

Nutritional Properties, Antioxidant and Antihaemolytic Activities of the Dry Fruiting Bodies of Wild Edible Mushrooms Consumed by Ethnic Communities of Northeast India

Merilin Kakoti^{1,2} , Dibya Jyoti Hazarika^{1,3} , Assma Parveen¹ , Samim Dullah¹ ,
Alokesh Ghosh¹ , Dipankar Saha¹ , Madhumita Barooah¹ , Robin Chandra Boro^{1*} 

¹Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat – 785013, Assam, India

²Department of Bioengineering and Technology, Gauhati University Institute of Science and Technology, Gauhati University, Guwahati – 781014, Assam, India

³DBT-North East Centre for Agricultural Biotechnology, Assam Agricultural University, Jorhat – 785013, Assam, India

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A variety of cultivated mushrooms in Northeast India are well known for their taste, nutritional and medicinal benefits. Many wild-growing mushrooms are also consumed due to their exotic flavours and tastes; however, the scientific exploration of their nutritional and bioactive properties is still negligible. In the present study, the 32 wild edible mushroom samples of 11 species collected from different parts of Northeast India were evaluated for their proximate composition, mineral and vitamin (ascorbic acid and riboflavin) contents, antioxidant and antihaemolytic activity, and profiles of organic and phenolic acids. *Lentinus sajor-caju* and *Lentinus squarrosulus* had the highest carbohydrate content (49.80 g/100 g dry weight (d.w.) and 46.36 g/100 g d.w., respectively), crude protein content (20.72 g/100 g d.w. and 20.54 g/100 g d.w., respectively) and a considerable content of minerals. The highest fat content was determined in *Lentinus velutinus* (7.17 g/100 g d.w.). Among the minerals, potassium was found as the most abundant in all the samples. The extracts of *L. sajor-caju*, *L. squarrosulus*, and *Pleurotus pulmonarius* were characterized by the highest antioxidant activity, while these of *L. sajor-caju*, *Pleurotus ostreatus*, *P. pulmonarius* and *Agaricus bisporus* showed the highest antihaemolytic potential. The HPLC analysis allowed determining the high contents of ascorbic acid and a few organic and phenolic acids such as lactic acid, gallic acid, 3,4-dihydroxybenzoic acid and *trans*-cinnamic acid in the tested mushrooms. Other compounds *viz.* citric acid, caffeic acid, riboflavin, vanillic acid, pyruvic acid, and *p*-coumaric acid were detected with variations. This study established the nutritional and health benefits of wild edible mushrooms of Northeast India region for consumption as functional foods in the human diet.

INTRODUCTION

Mushrooms (including the members of Basidiomycota and the fruiting body forming Ascomycota) are considered to be one of the important components of the forest ecosystem. They have been gaining in importance since ancient times due to their edibility, psychotropic properties, poisonous nature, and mycorrhizal or parasitic associations with the forest trees. With an estimation of around 1.5 million fungal species on earth [Hawksworth, 2001], more than 31,000 species of Basidiomycota (which form the fruiting bodies) and more than 66,000 species of Ascomycota (a small fraction of which forms the fruiting bodies) are well-characterized [Martins, 2017; Taylor *et al.*, 2015]. Among the discovered mushroom species, there are abundant numbers of wild edible mushrooms which are consumed world-wide. These mushrooms need to be evaluated for their nutritional composition and bioactive metabolites.

Many mushrooms are rich in nutrients, medicinal, and plant growth-promoting compounds [Ghate & Sridhar, 2016], whereas, many others contain toxic metabolites. Edible mushrooms contain considerable amount of nutritional compounds including carbohydrates (especially non-reducing sugars), proteins, minerals and vitamins. Presence of phenolics, tocopherols, carotenoids and ascorbic acid in mushroom fruiting bodies make them a good source of natural antioxidants [Sánchez, 2017]. These antioxidant molecules provide biochemical support to the growth of fruiting bodies by neutralizing the oxidative stresses provided by reactive oxygen species and free radicals. Likewise, consumption of foods that are rich in natural antioxidants provide excellent health benefits and protects our body against oxidative stresses and aging signs [Chang, 1996; Lindequist *et al.*, 2005]. Recent studies have demonstrated the antihaemolytic potential of a few mushroom species [Madhanraj *et al.*, 2019; Sharif *et al.*, 2017]. Antihaemolytic compounds are antioxidants that

* Corresponding Author:

E-mail: robin.boro@aaau.ac.in (R.Ch. Boro)

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inhibit the lysis of red blood cells caused by oxidative agents [Shabbir *et al.*, 2013].

The Northeast (NE) India possesses a richness in forests, with an abundance of many tree species and other woody plants. The biodiversity of woody plants can be correlated with an equally diverse mycoflora. Ethnic communities inhabiting different regions of Northeast India regularly consume edible mushrooms collecting from the wild based on their traditional knowledge on mushroom identities and their nutritional benefits. However, the diversity of wild mushrooms from this region is not well documented in terms of nutritional properties and bioactive properties. Earlier studies have identified some of the wild edible mushrooms from the states of NE India including Assam, Arunachal Pradesh, Meghalaya, and Nagaland [Khaund & Joshi, 2013; Parveen *et al.*, 2017; Sarma *et al.*, 2010]. However, nutritional profiling of majority of these wild edible mushrooms are not well investigated. In our recent study, a molecular genetic analysis was conducted to identify 50 wild mushrooms collected from different regions of five Northeastern states of India, out of which 32 edible samples belonging to 11 different species were detected based on their morphological characters as well as genetic information of the internal transcribed spacer (ITS) region [Kakoti *et al.*, 2021]. Most of these edible mushrooms are part of the regular diet of various tribal and non-tribal communities. Therefore, the present investigation was conducted to evaluate the species-wise nutritional profiling including proximate composition, mineral and vitamin (ascorbic acid and riboflavin) contents, bioactivity (antioxidant and anti-haemolytic activities) and contents of organic and phenolic acids of those 32 wild edible mushrooms to establish their edibility as functional food.

MATERIALS AND METHODS

Collection of the fruiting bodies of mushrooms

The fruiting bodies of different mushrooms were collected from different locations of five North-Eastern states of India (Assam, Arunachal Pradesh, Manipur, Meghalaya, and Nagaland). The fruiting bodies were cleaned at the site of collection with distilled water and immediately taken to the laboratory by packing inside the collection bags. A total of 32 wild-edible mushroom samples were used in this study and their fundamental sampling information are described in Table 1. These information are also available online at Barcode of Life Data (BOLD) system (<http://www.boldsystems.org/>). Morphological description of molecular identities (ITS barcode details) of the samples was provided previously [Kakoti *et al.*, 2021]. BOLD submission IDs and GenBank Accession numbers are provided in Table 1.

Preparation of dry powder

The mushroom fruiting bodies were initially shade-dried with dry air to remove the excessive moisture from the samples and placed in a hot air oven at 45°C until the residual moisture was removed. This process took 16–24 h depending on the sample characteristics. Dry mushrooms were then powdered using a grinder and sieved through 0.5 mm net.

Determination of moisture content

Moisture content of the fresh mushrooms was determined using the Association of Official Analytical Chemists (AOAC) standard protocol [AOAC, 1996]. Briefly, about 20 g of freshly collected samples were weighed, shade-dried at room temperature for 2 h inside a laminar air flow hood (to remove the excessive moisture), and placed in a hot-air oven at 105°C for 5 h. The dishes were later cooled in a desiccator and weighed with the lid on. The moisture content of the mushrooms was estimated using the formula:

$$\begin{aligned} &\text{Moisture content of fresh sample (g/100 g)} \\ &= \left(\frac{\text{Fresh weight (g)} - \text{Dry weight (g)}}{\text{Fresh weight (g)}} \right) \times 100 \end{aligned}$$

The residual moisture content in the dry fruiting powders was determined from 1 g dry powder by placing in a hot-air oven at 105°C for 5 h.

$$\begin{aligned} &\text{Moisture content of dry powder (g/100 g)} \\ &= \left(\frac{\text{Initial dry weight (g)} - \text{final dry weight (g)}}{\text{Initial dry weight (g)}} \right) \times 100 \end{aligned}$$

Moisture content of the dry powder was used to calculate the actual dry weight of the samples.

