Investigation of the nutritional value and antioxidant activities of common Bangladeshi edible mushrooms

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Abstract

Background: Mushrooms have been recognized both as medicine and nutritional food in many countries for thousands of years, owing to the presence of significant amounts of carbohydrates, proteins, lipids, fibers, and antioxidants. This study aims at evaluating the nutritional contents and antioxidant potentials of seven types of mushrooms cultivated in Bangladesh.

Methods: Proximate composition analysis of the collected mushrooms was performed to determine moisture content, pH, lipid, crude fibre, total ash, protein, carbohydrate content, and calorific value. Antioxidant potential of collected mushrooms were evaluated by mean of eight different methods including total flavonoid content, phenolic content, tannin content, total antioxidant activity, hydroxyl radical scavenging activity, DPPH assay and reducing power capacity.

Results: The results demonstrated that investigated mushrooms were found rich in proteins (20–45 g/100 g), carbohydrate (11–61 g/100 g in dry sample) and fibre (5–40 g/100 g). The ash content was found 6–10 g/100 g and glucose content 54–146 mg/100 g. However, all the mushrooms showed a lower content of lipid (1–4%). Results also revealed that the total antioxidant capacity of the extracts were found in the concentration range of 0.08–0.21 mg/mL, whereas the hydroxyl radical and DPPH radical scavenging activity were 0.88–1.40 and 0.05–0.63 mg/mL.

Conclusion: The findings of the current investigation proved that the studied Bangladeshi mushrooms are good source of nutritional and antioxidant components. Therefore, this study can help spreading awareness among Bangladeshi people regarding consumption of mushrooms as functional foods.

Keywords: Mushroom, Nutrition, Protein, Lipid, Free radical, Antioxidants

Introduction

In many countries around the world, food security has been a concern and a critical issue for decades. According to the World Health Organization (WHO) malnutrition is the main cause of death and diseases around the world. Recent statistics reported that millions of peoples in the world are suffering from malnutrition and about 45% of deaths among children (<5 yrs. of age) in low and middle income countries are linked to malnutrition [1]. Bangladesh is one of the smallest and highly populated countries in South-East Asia. Because of its huge population/density demand, the nation always struggled against poverty and starvation. Malnutrition in Bangladesh is one of the highest in the world and approximately 15–16% children under 5 yrs. old are acutely undernourished [2]. Around one third of Bangladeshi women suffering from chronic nutritional deficiency [3]. Household level double burden of malnutrition is reported to be present in 6.5% households in Bangladesh.
People suffering from malnutrition tend to have a weak immune system and increased cellular oxidative stress which lead to more vulnerable to infections and many other diseases [5]. Mushrooms have been used both as medicine and nutritional food in many countries for thousands of years [6]. It is estimated that no less than 140,000 species of mushrooms exist, but only 10% are catalogued, with approximately 3000 of these mushroom species considered as edible throughout the world [7]. World-wide mushroom consumption is constantly on the rise as more people recognize these as a valuable source of healthy food; it is rich in carbohydrates, proteins, minerals, and vitamins, but low in calorie and fat [8]. Furthermore, mushrooms have shown anti-tumor, antibacterial, antiviral, antiallergenic, immunomodulatory, anti-inflammatory, antiatherogenic, hypoglycaemic, and hepatoprotective activity [9]. Different pharmacological activities of mushrooms were due to their identified various bioactive constituents including common phenolics (gallic acid, protocatechuic acid, catechin, caffeic acid, ferulic acid and myricetin), terpenes (ganodermic acid, ganoderic acids, ganoderals, ganodermanontriol, lucidone, ganodermanodiol, confluentin, grifolin, neogrifolin, ganomycin A and B), sesquiterpenes (enokipodins A, B, C and D), anthraquinones (6-Methylxanthopurin-3-O-methyl ether, (1S,3S)-austrocortilutein, (1S,3R)-austrocortilutein, (1S,3S)-austrocortirubin, physcion, erthroglucain and emodin), peptides (plectasin, peptaibol boletusin, peptaibol chrysospermin 3 and peptaibol chrysospermin 5), fungal immunomodulatory proteins (FIP-fve, Ling-Zhi-8, FIP-gts, FIP-gsi, FIP-pcp, FIP-vvo, FIPvl, FIP-aca, FIP-gia, FIP-gmi and FIP-tvc) and polysaccharides (particularly lentinan, a b-1,3-D-glucan with b-1,6 branches) [10–12]. As a whole food, mushrooms are recognised as having anti-carcinogenic, anti-cholesterolaemic, antiviral, and prophylactic properties against neurodegenerative diseases, diabetes, coronary heart diseases and hypertension [13, 14]. Therefore, edible mushrooms (both cultivated and wild) have a great potential to contribute enormously to food value in our daily diet.

