Limited, Bangalore, India) at 40°C to obtain the dry extracts of various mushroom samples. The extracts were placed in plastic bottles, flushed with nitrogen and stored at 40°C in a refrigerator.

Radical scavenging activity (RSA)

The free radical scavenging activity of mushroom in terms of ability to annihilate 1-diphenyl-2-picryl-hydrazil (DPPH) was measured (Blois, 1958). The stock solution was prepared by dissolving 0.1 g extract in 100 ml methanol to obtain a concentration of 1mg/ml. Hundred ml of 0.1 mM solution of DPPH was prepared in methanol and 1 ml of this solution was added to 3 ml of mushroom extracts with 180 µg/ml concentration. The mixture was shaken in a horizontal shaker and allowed to stand at room temperature for 30 min. The absorbance of the reaction mixture was then measured in a Cary 50 UV-VIS spectrophotometer (Agilent Technologies, Santa Clara, USA) at 517 nm. The higher RSA was indicated by lower absorbance. The RSA was calculated using the following equation:

$$\text{RSA (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where, A_0 - Absorption of blank sample, A_1 - Absorption of tested solution

Reducing power

The reducing power of methanolic extract of mushroom was determined according to Oyaizu (1986) with some modifications. Methanolic extract of 200 µg/ml concentrations was prepared in methanol. The reaction mixture was prepared with 2.5 ml of 0.2M phosphate buffer (pH 6.6), 2.5 ml of 1% potassium ferricyanide and the extract. The mixture was then incubated at 50°C for

20 minutes, cooled to room temperature, 2.5 ml of 10% trichloroacetic acid added and finally centrifuged at 1000g for 10 min. Then, 2.5 ml of distilled water, 0.5 ml of 0.1% ferric chloride and 2.5 ml of the supernatant were combined. The absorbance of the mixture was measured in a Cary 50 UV-Vis at 700 nm.

Chelating effect on ferrous ion

The chelating effect on ferrous ions was estimated according to the method described by Dinis *et al.* (1994). From the stock solution prepared for estimation of RSA, 200 µg/ml concentration of methanolic extract of mushroom species was prepared in methanol, and then 0.05 ml of 2 mM ferrous chloride was added to each of the test tubes followed by addition of 0.2 ml of 5 mM ferrozine. The mixture was shaken vigorously in a horizontal shaker and allowed to stand for 10 min at room temperature. The absorbance was measured, once the mixture reached equilibrium after 10 min, in a Cary 50 UV-VIS spectrophotometer at 562 nm. The percentage of inhibition of ferrozine- Fe^{2+} complex was measured by the formula:

$$\text{Per cent inhibition} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100$$

Total phenolics

Dried powdered sample, about 0.5 g of each mushroom species, was weighed accurately and mixed with 5 ml of 80% ethanol in a 100 ml conical flask and put on a shaker for about 24 hrs at 150 rpm and then centrifuged at 10,000 rpm for 10-15 min. The supernatant was taken and evaporated to dryness on water bath and the residue was dissolved in a known volume of water for further analysis.

Total phenolic content was determined according to the method of Bray and Thorpe (1954) Aliquot (50 µl) was taken out from each sample into test tubes and the volume was made up to 3 ml by water. Then, 0.5 ml of Folin-ciocalteu reagent was added and allowed to stand for three min. Then, 2ml of 20% sodium carbonate was added, thoroughly mixed and the test tubes were placed in boiling water for 1 min, cooled and the absorbance measured in a Cary 50 UV-Vis spectrophotometer at 650 nm against a reagent blank. Catechol was used as standard during the analysis.

Total flavonoid content

Dried 0.5 g mushroom powder was mixed with 5 ml of 80% ethanol in 100 ml of conical flask and put on a shaker at 200 rpm for 24 h. The extracts were filtered through Whatman No. 42 filter paper. The volume of the filtrate was adjusted to 5 ml with 80% ethanol and was used for further analysis.