Determination of ash content

The ash content of mushrooms was determined from the dried, fine powders of the mushroom fruiting bodies. One gram of powder was weighed into a crucible, which was placed in a muffle furnace initially at 130°C for 1 h, and finally the temperature was increased to 600°C for about 6 h. The powder was cooled in a desiccator and weighed. The ash content was calculated using the following equation:

$$\begin{aligned} &\text{Ash content (g/100 g)} \\ &= \left(\frac{\text{Weight of ash (g)}}{\text{Weight of dry mushroom (g)}} \right) \times 100 \end{aligned}$$

Finally, ash content of dried mushrooms was expressed as g per 100 g of powder dry weight (d.w.).

Determination of crude protein content

The crude protein in the dried and powdered mushroom tissue was determined using the macro-Kjeldhal method [method 984.13; AOAC, 1990] with necessary modifications. Briefly, 100 mg of the mushroom powder was subjected to acid digestion in a KelPlus digestion apparatus (Pelican equipment, Chennai, Tamil Nadu, India). The digested sample was distilled following the alkali treatment and the released ammonia was extracted in 2.5% boric acid using the KelPlus automatic distiller (Pelican equipment). The resultant solution was then titrated manually against 0.02 N sulfuric acid to determine the nitrogen content. The crude protein content was calculated from the nitrogen content by multiplying with a factor of 4.38 [Reis *et al.*, 2012]. The results were expressed as g per 100 g of d.w. of mushroom powders.

Determination of total carbohydrate content

Dried mushroom powder (100 mg) was mixed with 2.5 N HCl and boiled in a water bath for 3 h. The hydrolysate was neutralized with sodium carbonate. The volume was made up

to 100 mL and supernatant was collected by centrifugation. Carbohydrate content in the supernatant was determined using the anthrone method [Sadasivam & Manickam, 1996]. Total carbohydrate content of mushroom powders was expressed as g per 100 g of d.w.

Determination of fat content

The total fat content of dried mushroom fruiting bodies was determined using the gravimetric method [AOAC, 2007]. Fat was extracted with ethanol : diethyl ether : petroleum ether (5:12:12, v/v/v) after hydrolysis of the dry mushroom powder with concentrated HCl. The petroleum ether layer was separated after proper mixing and dried to obtain the fat, which was further weighed. Total fat content in dried mushrooms was calculated as follows:

$$\text{Fat content (g/100 g)} = \left(\frac{\text{Weight of the extracted fat (g)}}{\text{Sample weight (g)}} \right) \times 100$$

The results were expressed as g per 100 g of d.w. of mushroom powders.

Determination of mineral content

The contents of minerals *viz.* calcium, magnesium, potassium, sodium, zinc, iron, copper and manganese, in the dried mushroom fruiting bodies were determined using an iCE3000 atomic absorption spectrometer (Thermo Scientific, Waltham, MA, USA). Extracts were prepared by digesting the powdered mushroom samples in nitric acid and hydrogen peroxide as described earlier [Soylak *et al.*, 2005]. The phosphorus content was estimated using the molybdovanadate method [method 965.17; AOAC, 1990]. The content of each element was determined using a calibration curve plotted with known concentrations of the respective standards. Results were expressed based on d.w. of mushroom powders.

Extract preparation from the fruiting bodies

Dry powdered fruiting bodies (1 g) were extracted overnight using 100 mL of methanol and the supernatant was carefully filtered through Whatman No. 42 filter paper (GE Healthcare, Chicago, IL, USA) taking the care that minimal residue was transferred to the filter paper. Supernatant was collected and the residue was extracted with another 100 mL of methanol as described above. For the determination of total phenolic content, the filtered supernatant was directly used in the assay. For antioxidant and antihaemolytic activities analysis, the extracts were evaporated to dryness using a rotary evaporator and re-dissolved in a required volume (to prepare the working solutions) of methanol or phosphate buffered saline (PBS; 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, 137 mM NaCl and 2.7 mM KCl, pH 7.4), respectively, as per the requirements for further experiments.

Determination of total phenolic content

Total phenolic content in the methanol extracts of mushroom samples was estimated spectrophotometrically, based on the procedure described by Singleton & Rossi [1965] with some modifications. First, 1 mL of the extract was mixed with 1 mL of a Folin-Ciocalteu's phenol reagent. After 3 min,

1 mL of a saturated sodium carbonate solution was added to the mixture and adjusted to the total volume to 10 mL with distilled water. The reaction mixture was kept in dark for 90 min, after that the absorbance was recorded at 725 nm. Known concentrations of gallic acid were used to prepare the standard curve. The total phenolic content of the samples was calculated based on the graph and expressed as mg gallic acid equivalents (GAE) per 100 g of d.w. of mushroom powders.

DPPH radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma, Saint Louis, MO, USA) radical scavenging activity was determined by the Blois's method [Blois, 1958] with minor modifications. The extract and reference standard solutions in methanol (1 mL) were prepared in different concentrations and mixed individually with 0.5 mL of 0.15 mM DPPH[•] solution. α -Tocopherol was used as the reference standard [Boonsong *et al.*, 2016]. The percentage of inhibition of DPPH[•] was obtained by measuring the absorbance at 517 nm using an Evolution 202 UV-Vis double beam spectrophotometer (Thermo Scientific) and calculation using the following formula:

$$\% \text{ Inhibition of DPPH radical} = \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100$$

The % inhibition data was used to calculate the IC₅₀ value – concentration of extract that could scavenge 50% of DPPH radicals. Additionally, the results were expressed as the tocopherol equivalent antioxidant activity (TEAA) in mg tocopherol equivalent per 100 g of d.w. of mushroom powder.

Determination of antihaemolytic activity

The antihaemolytic activity of the dried mushrooms was evaluated using the spectrophotometric method described previously by Shabbir *et al.* [2013] with minor modifications. Briefly, the reaction mixture consisted 0.5 mL of mushroom extract with varying concentrations *viz.*, 100, 250, 500, 750 and 1000 μ g/mL in PBS and 0.5 mL of a red blood cell (RBC) suspension, and the mixture was incubated at room temperature for 20 min. After incubation, 0.5 mL of hydrogen peroxide (H₂O₂) was supplemented to the mixture for induction of the oxidative degradation of membrane lipids. A control was prepared with a similar volume of the reaction mixture without adding the extract. The reaction mixture was then centrifuged at 500 \times g, 4°C for 5 min and the antihaemolytic activity was assessed spectrophotometrically at 540 nm. The percent of haemolysis was calculated using the following formula:

$$\% \text{ Inhibition of haemolysis} = \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100$$

The % inhibition data was used to calculate the IC₅₀ value – concentration of extract that could inhibit the haemolysis of RBC by 50%.

Detection of major organic acids and antioxidant compounds

The organic and phenolic acids and some other metabolites were initially extracted from powdered mushroom

TABLE 1. Sampling details of the wild edible mushrooms collected in Northeast India.