Climate of Bangladesh is one of the most favourable in the world for mushroom cultivation [15]. Although consumption of mushrooms around the globe for mushroom cultivation has a long history, cultivation and consumption of mushrooms are very recent in Bangladesh [16], since people are still largely unaware of the nutritional and medicinal importance of this food item. Though mushroom cultivation was introduced in late 1980’s by Bangladesh Agricultural Research Council (BARC), vigorous training by National Mushroom Extension and Development Centre (NMEDC) of Bangladesh has made it more popular in recent days with the yearly national production increasing from 10,500 metric tons in 2009–2010 to 40,000 metric tons in 2018–2019 [17, 18]. About 20 mushroom species currently grow in Bangladesh, including some poisonous species [19]. Approximately 9 species of edible mushrooms are currently cultivated commercially in Bangladesh by small-scale mushroom production farms [15]. These mushrooms offer an alternative functional food source which could play an important role in improving the nutritional status and prevent a number of common diseases in Bangladeshi people. Several studies has claimed that different species of mushrooms grown in Bangladesh are good source of nutritional components and minerals with marked anti-oxidant potential [6, 16, 20–30]. Besides several pharmacological activities including anti-tyrosinase, cytotoxic, anti-hyperglycemic and antimicrobial potential of Bangladeshi mushrooms have been scientifically demonstrated [28–31]. However, many of the mushrooms that cultivated in Bangladesh are still unexplored. Therefore, this study was conducted to investigate the comprehensive nutritional and antioxidant potential of seven edible mushrooms (Pleurotus ostreatus, Pleurotus djamor, Pleurotus citrinopileatus, Pleurotus ostreatus WS, Pleurotus eryngii, Ganoderma lucidum and Auricularia auricular) cultivated in Bangladesh, to highlight the nutritional value of the studied mushrooms and to shed light on their role in combatting malnutrition and diseases.

Materials and methods
Collection, identification and extraction
Mushroom species, Auricularia auricular (AAU), Ganoderma lucidum (GL), Pleurotus citrinopileatus (PC), Pleurotus djamor (PD), Pleurotus eryngii (PE), Pleurotus ostreatus (PO), and Pleurotus ostreatus WS (PO-WS), belonging to three families (Table 1) were collected from National Mushroom Extension and Development Centre (NMEDC), Savar, Dhaka, and Daulatpur Mushroom Sub-centre, Khulna, Bangladesh between April 2015 and June, 2015 based on their availability, scientific data available, and popularity in consumption among local people. All samples were identified and authenticated by Dr. Akhter Jahan Kakon, Mushroom Specialist, NMEDC, Savar, Bangladesh. Some mushroom samples were collected dry and ready to use, whereas the remaining samples were collected as raw whole material. Samples which required drying were shade-dried and kept in air tight containers. All mushroom material (20–25 g) were extracted by maceration overnight using ethanol (EtOH). Following the extraction, extracts were filtered and evaporated to dryness under reduced pressure by means of rotary evaporation.
Proximate composition analysis

Proximate composition analysis of the collected mushrooms was performed to determine moisture content, pH, lipid, crude fibre, total ash, protein, carbohydrate content, and calorific value. Moisture and lipid contents of the mushrooms were determined according to the method described by the Association of Official Analytical Chemist [32], whereas the pH was measured as per the procedure outlined by Konuk et al. [33]. We obtained the total nitrogen content of the collected edible mushroom using the micro Kjeldahl method and from this (nitrogen content) we determined the crude protein contents using the formula of $N \times 6.25$ [32] where $N$ is the average nitrogen content. On the other hand, the total ash and crude fibre were measured using established protocols, while the total carbohydrate was obtained by subtracting the sum of moisture, ash, total lipid, protein, and fiber contents from the weights of the samples. Finally, the calorific values were obtained by multiplying the values of total carbohydrate, lipid, and protein with the factors 4, 9, and 4, respectively [34].