The total flavonoid was determined according to Woisky and Salatino (1998) with minor modification. In a test tube, 1.5 ml 95% ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of

Fig. 1. Radical scavenging activity of the mushroom species

distilled water were added to 0.5 ml of extract. After incubation for 30 min at room temperature, the absorbance was measured at 415 nm in a Cary 50 UV-Vis spectrophotometer. Standard was prepared by dissolving 10 mg of quercetin in 100 ml of 80% ethanol, whose final concentration was 100 μ g/ml.

Statistical analysis

CRD was employed for the statistical analysis of the data. The data collected were subject to statistical analysis by Fisher's method of analysis of variance. Significance of variance among the data was analysed by calculating the F value and comparing it with the tabulated value of "F" at 5% level of probability.

RESULTS

Radical scavenging activity

The comparison among the six oyster mushroom species for radical scavenging activity revealed that it was highest in *P. djamor* which was 34.56% followed by *P. ostreatus*, *P. sajor-caju*, *P. citrinopileatus*, *P. florida* and *P. sapidus* which were 30.90, 29.01, 27.90, 24.87 and 23.12% respectively at 180 μ g/ml of mushroom extract (Fig. 1).

Reducing power

In our study, the highest reducing power was exhibited by *P. djamor* (0.51) followed by *P. ostreatus*, *P. sajor-caju*, *P. florida*, *P. citrinopileatus* and *P. sapidus* (0.40, 0.33, 0.26, 0.20 and 0.16 respectively) at 200 μ g/ml concentration of extract (Fig. 2).

The reducing power was measured in terms absorbance of various mushroom samples, higher the absorbance more is the reducing power.

Chelating effect

The chelating effect on ferrous ions was also highest in *P. djamor* (27.30%) followed by *P. ostreatus*, *P. sajor-caju*, *P. citrinopileatus*, *P. florida* and *P. sapidus* (24.61, 21.05, 20.39, 18.78 and 16.81% respectively) (Fig. 3).

Total phenolics

In the present study, the total phenolic content was highest in *P. djamor* (14.35mg catechol equivalent/g) with the lowest in *P. sapidus* (9.64mg) (Fig. 4). According to phenolic content, mushroom species are arranged in descending order: *Pleurotus djamor* > *P. ostreatus* > *P. sajor-caju* > *P. florida* > *P. citrinopileatus* > *P. sapidus*, respectively.

Total flavonoids

In the present investigation, the highest flavonoid was found in pink mushroom *P. djamor* (2.85 mg quercetin equivalent/g on dry weight basis) followed by *P. ostreatus*, grey *P. sajor-caju*, and white strains *P. florida*, *P. citrinopileatus* and *P. sapidus* (Fig. 5).

DISCUSSION

Several investigations for antioxidant activity have been conducted during the past decade in many fruits, vegetables, herbs, cereals, sprouts and seeds. Natural antioxidants are known to protect organisms and cells from damage by oxidative stress (Kozarski *et al.*, 2015). Antioxidant capacity of plant tissue is closely associated with the activity of enzymes responsible for scavenging free radicals (Khatua *et al.*, 2013). The radical scavenging activities of oyster mushroom are higher in mushrooms producing coloured fruits bodies. This was established in our study, as the highest radical scavenging activity

Fig. 2. Reducing power of the mushroom species

Fig. 3. Chelating effect of the mushroom species on ferrous ion

was found in pink mushroom *P. djamor*. The 30.90% RSA of *P. ostreatus* at 180 µg/ml concentration was lower on the basis of per cent inhibition. This shows that with the increase in concentration, the RSA in mushroom increases. Our result on *P. ostreatus* was higher than 29.0% at 2 mg/ml of concentration as reported by Kim *et al.* (2009). The results obtained for *P. sajor-caju* and *P. florida* were higher than the findings of Ramkumar *et al.* (2010), whereas for *P. citrinopileatus*, the result was at par with that of Lee *et al.* (2007), based on the concentration as discussed above for *P. ostreatus*. Although, the RSA of *P. sapidus* was the lowest among all the species under investigation, the finding has

importance since no earlier report on this species was available elsewhere.

The reducing power of mushroom species is positively influenced by their hydrogen-donating ability (Khatua *et al.*, 2013). This was true as higher reducing power was found in pigmented mushroom *Pleurotus djamor*. Therefore, pigmented mushroom might contain higher amount of reductants than other mushroom, which could terminate radical chain reaction reacting with free radicals (Ramkumar *et al.*, 2010; Kim *et al.*, 2009).