| No. | Species name | Sample ID | Habitat | Sampling location | Date of collection | Collected by | GenBank Accession No. | BOLD submission ID |
|-----|--|-----------|------------------------------|--|--------------------|----------------------------------|-----------------------|--------------------|
| 1 | <i>Agaricus bisporus</i> (J.E. Lange) Imbach 1946 | MLS2 | Soil containing plant litter | East Khasi Hill, Shillong, Meghalaya | 11.04.2018 | M. Kakoti, D. J. Hazarika | MK855508 | ITSA051–20 |
| 2 | <i>Auricularia auricula-judae</i> (Bull.) Quéf. 1886 | DIM1 | Living tree | Dimapur, Nagaland | 30.04.2018 | M. Kakoti | MK855509 | ITSA040–20 |
| 3 | <i>Lentinus sajor-caju</i> (Fr.) Fr. 1838 | APK5 | Wood surface | Koronu, Lower Dibang Valley, Arunachal Pradesh | 26.03.2018 | M. Kakoti, D. J. Hazarika | MK851527 | ITSA014–19 |
| | | DIM3 | Decaying wood | Dimapur, Nagaland | 30.04.2018 | M. Kakoti | MK851526 | ITSA016–19 |
| | | DH3 | Decaying wood | Haflong, Dima Hasao, Assam | 03.07.2017 | M. Kakoti | MK851528 | ITSA015–19 |
| | | MIS2 | Decaying wood | Missamara, Golaghat, Assam | 25.06.2018 | M. Kakoti, D. J. Hazarika | MK851531 | ITSA017–19 |
| | | MIS7 | Fully decayed wood | Missamara, Golaghat, Assam | 03.07.2018 | M. Kakoti, D. J. Hazarika | MK851530 | ITSA018–19 |
| | | MP3 | Decaying wood | Kohima, Manipur | 01.02.2018 | M. Kakoti | MK851529 | ITSA019–19 |
| | | AAU1 | Decaying wood | Assam Agricultural University Campus Barbheta, Jorhat, Assam | 15.06.2017 | M. Kakoti | MK851539 | ITSA020–19 |
| 4 | <i>Lentinus squarrosulus</i> Mont. 1842 | AAU2 | Decaying wood | Assam Agricultural University Campus Barbheta, Jorhat, Assam | 15.06.2017 | M. Kakoti | MK851538 | ITSA021–19 |
| | | AAU3 | Decaying wood | Assam Agricultural University Campus Barbheta, Jorhat, Assam | 15.06.2017 | M. Kakoti | MK851536 | ITSA022–19 |
| | | AAU4 | Decaying wood | Assam Agricultural University Campus Barbheta, Jorhat, Assam | 15.06.2017 | M. Kakoti | MK851535 | ITSA023–19 |
| | | AAU5 | Soil | Assam Agricultural University Campus Barbheta, Jorhat, Assam | 15.06.2017 | M. Kakoti | MK851534 | ITSA024–19 |
| | | AAU6 | Soil | Assam Agricultural University Campus Barbheta, Jorhat, Assam | 15.06.2017 | M. Kakoti | MK851533 | ITSA025–19 |
| | | AAU7 | Soil | Assam Agricultural University Campus Barbheta, Jorhat, Assam | 15.06.2017 | M. Kakoti | MK851532 | ITSA026–19 |
| | | DH1 | Decaying wood | Haflong, Dima Hasao, Assam | 03.07.2017 | M. Kakoti | MK851537 | ITSA027–19 |
| 5 | <i>Lentinus velutinus</i> Fr. 1830 | DIM2 | Decaying wood | Dimapur, Nagaland | 30.04.2018 | M. Kakoti | MK855509 | ITSA041–20 |
| | | KB2 | Dead tree | Bokajan, Karbi Anglong, Assam | | M. Kakoti, D. J. Hazarika | MK851540 | ITSA028–19 |
| | | KM5 | Soil | Kanubari Tea Estate, Sivasagar, Assam | 03.05.2017 | M. Kakoti, S. Dullah, A. Parveen | MK855514 | ITSA045–20 |
| | | BP9 | Soil | Barpeta, Assam | 31.08.2018 | M. Kakoti, A. Ghosh | MK851544 | ITSA003–19 |
| | | DIM8 | Soil | Dimapur, Nagaland | 17.06.2018 | M. Kakoti | MK851545 | ITSA004–19 |
| | | KM7 | Grassland soil | Kanubari Tea Estate, Sivasagar, Assam | 03.05.2017 | M. Kakoti, S. Dullah, A. Parveen | MK851546 | ITSA005–19 |
| 6 | <i>Lycoperdon scabrum</i> | RB6 | Soil | Rongbong, Golaghat, Assam | 18.06.2018 | M. Kakoti, D. J. Hazarika | MK851547 | ITSA006–19 |

TABLE 1. Continued

| No. | Species name | Sample ID | Habitat | Sampling location | Date of collection | Collected by | GenBank Accession No. | BOLD submission ID |
|-----|---|-----------|------------------------|--|--------------------|----------------------------------|-----------------------|--------------------|
| 7 | <i>Panus lecomtei</i> (Fr.) Corner 1981 | APBN3 | Wood surface | Bhismaknagar, Roing, Arunachal Pradesh | 26.03.2018 | M. Kakoti, D. J. Hazarika | MK851549 | ITSA036-19 |
| 8 | <i>Pleurotus giganteus</i> (Berk.) Karun. & K.D. Hyde 2011 | KM1 | Soil with plant litter | Kanubari Tea Estate, Sivasagar, Assam | 03.05.2017 | M. Kakoti, S. Dullah, A. Parveen | MK851552 | ITSA007-19 |
| | | KM2 | Soil with plant litter | Kanubari Tea Estate, Sivasagar, Assam | 03.05.2017 | M. Kakoti, S. Dullah, A. Parveen | MK855519 | ITSA044-20 |
| 9 | <i>Pleurotus ostreatus</i> (Jacq.) P. Kumm. 1871 | MP1 | Decaying wood | Kohima, Manipur | 01.02.2018 | M. Kakoti | MK855520 | ITSA046-20 |
| | <i>Pleurotus pulmonarius</i> (Fr.) Quél. 1872 | MLS1 | Decaying wood | East Khasi Hill, Shillong, Meghalaya | 11.04.2018 | M. Kakoti, D. J. Hazarika | MK851551 | ITSA008-19 |
| 10 | [synonym. <i>Pleurotus ostreatus</i> var. <i>pulmonarius</i> (Fr.) Iordanov, Vanev & Fakirova 1979] | MP2 | Dead tree surface | Kohima, Manipur | 01.02.2018 | M. Kakoti | MK855521 | ITSA047-20 |
| | <i>Polyporus arcularius</i> (Batsch) Fr. 1821 | APBN4 | Decaying wood | Bhismaknagar, Lower Dibang Valley, Arunachal Pradesh | 26.03.2018 | M. Kakoti, D. J. Hazarika | MK851553 | ITSA030-19 |
| 11 | [synonym. <i>Lentinus arcularius</i> (Batsch) Zmitr. 2010] | DH2 | Decaying wood | Halfong, Dima Hasao, Assam | 03.07.2017 | M. Kakoti | MK851555 | ITSA031-19 |
| | | MLS6 | Decaying wood | East Khasi Hill, Shillong, Meghalaya | 23.08.2018 | M. Kakoti, D. J. Hazarika | MK851554 | ITSA032-19 |

BOLD: Barcode of Life Data.

fruiting body (1 g) with 25 mL of 80% (v/v) acetone in water [Barros *et al.*, 2009] for 6 h and filtered through Whatman No. 42 filter paper (GE Healthcare) taking the care that minimal residue was transferred to the filter paper. The precipitate was re-extracted with another 25 mL of 80% (v/v) acetone as described above. The crude extracts were concentrated under vacuum and re-dissolved in 20 mL of 50% (v/v) methanol. The extracts were then filtered using a membrane syringe filter and a 20 μ L of sample was separated through a Cosmosil C-18 column (300 \times 4.6 mm, pore size 5 μ m; Nacalai Tesque Inc., Kyoto, Japan) installed in a Hitachi Chromaster 3000 series HPLC system with a diode array detector (Hitachi, Tokyo, Japan). The mobile phase used consisted of acetonitrile (A) and 0.1% (v/v) phosphoric acid (H₃PO₄) in water in a gradient mode: 5% of A at 0–2 min, 15% of A at 2–5 min, 40% of A at 5–10 min, 60% of A at 10–15 min, 90% of A at 15–18 min, reverting to 5% of A at 20 min and equilibration with 5% of A till 25 min. Detection of compounds was performed in the range of 200–400 nm. The peaks were compared with individual standards of 11 organic acids and antioxidants *viz.*: ascorbic acid (Sigma-Aldrich, Saint Louis, MO, USA), caffeic acid (Sigma-Aldrich), citric acid (Himedia, Mumbai, Maharashtra, India), 3,4-dihydroxybenzoic acid (Sigma-Aldrich), gallic acid (Sigma-Aldrich), lactic acid (Sigma-Aldrich), pyruvic acid (Himedia), *p*-coumaric acid (Sigma-Aldrich), riboflavin (Sigma-Aldrich), *trans*-cinnamic acid (Sigma-Aldrich) and vanillic acid (Sigma-Aldrich). The contents of compounds in mushrooms were calculated from the linear portion of the regression curve prepared from the peak areas of individual reference standards.