Determination of the sugar content

The sugar content was determined (mainly hexoses and pentoses) using the old phenolic-sulphuric acid method [35]. Briefly, 2.0 g of dried mushroom powder in a mortar was mixed thoroughly with 10 mL distilled water. The mixture was poured into three test-tubes (about 3 mL each), and the volume was adjusted to 10 mL with water. Mixtures were centrifuged at 3000 rpm for 5 min and then filtered, and the filtrate was treated with pure sulphuric acid and phenol (5%). Absorbance of the resulting solutions was measured at 480, 488, and 490 nm for pentose, hexose, and glucose, respectively, and quantified from standard curves of hexose (standard mixture), pentose (standard mixture) and glucose, respectively.

Determination of antioxidant components (total phenol, flavonoid, and tannin)

Total phenolic content of the mushroom samples was determined by means of the Folin–Ciocalteu’s method using a standard curve of gallic acid. Similarly, the total flavonoid content of the samples was found using quercetin as the standard [36]. On the other hand, the tannin content of the collected mushroom samples was determined by the Folin-Ciocalteu’s method using a standard curve of tannic acid [37]. Results of total phenolic, flavonoid, and tannin contents were expressed as gallic acid equivalent (mg GAE/g dry matter), quercetin equivalent (mg QE/g dry matter), and tannic acid equivalent (mg TAE/g dry matter), respectively, as calculated from the prepared standard curves.

Determination of total antioxidant potential

Total antioxidant capacity of the mushrooms was evaluated using the ammonium molybdate reduction method with some modification [38]. In the previous study, the test sample and reagent mixture was taken 1:1 ratio whereas in this study we have used 1:10 ratio to make sure all the antioxidant components in mushroom sample reacted completely with the reagent solution. Briefly, in this assay 0.3 mL of the test sample (100–500 μg/mL) was mixed with 3 mL of the reagent mixture (4 mM ammonium molybdate, 0.6 M H$_2$SO$_4$, and 28 mM Na$_3$PO$_4$) and incubated at 90 °C for 90 min. After cooling to room temperature, the absorbance of each mixture was measured at 695 nm. The half-minimal inhibitory concentration (IC$_{50}$) value was calculated by comparing the blank absorbance with the function of the sample concentration, using ascorbic acid (AA) as a positive control.

Determination of hydroxyl radical (•OH) scavenging ability

Hydroxyl radical (•OH) scavenging ability of the collected edible mushrooms was determined according to the procedure outlined by Sharma et al. [39]. Briefly, 1 mL of FeSO$_4$ solution (1.5 mM) was added to 1 mL extract/standard (ascorbic acid) solution of each concentration (0.5–2.5 mg/mL). The reaction mixture was then added to a solution containing 0.7 mL of H$_2$O$_2$ (6 mM) and 0.3 mL of sodium salicylate, followed by incubation for 1 h at 37 °C. Absorbance of the hydroxylated salicylate complex was measured at 562 nm, and the

| Name                    | Local name                 | Code   | Family               |
|-------------------------|----------------------------|--------|----------------------|
| Pleurotus ostreatus     | Tree Oyster                | PO     | Pleurotaceae         |
| Pleurotus eryngii       | King Oyster                | PE     | Pleurotaceae         |
| Pleurotus djamor        | Pink Oyster                | PD     | Pleurotaceae         |
| Pleurotus citrinopleatus| Golden Oyster              | PC     | Pleurotaceae         |
| Pleurotus ostreatus-WS  | Tree Oyster-WS strain      | PO-WS  | Pleurotaceae         |
| Ganodermata lucidum     | Reishi mushroom            | GL     | Ganodermaaceae       |
| Auricularia auricular   | Ear mushroom               | AAU    | Auriculariaeae       |
percentage of scavenging activity was calculated using the following equation:

\[
\% \text{ radical scavenging capacity} = \left[ \frac{1 - (A_1 - A_2)/A_0}{} \right] \times 100\% 
\]

Where, \( A_0 \), \( A_1 \), and \( A_2 \) are the absorbance of the control (without extract), presence of extract, and without sodium salicylate, respectively.