Phenolic compounds such as anthocyanins and carotenoids are free radical scavengers, due to their

Fig. 4. Total phenolic content of the mushroom species

Fig. 5. Total flavonoid content of the mushroom species

ability to donate hydrogen atoms or electrons (Cui *et al.*, 2013; Mazandarani *et al.*, 2011). The variation in total phenolics in all the 6 oyster mushrooms under investigation manifested the differences in antioxidant activities of the mushrooms species in terms of radical scavenging, reducing power, and chelating effects. Earlier studies have established that the association of phenolic contents with their antioxidant activities due to their redox potentials allowing them to act as reducing agents or hydrogen donors (Kozarski *et al.*, 2015). Similar results were reported earlier by Lee *et al.* (2007) in *P. citrinopileatus*.

There has been a growing interest in pigment components of natural food, belonging to flavonoid group, which may promote human health or lower the risk for disease. Deep pigmented fruits, vegetables or foods are recognized as healthier to human body (Lin and Tang, 2007). Similar results were also reported by earlier workers (Gogavekar *et al.*, 2014; Sudha *et al.*, 2012).

A correlation between total phenols and antioxidant property of mushroom has already been established (Turkoglu *et al.*, 2007). According to our results, though *P. citrinopileatus* had lower amount of phenol than *P.*

florida, it showed higher radical scavenging activity and chelating effect on ferrous ion than *P. florida*. Thus, it can be concluded that other factors might be involved for these properties. However, data in the literature about the relationship of phenolics and antioxidant activity are contradictory (Othman *et al.*, 2007). While some authors had observed high correlation (Kozarski *et al.*, 2015), others did not find any correlation with the antioxidant property (Othman *et al.*, 2007), which might be attributed to other water and alcohol soluble nonphenolics compounds like tocopherols, β -carotene, ascorbic acid, selenium etc.

The investigation was a preliminary study on the antioxidant properties of some common edible oyster mushrooms of Assam. It was seen that *P. djamor* and *P. ostreatus* had high health-promoting antioxidant properties. Pigmented mushrooms have high amounts of phenolic compounds, and consequently have higher antioxidant and radical scavenging activity. Presently, white mushroom species like *P. florida*, *P. sapidus* and *P. citrinopileatus*, and grey species *P. sajor-caju* are commonly cultivated by farmers in Assam. Our investigation has revealed that the pigmented species like *P. djamor* and white species *P. ostreatus* are healthier than the other species, which should be popularized and brought into cultivation. These two species have the potential for their inclusion in a breeding programme for improvement of the species already in cultivation in Assam.