Statistical analysis

All the statistical analyses were performed using IBM SPSS software, version 25 (Armonk, NY, USA). To test the significant differences among the samples, one-way analysis of variance (ANOVA) with Duncan's multiple range test was used, while non-parametric Kruskal-Wallis test was used for species-wise comparison. Results were considered to be significant with 95% confidence level and $p < 0.05$. Pearson's correlation analysis was performed to calculate the correlation coefficient among total phenolic content, antioxidant activity and antihemolytic activity. All the experiments, including the preparation of extracts, determination of proximate compositions and bioactivities were performed with three independent replicates for each sample. Three data points were generated from three independent replications for statistical analysis.

RESULTS AND DISCUSSION

Nutritional properties of the wild edible mushrooms

In this study, 32 wild edible mushroom samples collected from different locations of Northeast India were assessed for their nutritional properties. Sample-wise as well as species-wise comparisons of the moisture content among different edible samples are represented in Table 2. The highest moisture content (90.35 g/100 g) was recorded in *Auricularia auricula-judae*, while *Polyporus arcularius* (synonym. *Lentinus arcularius*) was recorded with the lowest moisture content

TABLE 2. Sample-wise and species-wise comparison of proximate compositions of the mushroom fruiting bodies.

| No. | Species name & sample code | Moisture content of the fresh fruiting bodies (g/100 g) | | Moisture content of the dried powder (g/100 g) | | Ash (g/100 g dry weight) | | Carbohydrate (g/100 g dry weight) | | Crude protein (g/100 g dry weight) | | Fat (g/100 g dry weight) | |
|-----|-----------------------------------|---|---------------------------------|--|---------------------------------|---------------------------------|-------------------------------|-----------------------------------|-------------------------------|------------------------------------|-------------------------------|---------------------------------|-------------------------------|
| | | Average of samples | Average of species | Average of samples | Average of species | Average of samples | Average of species | Average of samples | Average of species | Average of samples | Average of species | Average of samples | Average of species |
| 1. | <i>Agaricus bisporus</i> | 84.50±0.44^c | 2.60±0.10^d | 9.23±0.38^a | 36.52±2.22^f | 18.65±0.80^{lm} | 2.46±0.18^{ef} | 18.65±0.80^{lm} | 2.46±0.18^{ef} | 18.65±0.80^{lm} | 2.46±0.18^{ef} | 18.65±0.80^{lm} | 2.46±0.18^{ef} |
| | MLS2 | 84.50±0.44 ^f | 2.60±0.10 ^{lm} | 9.23±0.38 ^a | 36.52±2.22 ^p | 18.65±0.80 ^{lm} | 2.46±0.18 ^{ef} | 18.65±0.80 ^{lm} | 2.46±0.18 ^{ef} | 18.65±0.80 ^{lm} | 2.46±0.18 ^{ef} | 18.65±0.80 ^{lm} | 2.46±0.18 ^{ef} |
| 2. | <i>Auricularia auricula-judae</i> | 90.35±0.41^a | 2.83±0.06^{hijl} | 5.68±0.39ⁿ | 42.75±2.42^{ghi} | 19.70±0.94^{hij} | 1.44±0.00^{hi} | 19.70±0.94^{hij} | 1.44±0.00^{hi} | 19.70±0.94^{hij} | 1.44±0.00^{hi} | 19.70±0.94^{hij} | 1.44±0.00^{hi} |
| | DIMI | 90.35±0.41 ^a | 2.83±0.06 ^{hijl} | 5.68±0.39 ⁿ | 42.75±2.42 ^{ghi} | 19.70±0.94 ^{hij} | 1.44±0.00 ^{hi} | 19.70±0.94 ^{hij} | 1.44±0.00 ^{hi} | 19.70±0.94 ^{hij} | 1.44±0.00 ^{hi} | 19.70±0.94 ^{hij} | 1.44±0.00 ^{hi} |
| 3. | <i>Lentinus sajor-caju</i> | 81.12±1.17^c | 2.63±0.15^d | 7.19±0.50^c | 49.80±3.34^a | 20.72±1.97^a | 0.62±0.55^e | 20.72±1.97^a | 0.62±0.55^e | 20.72±1.97^a | 0.62±0.55^e | 20.72±1.97^a | 0.62±0.55^e |
| | APK5 | 82.20±0.66 ^{gh} | 2.53±0.15 ^{mn} | 7.61±0.31 ^{dehgh} | 47.76±2.39 ^{de} | 19.93±0.20 ^{ghi} | 0.24±0.06 ⁿ | 19.93±0.20 ^{ghi} | 0.24±0.06 ⁿ | 19.93±0.20 ^{ghi} | 0.24±0.06 ⁿ | 19.93±0.20 ^{ghi} | 0.24±0.06 ⁿ |
| | DH3 | 80.22±0.33 ^{lmn} | 2.73±0.06 ^{klmn} | 7.05±0.12 ^{jk} | 52.09±2.32 ^{ab} | 19.05±0.33 ^{ijkl} | 0.24±0.06 ⁿ | 19.05±0.33 ^{ijkl} | 0.24±0.06 ⁿ | 19.05±0.33 ^{ijkl} | 0.24±0.06 ⁿ | 19.05±0.33 ^{ijkl} | 0.24±0.06 ⁿ |
| | DIM3 | 82.72±0.46 ^g | 2.77±0.15 ^{klmn} | 7.36±0.37 ^{ghij} | 51.63±1.12 ^{ab} | 22.04±0.42 ^{okle} | 0.75±0.22 ^{jk} | 22.04±0.42 ^{okle} | 0.75±0.22 ^{jk} | 22.04±0.42 ^{okle} | 0.75±0.22 ^{jk} | 22.04±0.42 ^{okle} | 0.75±0.22 ^{jk} |
| | MIS2 | 81.36±0.48 ^{hij} | 2.67±0.12 ^{klmn} | 6.64±0.32 ^{kl} | 49.88±1.29 ^{bcd} | 23.11±0.32 ^{ab} | 0.31±0.10 ^{mn} | 23.11±0.32 ^{ab} | 0.31±0.10 ^{mn} | 23.11±0.32 ^{ab} | 0.31±0.10 ^{mn} | 23.11±0.32 ^{ab} | 0.31±0.10 ^{mn} |
| | MIS7 | 80.49±0.48 ^{kl} | 2.43±0.06 ⁿ | 7.74±0.41 ^{bcdef} | 44.39±1.04 ^{gh} | 17.86±0.45 ^{nm} | 1.71±0.21 ^{gh} | 17.86±0.45 ^{nm} | 1.71±0.21 ^{gh} | 17.86±0.45 ^{nm} | 1.71±0.21 ^{gh} | 17.86±0.45 ^{nm} | 1.71±0.21 ^{gh} |
| | MP3 | 79.72±0.21 ^{lm} | 2.63±0.12 ^{klmn} | 6.73±0.21 ^{kl} | 53.05±0.85 ^a | 22.31±0.32 ^{bcd} | 0.48±0.06 ^{klm} | 22.31±0.32 ^{bcd} | 0.48±0.06 ^{klm} | 22.31±0.32 ^{bcd} | 0.48±0.06 ^{klm} | 22.31±0.32 ^{bcd} | 0.48±0.06 ^{klm} |
| 4. | <i>Lentinus squarrosulus</i> | 80.48±1.40^c | 2.92±0.36^{bc} | 7.07±0.41^c | 46.36±3.71^b | 20.54±1.72^a | 2.69±0.69^b | 20.54±1.72^a | 2.69±0.69^b | 20.54±1.72^a | 2.69±0.69^b | 20.54±1.72^a | 2.69±0.69^b |
| | AAU1 | 82.17±0.72 ^{gh} | 2.83±0.12 ^{hijl} | 7.57±0.30 ^{efgh} | 50.53±1.08 ^{abc} | 19.49±0.42 ^{ijk} | 2.57±0.00 ^{ef} | 19.49±0.42 ^{ijk} | 2.57±0.00 ^{ef} | 19.49±0.42 ^{ijk} | 2.57±0.00 ^{ef} | 19.49±0.42 ^{ijk} | 2.57±0.00 ^{ef} |
| | AAU2 | 80.89±0.65 ^{ijk} | 2.53±0.12 ^{mn} | 6.74±0.23 ^{kl} | 49.69±0.56 ^{bcd} | 22.87±0.27 ^{abc} | 2.36±0.00 ^f | 22.87±0.27 ^{abc} | 2.36±0.00 ^f | 22.87±0.27 ^{abc} | 2.36±0.00 ^f | 22.87±0.27 ^{abc} | 2.36±0.00 ^f |
| | AAU3 | 78.25±1.06 ⁿ | 2.90±0.10 ^{ghij} | 6.53±0.24 ^{lm} | 48.05±0.59 ^{cd} | 21.15±0.25 ^{ef} | 3.26±0.06 ^e | 21.15±0.25 ^{ef} | 3.26±0.06 ^e | 21.15±0.25 ^{ef} | 3.26±0.06 ^e | 21.15±0.25 ^{ef} | 3.26±0.06 ^e |
| | AAU4 | 79.32±0.79 ^m | 2.83±0.06 ^{hijl} | 7.30±0.13 ^{efgh} | 43.06±1.17 ^{ghi} | 20.67±0.25 ^{fg} | 3.95±0.26 ^b | 20.67±0.25 ^{fg} | 3.95±0.26 ^b | 20.67±0.25 ^{fg} | 3.95±0.26 ^b | 20.67±0.25 ^{fg} | 3.95±0.26 ^b |
| | AAU5 | 81.62±0.42 ^{hi} | 2.73±0.06 ^{klmn} | 7.02±0.16 ^{ijk} | 45.29±1.99 ^{efg} | 23.30±0.45 ^a | 3.19±0.10 ^e | 23.30±0.45 ^a | 3.19±0.10 ^e | 23.30±0.45 ^a | 3.19±0.10 ^e | 23.30±0.45 ^a | 3.19±0.10 ^e |
| | AAU6 | 80.82±0.45 ^{ijk} | 2.43±0.49 ⁿ | 6.75±0.14 ^{kl} | 39.96±0.36 ^{klmn} | 21.55±0.12 ^{def} | 2.29±0.06 ^f | 21.55±0.12 ^{def} | 2.29±0.06 ^f | 21.55±0.12 ^{def} | 2.29±0.06 ^f | 21.55±0.12 ^{def} | 2.29±0.06 ^f |
| | AAU7 | 82.11±0.71 ^{gh} | 3.53±0.06 ^a | 6.67±0.23 ^{kl} | 43.29±1.88 ^{ghi} | 20.61±0.32 ^{gh} | 2.49±0.27 ^{ef} | 20.61±0.32 ^{gh} | 2.49±0.27 ^{ef} | 20.61±0.32 ^{gh} | 2.49±0.27 ^{ef} | 20.61±0.32 ^{gh} | 2.49±0.27 ^{ef} |
| | DH1 | 80.93±0.60 ^{ijk} | 3.33±0.06 ^{abc} | 7.28±0.17 ^{efgh} | 45.00±2.48 ^{efgh} | 19.04±0.33 ^{ijkl} | 3.03±0.06 ^{cd} | 19.04±0.33 ^{ijkl} | 3.03±0.06 ^{cd} | 19.04±0.33 ^{ijkl} | 3.03±0.06 ^{cd} | 19.04±0.33 ^{ijkl} | 3.03±0.06 ^{cd} |
| | DIM2 | 79.44±0.52 ^m | 3.13±0.06 ^{cd} | 7.66±0.10 ^{defg} | 51.00±1.10 ^{ab} | 18.70±0.26 ^{klm} | 2.37±0.27 ^f | 18.70±0.26 ^{klm} | 2.37±0.27 ^f | 18.70±0.26 ^{klm} | 2.37±0.27 ^f | 18.70±0.26 ^{klm} | 2.37±0.27 ^f |
| | KB2 | 79.25±0.34 ^{lm} | 2.93±0.06 ^{efgh} | 7.21±0.09 ^{efgh} | 47.76±1.68 ^{de} | 17.99±0.65 ^{nm} | 1.41±0.46 ^{hi} | 17.99±0.65 ^{nm} | 1.41±0.46 ^{hi} | 17.99±0.65 ^{nm} | 1.41±0.46 ^{hi} | 17.99±0.65 ^{nm} | 1.41±0.46 ^{hi} |
| 5. | <i>Lentinus velutinus</i> | 80.59±0.48^c | 3.37±0.06^{ab} | 8.16±0.20^{ab} | 41.22±1.20^{kl} | 15.36±0.13^q | 7.17±0.15^a | 41.22±1.20^{kl} | 7.17±0.15^a | 41.22±1.20^{kl} | 7.17±0.15^a | 41.22±1.20^{kl} | 7.17±0.15^a |
| | KM5 | 80.59±0.48 ^{kl} | 3.37±0.06 ^{ab} | 8.16±0.20 ^b | 41.22±1.20 ^{kl} | 15.36±0.13 ^q | 7.17±0.15 ^a | 41.22±1.20 ^{kl} | 7.17±0.15 ^a | 41.22±1.20 ^{kl} | 7.17±0.15 ^a | 41.22±1.20 ^{kl} | 7.17±0.15 ^a |