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

DPPH free radical scavenging activity of the investigated mushrooms was evaluated by a standard published method [40]. An aliquot of (50 μL) of the extract solution (0.98–500 μg/mL) was mixed with 5 mL of DPPH solution (40 μg/mL) in ethanol, and the reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. Absorbance of the mixtures was then measured spectrophotometrically at 517 nm while acetic acid was used as positive control. We calculated the percentages of inhibition using the following equation:

\[
\% \text{ radical scavenging capacity} = \left[ \frac{1 - (Abs_{sample}/Abs_{control})}{} \right] \times 100\% 
\]

Where, \( Abs_{sample} \) and \( Abs_{control} \) stand for the absorbance of the sample and the control, respectively.

**Determination of reducing power capacity**

We assessed the extracts of collected mushrooms in terms of their capacity to reduce ferric to ferrous ions according to published procedures [36]. Different concentrations (0.01–0.1 mg/mL) of the extract (1 mL) were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%), followed by incubation at 50 °C in a water bath for 20 min. Then 2.5 mL of trichloroacetic acid (10%) was added to terminate the reaction. The upper portion of the solution (2.5 mL) was mixed with 2.5 mL distilled water, and 0.5 mL FeCl₃ solution (0.1%). After keeping it at room temperature for 10 min, the absorbance was measured at 700 nm against an appropriate blank solution, while AA was used as a standard.

**Results and discussion**

**Proximate analysis**

Proximate analysis is the analysis of macronutrients in foods. Proximate values is one of the key commercial criteria to ensure quality food as well as it showed the differences in nutrient composition between food samples. In this study, the proximate analysis of Bangladeshi mushrooms to determine the macronutrient values of these samples. Displayed in Table 2 are results of the proximate composition of the investigated mushrooms. Dry matter/moisture content is one the most important factors which directly affect the nutrient content of mushrooms [41]. Similarly, the moisture content of food material is also important in terms of consumption, freshness, and stability of the food. Our findings indicate that the moisture content of the seven mushroom species studied varies from 4.80 (AAU) to 11% (PE). Additionally, our results show that the studied mushrooms have moderate moisture content, and are therefore high in their dry matter content. These results are in agreement with a previous reported analysis of Indian edible *Pleurotus* species which also contained about 90% dry matter and 10% moisture [42]. On the other hand, an imbalanced diet, high in acid-forming foods, and alkaline foods put pressure on the body’s regulating systems to maintain pH neutrality, causing different problems. Our findings indicate that *Pleurotus* mushrooms showed consistent results around pH 6–7. In this context, Valverde and colleagues reported that mushrooms contain slightly acidic or neutral constituents and is included in a list of neutral or slightly acidic foods [43]. Our results support this statement and confirm that the selected mushrooms are neither too acidic nor basic and are therefore ‘safe’ as a food as it can be compared with the pH of most vegetables, meat and dairy products [44].

Natural lipids play important roles in the storage of energy, forming cell membranes, and in the intracellular signalling and local hormonal regulation [45]. Research findings have shown several health benefits associated with the consumption of fatty acids (such as omega-3) from plant or animal sources in infant development as well as to prevent cancer, cardiovascular diseases, and various mental illnesses [46]. Mushrooms contain different classes of lipids, including free fatty acids, triglycerides, sterols, esters, and phospholipids [41]. In this study, our results show that the lipid content of the selected mushroom samples varied from one species to another and ranged from 1.10 (AAU) to 4.10 (PO) g/100 g. Previous lipid analysis of different *Pleurotus* species cultivated in Bangladesh showed that the lipid content of PO and PC are in agreement with our results [16].

Ash is the completely un-burnable inorganic salts in a sample, with potassium (K) and phosphorus (P) recognised as the main ash constituents in mushrooms [41]. Researchers have reported that mushrooms are rich in mineral content and can act as a better source of minerals than vegetables [41]. In this investigation, results revealed that the total ash content, determined as dry matter basis (d.w.), ranges from 6.50 (PE) to 9.60 (GL) g/100 g. In this regard, it is worth mentioning that this is the first ever report on the ash content of PO-WS and AA cultivated in Bangladesh. In a similar fashion, the dietary fibre contents of the collected mushrooms were found to be in the range of 5.76 (AAU) to 40.63 g/100 g.
and these mushrooms can, therefore, make a significant contribution towards healthy dietary calorific requirements.