REFERENCES

- Blois MS (1958). Antioxidant determinations by the use of a stable free radical. *Nature* 181: 1199-1200.
- Bray HG and Thorpe WV (1954). Analysis of phenolic compounds of interest in metabolism. In: *Methods of Biochemical Analysis*, (Ed. Glick D), John Wiley & Sons, Inc., Hoboken, NJ, USA.
- Chang ST, Wu JH, Wang SY, Kang PL, Yang NS and Shyur LF (2001). Antioxidant activity of extracts from *Acacia confuse* bark and heartwood. *J. Agric. Food Chem.* 49: 3420-3424.
- Cui C, Zhang S, You L, Ren J, Luo W, Chen W and Zhao M (2013). Antioxidant capacity of anthocyanins from *Rhodomyrtus tomentosa* (Ait.) and identification of the major anthocyanins. *Food Chem.* 139: 1-8.
- Dehariya P and Vyas D (2013). Effect of different agro-waste substrates and their combinations on the yield and biological efficiency of *Pleurotus sajor-caju*. *IOSR J. Pharm. Biol. Sci.* 8: 60-64.
- Denis TCP, Madeira VMC and Almeida LM (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch. Biochem. Biophys.* 315: 161-169.
- Elmastaş M, Isildak O, Turkecul I and Temur N (2007). Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. *J. Food Comp. Anal.* 20: 337-345.
- Gogavekar SS, Rokade SA, Ranveer RC, Ghosh JS, Kalyani DC and Sahoo AK (2014). Important nutritional constituents, flavour components, antioxidant and antibacterial properties of *Pleurotus sajor-caju*. *J. Food Sci. Technol.* 51: 1483-1491.
- Jayakumar T, Ramesh E and Geraldine P (2006). Antioxidant activity of the oyster mushroom, *Pleurotus ostreatus*, on CCl₄-induced liver injury in rats. *Food Chem. Toxicol.* 44: 1989-1996.
- Jedinak A, Dudhgaonkar S, Jiang J, Sandusky G and Sliva D (2010). *Pleurotus ostreatus* inhibits colitis-related colon carcinogenesis in mice. *Int. J. Mol. Med.* 26: 643-650.
- Khatua S, Paul S and Acharya K (2013). Mushroom as the potential source of new generation of antioxidant: A review. *Res. J. Pharm. Tech.* 6: 495-505.
- Khatun S, Islam A, Cakilcioglu U, Guler P and Chatterjee NC (2015). Nutritional qualities and antioxidant activity of three edible oyster mushrooms (*Pleurotus* spp.). *NJAS – Wageningen. J. Life Sci.* 72-73: 1-5.
- Kim JH, Kim SJ, Park HR, Choi JI, Ju YC, Nam KC, Kim SJ and Lee SC (2009). The different antioxidant and anticancer activities depending on the color of oyster mushrooms. *J. Med. Plant Res.* 3: 1016-1020.
- Kozarski M, Klaus A, Jakovljevic D, Todorovic N, Vunduk J, Petrović P, Niksic M, Vrvic MM and Griensven LV (2015). Antioxidants of edible mushrooms. *Mol.* 20: 19489-19525.
- Lee YL, Huang GW, Liang ZC and Mau JL (2007). Antioxidant properties of three extracts from *Pleurotus citrinopileatus*. *LWT* 40: 823-833.
- Lin JY and Tang CY (2007). Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chem.* 101: 140-147.
- Mazandarani M, Moghaddam PZ, Baiat H, Zolfaghari MR, Ghaemi EA and Hemati H (2011). Antioxidant activity, phenol, flavonoid and anthocyanin contents in various extracts of *Onosma dichroanthum* Boiss. in north of Iran. *Iran. J. Plant Physiol.* 1: 169-176.
- Othman A, Ismail A, Ghani NA and Adenan I (2007). Antioxidant capacity and phenolic content of cocoa beans. *Food Chem.* 100: 523-530.
- Oyaizu M (1986). Studies on products of browning reaction: Antioxidative activity of products of browning reaction prepared from glucosamine. *Jpn. J. Nutr.* 44: 307-315.
- Ramkumar L, Ramanathan T, Thirunavukkarasu P and Arivuselvan N (2010). Antioxidant and radical scavenging activity of nine edible mushroom extract. *Int. J. Pharmacol.* 6: 950-953.
- Singh MN and Chhetry GKN (2010). Biodiversity of macrofungi in Imphal, India-I. *Indian Phytopath.* 63: 79-82.
- Sudha G, Vadivukkarasi S, Shree RBI and Lakshmanan P (2012). Antioxidant activity of various extracts from an edible mushroom *Pleurotus eous*. *Food Sci. Biotechnol.* 21: 661-668.
- Thakur MP (2014). Present status and future prospects of tropical mushroom cultivation in India: a review. *Indian Phytopath.* 67: 113-125.
- Turkoglu A, Duru ME, Mercan N, Kivrak I and Gezer K (2007). Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull.) Murrill. *Food Chem.* 101: 267-273.
- Visioli F, Bellomo G and Galli C (1998). Free radical-scavenging properties of olive oil polyphenols. *Biochem. Biophys. Res. Comm.* 247: 60-64.
- Woisk, RG and Salatino A (1998). Analysis of propolis: some parameters and procedures for chemical quality control. *J. Apic. Res.* 37: 99-105.
- Zhou CX, Kong LD, Ye WC, Cheng CHK and Tan RX (2001). Inhibition of xanthine and monoamine oxidases by stillbenoids from *Veratrum taliense*. *Plant. Med.* 6: 158-161.