TABLE 2. Continued

| No. | Species name & sample code | Moisture content of the fresh fruiting bodies (g/100 g) | | Moisture content of the dried powder (g/100 g) | | Ash (g/100 g dry weight) | | Carbohydrate (g/100 g dry weight) | | Crude protein (g/100 g dry weight) | | Fat (g/100 g dry weight) | |
|------------------------------|----------------------------|---|-----------------------------|--|------------------------------|--------------------------------|----------------------------|-----------------------------------|--------------------|------------------------------------|--------------------|--------------------------------|--------------------|
| | | Average of samples | Average of species | Average of samples | Average of species | Average of samples | Average of species | Average of samples | Average of species | Average of samples | Average of species | Average of samples | Average of species |
| <i>Lycoperdon scabrum</i> | | | | | | | | | | | | | |
| | | 87.42 ± 1.10^b | | 2.85 ± 0.13^c | | 5.62 ± 0.37^d | | 38.55 ± 1.17^{ef} | | 17.31 ± 0.74^c | | 0.57 ± 0.16^g | |
| | BP9 | 86.13 ± 0.24 ^{de} | 2.77 ± 0.06 ^{klm} | 5.47 ± 0.21 ⁿ | 38.43 ± 1.08 ^{nop} | 16.41 ± 0.26 ^p | 0.62 ± 0.18 ^{kl} | | | | | | |
| 6. | DIM8 | 88.71 ± 0.48 ^b | 2.87 ± 0.06 ^{hik} | 5.44 ± 0.17 ⁿ | 37.70 ± 1.55 ^{op} | 17.14 ± 0.25 ^{nop} | 0.41 ± 0.10 ^{lm} | | | | | | |
| | KM7 | 87.58 ± 1.10 ^c | 2.73 ± 0.06 ^{klm} | 6.16 ± 0.08 ^m | 39.44 ± 0.77 ^{lmn} | 18.08 ± 0.64 ^{lmn} | 0.51 ± 0.10 ^{lmn} | | | | | | |
| | RB6 | 87.27 ± 0.39 ^c | 3.03 ± 0.06 ^{efg} | 5.42 ± 0.30 ^p | 38.61 ± 1.01 ^{mnop} | 17.63 ± 0.39 ^{no} | 0.72 ± 0.10 ^k | | | | | | |
| <i>Panus lecomtei</i> | | | | | | | | | | | | | |
| 7. | APBN3 | 81.39 ± 0.77 ^{de} | 3.47 ± 0.12 ^{ab} | 7.21 ± 0.29 ^{ghj} | 41.44 ± 1.42 ^{jk} | 16.52 ± 0.90 ^p | 0.86 ± 0.06 ⁱ | | | | | | |
| <i>Pleurotus giganteus</i> | | | | | | | | | | | | | |
| | KM1 | 83.87 ± 0.56 ^f | 3.37 ± 0.06 ^{ab} | 8.88 ± 0.17 ^a | 39.52 ± 1.34 ^{lmn} | 17.18 ± 0.39 ^{nop} | 4.07 ± 0.26 ^b | | | | | | |
| 8. | KM2 | 81.98 ± 0.28 ^{gh} | 3.47 ± 0.06 ^{ab} | 8.10 ± 0.20 ^{bc} | 37.72 ± 1.89 ^{op} | 14.96 ± 0.62 ^q | 2.80 ± 0.65 ^{de} | | | | | | |
| <i>Pleurotus ostreatus</i> | | | | | | | | | | | | | |
| 9. | MP1 | 87.58 ± 0.42 ^c | 3.27 ± 0.06 ^{bcd} | 7.13 ± 0.13 ^{hijk} | 39.28 ± 1.04 ^{lmn} | 19.28 ± 1.09 ^{jk} | 1.79 ± 0.12 ^{gh} | | | | | | |
| <i>Pleurotus pulmonarius</i> | | | | | | | | | | | | | |
| | MLS1 | 87.06 ± 0.55 ^{cd} | 3.10 ± 0.10 ^{def} | 7.37 ± 0.35 ^{gh} | 40.58 ± 0.94 ^{lmn} | 19.29 ± 0.83 ^{jk} | 1.93 ± 0.06 ^g | | | | | | |
| 10. | MP2 | 85.81 ± 0.53 ^e | 2.87 ± 0.12 ^{hijk} | 6.98 ± 0.47 ^{ijkl} | 42.39 ± 1.03 ^{hij} | 17.56 ± 0.51 ^{no} | 2.37 ± 0.27 ^f | | | | | | |
| <i>Polyporus arcularius</i> | | | | | | | | | | | | | |
| | APBN4 | 58.67 ± 0.89 ^a | 2.73 ± 0.06 ^{klm} | 8.05 ± 0.19 ^{bcd} | 49.42 ± 0.93 ^{bcd} | 17.37 ± 1.01 ^{mnop} | 1.41 ± 0.06 ^{hi} | | | | | | |
| 11. | DH2 | 61.70 ± 0.63 ^o | 3.33 ± 0.06 ^{abc} | 8.13 ± 0.26 ^{bc} | 43.16 ± 0.44 ^{ghi} | 15.23 ± 0.37 ^a | 1.34 ± 0.10 ^{hi} | | | | | | |
| | MLS6 | 60.73 ± 0.40 ^p | 3.43 ± 0.06 ^{ab} | 7.95 ± 0.21 ^{bcd} | 47.34 ± 0.75 ^{def} | 16.65 ± 0.72 ^{op} | 1.28 ± 0.12 ⁱ | | | | | | |