**Determination of sugar content**

Sugar play an important role in food and provides the majority of energy of human being. The sugar content of the tested mushroom samples was determined mainly hexoses and pentose sugar. Shown in Table 3 are the sugar (hexose and pentose) contents of the selected Bangladeshi mushrooms. Results reveal that glucose was the main sugar in all collected mushroom samples and ranged from 54.90 (AAU) to 159.50 (GL) mg/100 g. Sucrose content of the samples was in the range 1 to 13.50 mg/100 g, whereas xylose ranged from 4 to 8.70 mg/100 g. These results suggest the studied mushroom species contain low amounts of sugar which is in agreement with results obtained by other researchers [51]. In addition, all of the investigated mushroom species

### Table 2 Proximate composition of selected Bangladeshi cultivated mushrooms

| Mushroom Sample | % Moisture | % Dry matter | pH | % Lipid | % Ash | % Fibre | % Protein | % Carbohydrate | Caloric values (kcal/100 g) |
|-----------------|------------|--------------|----|---------|-------|---------|-----------|-----------------|-----------------------------|
| PO              | 9.70 ± 0.30| 90.30 ± 0.30 | 6.02 ± 010 | 4.13 ± 025 | 7.86 ± 060 | 24.02 ± 010 | 31.63 ± 096 | 22.66 ± 050 | 253.97 ± 8.25 |
| PE              | 11.03 ± 0.30| 88.97 ± 0.30 | 6.70 ± 020 | 1.82 ± 016 | 6.49 ± 040 | 19.30 ± 016 | 22.90 ± 010 | 38.46 ± 030 | 261.98 ± 3.04 |
| PD              | 5.97 ± 0.60| 94.03 ± 0.60 | 6.90 ± 008 | 3.96 ± 016 | 8.38 ± 020 | 15.32 ± 018 | 27.18 ± 050 | 39.19 ± 030 | 301.24 ± 4.64 |
| PC              | 4.37 ± 0.30| 95.63 ± 0.30 | 6.16 ± 010 | 2.48 ± 009 | 8.74 ± 040 | 21.48 ± 030 | 35.88 ± 040 | 27.05 ± 030 | 274.32 ± 4.10 |
| PO-WS           | 6.12 ± 0.30| 93.88 ± 0.30 | 6.38 ± 010 | 2.70 ± 009 | 7.32 ± 040 | 27.08 ± 030 | 45.00 ± 040 | 51.78 ± 027 | 251.50 ± 3.66 |
| GL              | 10.70 ± 0.20| 89.30 ± 0.20 | 7.02 ± 010 | 2.35 ± 009 | 9.60 ± 040 | 40.63 ± 030 | 24.95 ± 040 | 11.70 ± 025 | 167.55 ± 5.00 |
| AAU             | 4.83 ± 0.30| 95.17 ± 0.30 | 5.45 ± 010 | 1.13 ± 008 | 7.24 ± 015 | 5.76 ± 020 | 20.14 ± 025 | 60.90 ± 018 | 334.17 ± 2.90 |

Values are mean ± SD (n = 3)

PO Pleurotus ostreatus, PE Pleurotus eryngii, PD Pleurotus djamor, PC Pleurotus citrinopleatus, PO-WS Pleurotus ostreatus-WS, GL Ganoderma lucidum, AAU Auricularia auricular

### Table 3 Sugar contents of selected Bangladeshi cultivated mushrooms

| Mushroom Sample | Glucose | Sucrose | Xylose |
|-----------------|---------|---------|--------|
| PO              | 81.35 ± 0.08| 1.05 ± 0.08| 4.83 ± 0.09 |
| PE              | 147.00 ± 0.13| 15.85 ± 0.08| 7.90 ± 0.01 |
| PD              | 69.25 ± 0.08| 3.88 ± 0.07| 4.50 ± 0.01 |
| PC              | 125.50 ± 0.10| 13.50 ± 0.13| 8.73 ± 0.01 |
| PO-WS           | 78.50 ± 0.13| 0.85 ± 0.08| 4.72 ± 0.01 |
| GL              | 159.50 ± 0.13| 10.65 ± 0.10| 7.30 ± 0.01 |
| AAU             | 54.93 ± 0.15| 1.72 ± 0.05| 3.98 ± 0.01 |

Values are mean ± SD (n = 3)

PO Pleurotus ostreatus, PE Pleurotus eryngii, PD Pleurotus djamor, PC Pleurotus citrinopleatus, PO-WS Pleurotus ostreatus-WS, GL Ganoderma lucidum, AAU Auricularia auricular
showed consistency in terms of glucose content. This result is consistent with reports that mushrooms are a popular low-calorie food, and are low in sugar [52].