All the sample-wise data are represented as average ± standard deviations (SD) of three independent replications, while species-wise data are represented as average ± SD of all the triplicate data belonging to a single species. The different lowercase letters (a, b, c, d, and so on) after each data in columns indicate the significant difference among the samples/species ($p < 0.05$).

(60.36 g/100 g). Moisture contents vary among species to species depending on their types of fruiting bodies. Although, differences in moisture content among the members of the same species may vary depending upon the environmental factors such as relative humidity, temperature, and relative amount of metabolic water [Crisan & Sands, 1978; Singdevsachan *et al.*, 2014]. Dry fruiting body powders of the mushrooms also retained the minimal amount of moisture ranging from 2.43 g/100 g to 3.53 g/100 g (Table 2). The highest ash content was determined in *Agaricus bisporus* (9.23 g/100 g d.w.), while the lowest one in *Lycoperdon scabrum* (5.62 g/100 g d.w.) and *A. auricula-judae* (5.68 g/100 g d.w.).

Total crude protein content of the edible dried mushrooms showed variations among different species (Table 2). Species-wise comparisons showed that the crude protein content was the highest in *Lentinus sajor-caju* (20.72 g/100 g d.w.), and *Lentinus squarrosulus* (20.54 g/100 g d.w.). Although, there were no significant differences ($p < 0.05$) observed in the crude protein content of these two species with that of *A. auricula-judae* (19.70 g/100 g d.w.), *Pleurotus ostreatus* (19.28 g/100 g d.w.) and *A. bisporus* (18.65 g/100 g d.w.). Our results were comparable to the previous findings on the protein contents of edible mushroom species [Kalač, 2013; Phan *et al.*, 2012; Reis *et al.*, 2012]. However, different researchers found differences in the protein contents based on the external growing parameters. For instance, the protein content in *Pleurotus pulmonarius* was reported to vary from ~14 g/100 g d.w. to 26 g/100 g d.w. depending on different carbon sources supplemented during their cultivation process [Smiderle *et al.*, 2012]. Therefore, it can be concluded that protein content in wild mushrooms may vary depending upon the substrates on which they grow.

Among the edible mushroom species, *L. sajor-caju* showed the highest carbohydrate contents (49.80 g/100 g d.w.), which was followed by *P. arcularius* (46.64 g/100 g d.w.), *L. squarrosulus* (46.36 g/100 g d.w.) and *A. auricula-judae* (42.75 g/100 g d.w.) (Table 2). Our results could be compared with the carbohydrate content of edible mushrooms as reported earlier [Johnsy *et al.*, 2011; Nwanze *et al.*, 2005]. Carbohydrates are the most abundant constituents of mushrooms, which include sugars (monosaccharides, their derivatives and oligosaccharides) as well as both reserved and construction polysaccharides [Kalač, 2013]. Compared to the small amount of reducing sugars present in mushrooms, chitin and starch constitute the major fraction of total carbohydrates [Manzi *et al.*, 2001]. Mushrooms contain digestible carbohydrates (such as glucose, glycogen, mannitol, and trehalose) as well as non-digestible carbohydrate (such as β -glucan, chitin and mannans). Both of these carbohydrate forms constitute the total carbohydrates in mushroom fruiting bodies [Ho *et al.*, 2020].

Species-wise comparison showed the highest total fat content in *Lentinus velutinus* (7.17 g/100 g d.w.), which was followed by *Pleurotus giganteus* (3.43 g/100 g d.w.) and *L. squarrosulus* (2.69 g/100 g d.w.). The lowest total fat content was determined in the samples of *L. scabrum* (0.57 g/100 g d.w.) and *L. sajor-caju* (0.62 g/100 g d.w.) (Table 2). Typically, mushrooms have been reported to have a low fat content compared to the carbohydrate and protein contents. Fruiting

bodies of edible mushrooms mostly contain *cis*-linoleic acid as a major fatty acid which varies from 22–65% in abundance of total fat. The other major fatty acids in mushrooms include *cis*-oleic acid, palmitic acid, and stearic acids [Günç Ergönül *et al.*, 2013].

The mineral content analysis of the edible dried mushrooms revealed that there were significant differences among the tested samples. Table 3 and Table 4 show the species-wise average contents of macro- and microelements, respectively, of the mushroom samples. Among all the minerals, potassium (K) content was the highest in all the samples. Species-wise comparison showed that potassium content varied among different species and the highest content was in *P. ostreatus* (2074.0 mg/100 g d.w.), along with *A. bisporus*, *P. pulmonarius*, *L. squarrosulus*, *L. sajor-caju*, *A. auricula-judae* and *Panus lecomtei* (Table 3). Previous studies also reported potassium as the predominant macroelement among different mushroom species [Dursun *et al.*, 2006; Gençcelep *et al.*, 2009]. The phosphorus content was the highest in *L. velutinus* (318.8 mg/100 g d.w.), which was followed by that in *P. pulmonarius* (294.3 mg/100 g d.w.) and *P. ostreatus* (285.2 mg/100 g d.w.). The highest calcium (Ca) content was found in *L. sajor-caju* (232.0 mg/100 g d.w.), which was non-significantly higher ($p \geq 0.05$) than in *A. auricula-judae* (222.3 mg/100 g d.w.), and few other species (Table 3). There were very little differences in the magnesium (Mg) content among different species. The average sodium (Na) content of *A. bisporus* was the highest among the analysed mushroom species (Table 3). On the other hand, the lowest sodium content was found in *P. lecomtei*. Four microelements, namely iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn), were also determined using atomic absorption spectroscopy (Table 4). The findings revealed exceptionally high iron content in *A. auricula-judae* (97.30 mg/100 g d.w.) compared to the samples of other species. Iron contents of *L. squarrosulus*, *L. sajor-caju*, *P. pulmonarius* and *A. bisporus* were also found high. Compared to iron contents, other three elements (Zn, Cu and Mn) were determined at lower levels, which was consistent with findings of other authors [Dursun *et al.*, 2006; Gençcelep *et al.*, 2009]. As suggested by previous findings, the mineral contents of mushrooms are greatly affected by geographical locations, growing substrates and several other internal and external factors including growth conditions and genetic factors [Gençcelep *et al.*, 2009; Mallikarjuna *et al.*, 2013; Uzun *et al.*, 2017]. It was also reported that bioavailability of some elements in mushrooms, especially copper, is low for human due to limited absorption from the small intestine [Schellmann *et al.*, 1980].

Total phenolic content (TPC) in the dry mushrooms

Phenolic compounds are a major class of secondary plant metabolites with an important role in the protection against oxidation processes [Croft, 1999]. Numerous studies have proved that mushrooms also contain many phenolics equivalent to plant phenolics with potent radical scavenging ability [Elmastas *et al.*, 2007; Turkoglu *et al.*, 2007]. Here, total phenolic contents of the wild edible mushrooms were determined spectrophotometrically using the Folin-Ciocalteu reagent after extraction with methanol. Methanol can be considered

as the most suitable solvent for the extraction of organic compounds including phenolics. Previously, it was reported that extraction of phenolics with methanol resulted in the highest TPC compared to ethanol, acetone and water [Do et al., 2020]. In the present study, the total phenolic content ranged from 59.2 to 1051.5 mg GAE/100 g d.w. for the dried mushrooms (Table 5). Species-wise comparisons showed that the highest TPC was found in the samples of *L. sajor-caju* (831.3 mg GAE/100 g of d.w.), which was followed by *P. lecomtei* (780.9 mg GAE/100 g of d.w.) and *P. pulmonarius*. Samples belonging to the species *P. arcularius* had the lowest TPC (109.1 mg GAE/100 g of d.w.) compared to those of other species (Table 5). There are extensive reports concerning the phenolic contents of mushrooms; however, comparison of findings is difficult due to diversity in research materials, environmental conditions, habitats, analytical methods or ways of expressing the findings [Nowacka et al., 2014]. Our study demonstrated higher total phenolic contents in wild mushrooms (such as *L. sajor-caju*, *P. ostreatus*, and *P. pulmonarius*) compared to the cultivated strains of those species described earlier [Jeena et al., 2014]; however, total phenolic contents as high as 2.17–36.19 mg/g d.w. have been described earlier for a few edible mushrooms [Boonsong et al., 2016].

Antioxidant activity

DPPH radical scavenging activity of the mushroom samples was evaluated and the species-wise results were compared based on the IC_{50} of the methanolic extracts, as well as the tocopherol equivalent antioxidant activity (TEAA). The results suggested that the samples of the species *L. sajor-caju*, *L. squarrosulus*, and *P. pulmonarius* exhibited higher antioxidant activity indicated by the lower IC_{50} and higher TEAA as compared to other species (Table 5). IC_{50} signifies the ability of the extract to scavenge the DPPH radical in a concentration-dependent manner. Based on the IC_{50} , our results were comparable with those of earlier reports, which suggested that 40–60% inhibition of DPPH radical occurred in the presence of ~500 μ g/mL mushroom extracts [Boonsong et al., 2016]; although, concentrations as high as 5–20 mg/mL for scavenging 40–60% of DPPH radical were also reported for few edible mushroom extracts [Cheung et al., 2003; Jeena et al., 2014; Wong & Chye, 2009]. Extraction solvents play a crucial role in the determination of antioxidant activity of biological samples. Previous studies reported a high extraction yield of antioxidants from mushrooms with high antioxidant properties using methanol and ethyl acetate [Akata et al., 2019; Lakshmi et al., 2004]. In our study, there was a positive correlation between total phenolic content and TEAA, with a correlation coefficient of 0.544 ($p < 0.01$), suggesting the major role of phenolic compounds in antioxidant activity of mushroom powders. It was earlier reported that the antioxidant properties of button mushrooms varied between 5.49 and 10.48 nmol Trolox equivalent/mg d.w. based on their growing stages, and the antioxidant activity of those samples correlated with the ergosterol content [Shao et al., 2010].

Antihaemolytic activity

Antihaemolytic activity of the extracts was tested in goat RBC cells in the presence of the haemolytic agent H_2O_2 .

Extracts from the mushroom powders showed inhibition of haemolytic activity, which was found to be increased with increasing concentrations of the extracts (data not shown). Table 4 shows sample-wise and species-wise comparisons of the antihemolytic activities of the dried fruiting body extracts. The samples belonging to the species *L. sajor-caju*, *P. ostreatus*, *P. pulmonarius* and *A. bisporus* showed the highest antihemolytic activity compared to the samples of other species. Few extracts of *L. squarrosulus* indicated low IC_{50} values, suggesting to have prominent antihemolytic activity. The antihemolytic activity of the mushroom extracts can be correlated to their antioxidative potential, or total phenolic content [Afsar et al., 2016]. Haemolytic agents, like H_2O_2 , oxidize the lipids in the plasma membrane of RBC cells, due to which haemoglobins release to the extracellular matrix. Phenolic compounds in the mushroom extracts inhibit the oxidation of lipids by H_2O_2 due to their antioxidant potential. There are plenty of reports demonstrating the antihemolytic activity of phytoconstituents from different plant species [Alinezhad et al., 2013; Besbas et al., 2020; Chansiw et al., 2018]; however, only few studies have described the antihemolytic potential of the extracts from the fruiting bodies of mushrooms. In an earlier study [Sharif et al., 2017], haemolytic inhibitory activity was evaluated in five different extracts (obtained using methanol, ethanol, ethyl acetate, *n*-hexane and water) of *Ganoderma lucidum* against human erythrocytes. The results suggested that two extracts (water and *n*-hexane) showed the antihemolytic activity but, the other two extracts (ethyl acetate and ethanol) were detected as toxic. The decrease in toxicity of the five extracts was found to be in the order of ethyl acetate > ethanol > methanol > *n*-hexane > water [Sharif et al., 2017].

Content of organic acids, phenolic acids and vitamins (ascorbic acid and riboflavin) in mushrooms

The presence of organic and phenolic acids and other metabolites in the mushroom extracts was determined using the HPLC analysis by comparing the retention times (t_R) and the absorption maxima of the separated peaks with these of reference standards (supplementary Table S1). The chromatograms of selected mushroom extract (DIM1) and 11 commercially available reference substances used for the identification of mushroom powder compounds are shown in Figure 1. Few phenolic acids, including 3,4-dihydroxybenzoic acid (t_R : 10.2 min), gallic acid (t_R : 8.5 min), and *trans*-cinnamic acid (t_R : 15.4 min), were identified in most of the tested samples (Table 6). Other non-phenolic acids, including ascorbic acids (t_R : 3.8 min) and lactic acid (t_R : 4.8 min), were also detected abundantly in most of the samples. Few other organic and phenolic acids, like citric acid (t_R : 6.0 min), caffeic acid (t_R : 9.7 min), vanillic acid (t_R : 10.6 min), pyruvic acid (t_R : 14.4 min), and *p*-coumaric acid (t_R : 14.6 min), showed species-specific variation in their presence and contents (Table 6). Riboflavin was detected with moderate to low content (9–65 mg/100 g d.w.) in few species only. The presence of compounds with antioxidant activities has supported the earlier findings of this study. Previous studies also reported some major non-phenolic and phenolic acids, such as ascorbic acids, citric acid, caffeic acid, vanillic acid, gallic acid, and *trans*-cinnamic acid, in edible mushroom

TABLE 3. Macroelement contents (mg/100 g dry weight) of the dried mushroom fruiting bodies.