Determination of antioxidant components (total phenol, flavonoid, and tannin)

Phenolics, flavonoids and tannins are the key secondary metabolites of mushrooms that are responsible for their antioxidant activity. In this study, total phenolics, total flavonoids and total tannins properties were measured of collected mushroom samples and was compared each other. Analyses of antioxidants (phenolic, flavonoid, and tannin) in the mushroom samples were determined using a regression equation, obtained from calibration curves of respective standards. Phenolic, flavonoid, and condensed tannin contents were found in the ranges 10.50–83.50 mg GAE/g, 51.90–184.80 mg QE/g, and 36.40–61.80 mg TAE/g, respectively (Table 4).

Consumption of foods high in phenolics and polyphenols can protect against a number of ailments, including heart diseases and cancer [53]. Phenolic constituents represent one of the major chemical groups in our diet, possessing antioxidant properties and acting as free radical scavengers. In the present study, significant phenolic content levels were observed in the mushroom samples under investigation. Our results show that the phenolic content in the mushroom samples follows the order: *Ganoderma lucidum* > *Pleurotus ostreatus* > *Pleurotus citrinopleatus* > *Pleurotus eryngii* > *Auricularia auricular* > *Pleurotus ostreatus-WS* > *Pleurotus djamor*. The highest level of phenolics was found in the GL extract (83.50 mg GAE/g) which very likely contributes to its free radical or *OH scavenging activity. GL had the highest free radical scavenging activity among all the mushroom samples tested. In a previous study, the phenolic content of GL grown in Turkey was found to be 69.80 mg GAE/g [54]. This is the first report of phenolic content of these mushroom species grown in Bangladesh, except for PO (collected from Chittagong) for which the phenolic content has been previously reported at 3.20 mg/mL [29]. The small difference in our observed values (83.50 mg GAE/g) from that of the reported values (69.80 mg GAE/g) could be due to changes in cultivation medium, differences in climate or the extraction procedure.

Flavonoids are considered as one of the most diverse and widespread group of natural phenolics, exhibiting the highest degree of antioxidant activity [53]. It is believed that the localization of flavonoids within the artificial and biological membranes along with interactions of flavonoids at the surface of the lipid bilayer can reduce the access of deleterious molecules (i.e., oxidants) and protect the structure and function of membranes from oxidants [55]. Therefore, the flavonoid contents of the investigated mushrooms were of interest when evaluating their antioxidant properties. Our findings showed that the flavonoid content of the mushroom species used in this study follows the order: *Ganoderma lucidum* > *Pleurotus eryngii* > *Pleurotus citrinopleatus* > *Pleurotus djamor* > *Pleurotus ostreatus-WS* > *Pleurotus ostreatus* > *Auricularia auricular*. However, two Malaysian mushroom samples from the *Pleurotus* genus (PD and PO) showed a total flavonoid content around 14 mg QE/g of dry extract [29] whereas in our study the values were in the range of 54.2 to 85.4 QE/g for all the *Pleurotus* species investigated. The cause of this significant difference (14 mg QE/g and 54.2–85.4 QE/g for Malaysian and Bangladeshi species, respectively) could be attributed to the cultivated medium, climate of cultivation country, as well as the extraction procedure.

Tannins, known for their antioxidant and other important bioactivities, are widely distributed in almost all plant species and thus are incorporated into our daily diet. It is well established that tannins are strong antioxidants and by virtue of this property, they reduce the risk of cancer and cardiovascular diseases [56]. In this study, all of the mushroom species were evaluated for their tannin content. Tannin content of the mushroom samples from *Pleurotus* genus were in the range of 36 to 40

| Mushroom Sample | Total phenolic content mg GAE/g of dry extract | Total flavonoid content mg QE/g of dry extract | Total tannin content mg TAE/g of dry extract |
|-----------------|-----------------------------------------------|-----------------------------------------------|---------------------------------------------|
| PO              | 68.00 ± 1.00                                  | 54.20 ± 2.30                                  | 36.70 ± 0.50                                |
| PE              | 39.70 ± 0.14                                  | 85.40 ± 1.20                                  | 36.40 ± 0.20                                |
| PD              | 10.50 ± 0.07                                  | 56.50 ± 2.30                                  | 38.30 ± 0.20                                |
| PC              | 63.40 ± 0.01                                  | 64.60 ± 1.20                                  | 37.80 ± 0.20                                |
| PO-Ws           | 24.20 ± 0.07                                  | 85.40 ± 1.20                                  | 40.00 ± 0.50                                |
| GL              | 83.50 ± 0.14                                  | 184.80 ± 1.10                                 | 61.80 ± 0.02                                |
| AAU             | 20.70 ± 0.35                                  | 51.90 ± 2.30                                  | 4.04 ± 0.02                                 |

Values are mean ± SD (n = 3)

PO *Pleurotus ostreatus*, PE *Pleurotus eryngii*, PD *Pleurotus djamor*, PC *Pleurotus citrinopleatus*, PO-WS *Pleurotus ostreatus-WS*, GL *Ganoderma lucidum*, AAU *Auricularia auricular*, GAE Gallic acid equivalent, QE Quercetin equivalent, TAE Tannic acid equivalent
mg TAE/g. Thus, the Pleurotus mushrooms showed higher content of flavonoids as compared to their tannin content. One recent report showed that Pleurotus mushrooms (PC, PO and PE) cultivated in culture flasks possess tannin contents in the range of 3–12 mg CE/g [57].

**Determination of total antioxidant capacity**

It is well established that consumption of food containing phytochemicals with potent antioxidant activity can reduce the progression of chronic diseases associated with oxidative stress [34]. Apart from providing basic nutrition, most vegetables and fruits serve as functional foods by virtue of their antioxidant capacity. In this investigation, the total antioxidant activities of the collected mushroom extracts were evaluated for their ability to reduce Mo (VI) to Mo (V). Shown in Table 4 are results of our study of the total antioxidant activity of the mushroom extracts and IC50 values which ranged between 0.087 (GL) and 0.21 mg/mL (PE). To the best of our knowledge, there were no other reports on the total antioxidant activity on Bangladeshi cultivated mushrooms. There are, however, several reports on antioxidant activity of cultivated Pleurotus or Ganoderma mushrooms grown in various parts of the world, along with their antioxidant properties. These reports displayed variations in the antioxidant activity which might be due to differences in cultivation conditions, age of the mushrooms at the time of collection, storage conditions, as well as method of detection [43]. For example, in our study GL showed strong total antioxidant activity (IC50 = 0.087 mg/mL), whereas the same mushroom species cultivated in India reportedly had an IC50 of 0.22 mg/mL by ABTS scavenging methods [58].

**Determination of *OH scavenging ability**

All the mushroom extracts were evaluated for their *OH scavenging activity through the inhibition of *OH generated from the reaction between FeSO4 and hydrogen peroxide. Displayed in Table 5 are results of our investigation of the *OH scavenging activity of all collected mushroom samples. Our findings indicate that PC has the highest *OH scavenging activity with an IC50 of 1.417 mg/mL, and GL has the lowest capacity with an IC50 of 0.886 mg/mL, using AA as the standard with an IC50 value of 0.572 mg/mL. Most of the Pleurotus species showed potent scavenging activity with IC50 values below 1.50 mg/mL. In contrast, an Indian *P. oyster* species was reported to have an IC50 of 8 mg/mL in *OH scavenging activity, which is less potent than our Bangladeshi cultivated *P. oyster* (IC50 of 1.21 mg/mL) [53]. Interestingly, this is the first report on the evaluation of *OH scavenging activity of selected Bangladeshi cultivated mushrooms. Variations between our results (IC50 of 1.21 mg/mL) and other reported values (IC50 of 8 mg/mL) for *P. oyster* could be attributable to variations in cultivation and storage conditions, the age at which the samples were collected, as well as to variation in the methods of detection [43].

**Determination of DPPH radical scavenging activity**

DPPH assay is used to evaluate free radical scavenging activity of antioxidant present in the sample. In this study, DPPH free radical scavenging assay was used to quantitative determination of antioxidant present in mushroom samples that can scavenge free radical. Listed in Table 5 are results of our study of the DPPH radical scavenging capacity of the mushroom samples. Our findings show that GL was the most potent in DPPH radical scavenging ability with an IC50 value of 0.05 mg/mL, whereas PC was the least with an IC50 of 0.63 mg/mL, among the tested mushroom extracts. Excess generation of free radicals within living systems can cause serious damage to tissues and organs, leading to life threatening diseases [47]. This is the first report of the free radical scavenging activity of these selected Bangladeshi cultivated mushroom species, except for PO which has been assayed previously with an IC50 value 0.10 mg/mL [29]. GL species showed the highest antioxidant potential

**Table 5** Free radical scavenging activity of the collected mushrooms

| Mushroom Sample | DPPH scavenging IC50 values (mg/mL) | Hydroxyl radical scavenging | Total antioxidant activity |
|-----------------|-------------------------------------|-----------------------------|---------------------------|
| PO              | 0.535                               | 1.219                       | 0.202                     |
| PE              | 0.390                               | 1.161                       | 0.212                     |
| PD              | 0.547                               | 1.147                       | 0.166                     |
| PC              | 0.639                               | 1.412                       | 0.175                     |
| PO-WS           | 0.457                               | 1.292                       | 0.189                     |
| GL              | 0.053                               | 0.886                       | 0.087                     |
| AAU             | 0.234                               | 0.975                       | 0.103                     |
| AA              | 0.011                               | 0.572                       | 0.011                     |

PO Pleurotus ostreatus, PE Pleurotus eryngii, PD Pleurotus djamor, PC Pleurotus citrinopleatus, PO-WS Pleurotus ostreatus-WS, GL Ganoderma lucidum, AAU Auricularia auricular, AA Ascorbic acid, IC50 Half-minimal inhibitory concentration
which is consistent with the value determined on a Turkish grown sample (IC$_{50}$: 0.055 mg/mL) [54] but significantly higher than the value determined on a Taiwanese GL sample (IC$_{50}$: 0.64 mg/mL).

Determination of reducing power capacity
Reducing capacity is considered as a significant index of antioxidant activity [59]. In this study, all mushroom extracts were evaluated for their reducing power through their ability to reduce ferric ions (Fe$_3^+$) to ferrous (Fe$_2^+$). All extracts showed increased reducing power with the increase of concentrations (Fig. 1). Among the tested mushrooms, the highest and lowest activity was observed for GL and AAU, respectively. The reducing capacity of a substance contributes towards its antioxidant potential along with other factors such as chain initiation, decomposition of peroxides, reducing capacity and radical scavenging ability [36].

Conclusions
Results from the present investigation demonstrated that out of the seven mushroom species studied, GL scored the highest levels of ash, fibre, and glucose contents, PO-WS were the top in terms of the protein level, whereas AAU possessed the highest level of total carbohydrate. Interestingly, all tested mushroom species showed comparatively low levels of lipid content. With regard to antioxidant assays, GL displayed the highest DPPH and *OH radical scavenging activity as well as total antioxidant capacity than the other investigated mushrooms. It additionally recorded the highest level of phenolic, flavonoid, and tannin contents which might contribute to its free radical scavenging or reducing power capacity. Results obtained clearly indicate that these seven Bangladeshi grown mushroom species are rich in protein, fibre, and carbohydrate as well as a good source of antioxidants. Therefore, these mushrooms can be promoted as a potential source of nutrients and antioxidants to combat malnutrition and other diseases.

Abbreviations
PO: Pleurotus ostreatus; PE: Pleurotus eryngii; PD: Pleurotus djamor; PC: Pleurotus citrinopleatus; PO-WS: Pleurotus ostreatus-WS; GL: Ganoderma lucidum; AAU: Auricularia auricular; NMDEC: National Mushroom Development and Extension Centre; BARC: Bangladesh Agricultural Research Council; GAE: Gallic acid equivalent; QE: Querctin equivalent; TAE: Tannic acid equivalent; DPPH: 2,2-diphenyl-1-picrylhydrazyl

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Authors’ contributions
NS and FL conducted the experimental works, acquisition and analysis of data. SJU designed the study and supervised NS and FL work. SJU, SMNKZ and MGH prepared the final draft of the manuscript. JAS, IDG and MSM reviewed and critically analysed the manuscript and English check. All authors have read and approved the final form of the manuscript.

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Not applicable.

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Authors declare that they have no conflict of interests.

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![Fig. 1 Reducing power ability of the collected mushroom extracts, where the absorbance is plotted against concentration of extract. (Values are mean ± SD (n = 3))](image-url)
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