| No. | Species name & sample code | Ca | | K | | Mg | | Na | | P | | | | | | | |
|------|------------------------------|-------------------------------|---------------------------|---------------------------------|--------------------------|------------------------------|---------------------------|-----------------------------------|---------------------------------|--------------------------------|---------------------------------|------------------------------|------------------------------|-----------------------------|------------------------------|------------------------------|-------------------------------|
| | | Average of samples | Average of species | Average of samples | Average of species | Average of samples | Average of species | Average of samples | Average of species | | | | | | | | |
| 1. | <i>Agaricus bisporus</i> | 158.6±10.2^g | | 1882.8±23.8^c | | 35.86±0.2^a | | 99.3±1.0^a | | 247.4±5.3^c | | | | | | | |
| | MLS2 | 158.6±10.2 ^g | 1882.8±23.8 ^c | 35.9±0.2 ^{bed} | 99.3±1.0 ^a | 247.4±5.3 ^c | 2. | <i>Auricularia auricula-judae</i> | 222.3±9.2^a | | 1625.2±17.5^a | | 36.43±0.1^a | | 97.6±0.1^b | | 199.9±7.5^k |
| DIMI | 222.3±9.2 ^{cd} | 1625.2±17.5 ⁱ | 36.4±0.1 ^a | 97.6±0.1 ^b | 199.9±7.5 ^k | 3. | | <i>Lentinus sajor-caju</i> | 232.0±142.1^{ab} | | 1702.1±269.4^a | | 34.82±0.7^b | | 47.9±8.0^{de} | | 201.3±17.7^d |
| APK5 | 155.8±4.0 ^{fg} | 1853.2±19.7 ^e | 35.5±0.0 ^e | 49.8±1.3 ⁱ | 177.1±6.6 ⁿ | | DH3 | 83.2±0.9 ^{kl} | 2008.8±11.7 ^d | 34.4±0.1 ^{jk} | 45.7±0.3 ^{mm} | 211.6±5.8 ^{ij} | DIM3 | 61.7±0.8 ^{lm} | 2003.4±49.0 ^d | 33.9±0.2 ⁱ | 49.3±0.4 ⁱ |
| MIS2 | 348.7±1.9 ^b | 1508.8±8.6 ⁱ | 34.4±0.0 ^{kl} | 62.8±0.3 ^{hi} | 188.5±5.6 ^{lm} | MIS7 | 421.4±0.3 ^a | 1461.1±28.1 ^k | 34.9±0.1 ^{gh} | 39.1±0.2 ^q | 197.8±6.42 ^{kl} | MP3 | 321.0±0.3 ^b | 1377.5±17.3 ⁱ | 35.8±0.1 ^{cde} | 40.6±0.5 ^{pq} | 203.9±3.2 ^{kl} |
| 4. | <i>Lentinus squarrosulus</i> | 135.9±93.5^a | | 1795.9±262.8^a | | 35.8±0.6^a | | 68.4±24.1^c | | 227.4±31.2^c | | | | | | | |
| | AAU1 | 157.3±1.7 ^{fg} | 1731.8±21.4 ^{gh} | 36.7±0.1 ^a | 87.6±0.6 ^d | 230.2±6.1 ^{fg} | AAU2 | 60.3±3.7 ^{lm} | 2371.8±12.4 ^a | 35.8±0.1 ^{cde} | 73.4±1.8 ^e | 198.2±3.45 ^{kl} | AAU3 | 47.7±1.1 ^{mm} | 2188.7±23.3 ^b | 35.5±0.1 ^e | 52.2±1.1 ^k |
| AAU4 | 114.8±1.3 ^{hij} | 1745.7±38.2 ^g | 35.8±0.1 ^{cde} | 93.9±0.18 ^c | 287.7±6.1 ^c | AAU5 | 118.8±7.1 ^{bi} | 1703.7±27.1 ^{gh} | 35.7±0.0 ^{cde} | 56.6±2.2 ⁱ | 265.3±2.4 ^d | AAU6 | 240.3±8.4 ^c | 1517.7±8.6 ⁱ | 35.8±0.3 ^{bede} | 71.7±0.7 ^f | 235.6±7.5 ^f |
| AAU7 | 58.8±2.2 ^{mm} | 1758.2±12.3 ^f | 36.0±0.1 ^{bc} | 99.4±1.4 ^a | 207.9±4.4 ^{hik} | DHI | 119.3±0.9 ^{hi} | 1599.1±29.2 ⁱ | 35.0±0.1 ^{fg} | 39.7±0.4 ^q | 182.7±4.0 ^{mm} | DIM2 | 94.9±2.8 ^{ijk} | 1756.0±20.8 ^f | 34.7±0.1 ^{hij} | 23.1±0.2 ^s | 199.2±3.0 ^k |
| KB2 | 346.8±98.7 ^b | 1586.3±24.7 ⁱ | 36.6±0.1 ^a | 86.2±1.2 ^d | 232.0±4.3 ^g | 5. | <i>Lentinus velutinus</i> | 42.7±1.7^c | | 1337.3±17.3^b | | 35.6±0.1^{ab} | | 95.9±0.6^c | | 318.8±7.6^a | |
| KMS5 | 42.7±1.7 ^{mm} | 1337.3±17.3 ⁱ | 35.6±0.1 ^{de} | 95.9±0.6 ^b | 318.8±7.6 ^a | | | | | | | | | | | | |

TABLE 3. Continued

| No. | Species name & sample code | Ca | | K | | Mg | | Na | | P |
|------------------------------|----------------------------|----------------------------------|----------------------------|-----------------------------------|--------------------------|---------------------------------|--------------------|----------------------------------|--------------------|---------------------------------|
| | | Average of samples | Average of species | Average of samples | Average of species | Average of samples | Average of species | Average of samples | Average of species | |
| <i>Lycoperdon scabrum</i> | | | | | | | | | | |
| | | 103.5 ± 29.6^b | | 1003.0 ± 110.4^e | | 34.5 ± 0.5^{bc} | | 47.3 ± 10.8^d | | 232.5 ± 16.4^f |
| | BP9 | 135.7 ± 0.18 ^{gh} | 862.6 ± 22.4 ^{pq} | 33.9 ± 0.1 ^l | 35.5 ± 0.6 ^r | 256.5 ± 6.3 ^{de} | | | | |
| 6. | DIM8 | 116.0 ± 0.1 ^{hij} | 1154.6 ± 43.8 ^m | 34.5 ± 0.1 ^{ijk} | 42.6 ± 0.4 ^o | 224.6 ± 1.7 ^{gh} | | | | |
| | KM7 | 58.6 ± 0.1 ^{lm} | 990.0 ± 16.1 ⁿ | 35.0 ± 0.1 ^{le} | 63.6 ± 0.0 ^h | 215.9 ± 6.7 ^{hi} | | | | |
| | RB6 | 103.7 ± 2.4 ^{hij} | 1004.9 ± 8.6 ⁿ | 34.5 ± 0.7 ^{ij} | 47.3 ± 0.6 ^m | 232.9 ± 3.9 ^g | | | | |
| <i>Panus lecontei</i> | | | | | | | | | | |
| 7. | | 156.1 ± 4.3^{ab} | | 1538.8 ± 17.1^a | | 34.2 ± 0.0^c | | 35.3 ± 0.2^f | | 197.8 ± 5.0^d |
| | APBN3 | 156.1 ± 4.3 ^{le} | 1538.2 ± 17.1 ^l | 34.2 ± 0.0 ^k | 35.3 ± 0.2 ^r | 197.8 ± 5.0 ^u | | | | |
| <i>Pleurotus giganteus</i> | | | | | | | | | | |
| | | 22.6 ± 2.3^d | | 968.3 ± 51.7^c | | 33.3 ± 2.0^{bc} | | 57.2 ± 4.8^{ste} | | 242.2 ± 13.9^r |
| 8. | KM1 | 20.7 ± 0.6 ⁿ | 1014.7 ± 11.2 ⁿ | 31.6 ± 0.1 ^m | 52.9 ± 0.9 ^k | 254.2 ± 4.0 ^r | | | | |
| | KM2 | 24.4 ± 1.8 ⁿ | 921.9 ± 10.0 ^o | 35.1 ± 0.1 ^f | 61.5 ± 0.3 ⁱ | 230.1 ± 5.8 ^g | | | | |
| <i>Pleurotus ostreatus</i> | | | | | | | | | | |
| 9. | | 187.2 ± 0.2^a | | 2074.0 ± 72.6^a | | 34.6 ± 0.0^{bc} | | 58.2 ± 0.4^{ste} | | 285.2 ± 9.2^b |
| | MP1 | 187.215 ± 0.2 ^{ef} | 2074.0 ± 72.6 ^c | 34.6 ± 0.0 ^{hij} | 58.2 ± 0.4 ⁱ | 285.2 ± 9.2 ^c | | | | |
| <i>Pleurotus pulmonarius</i> | | | | | | | | | | |
| | | 116.6 ± 54.1^{ab} | | 1774.5 ± 97.7^a | | 35.3 ± 0.9^{abc} | | 64.8 ± 24.7^{ste} | | 294.3 ± 12.5^b |
| 10. | MLS1 | 67.3 ± 3.1 ^{klm} | 1688.9 ± 23.3 ^h | 36.1 ± 0.1 ^b | 87.4 ± 0.7 ^d | 284.6 ± 9.9 ^e | | | | |
| | MP2 | 165.9 ± 0.8 ^{le} | 1860.2 ± 35.9 ^e | 34.6 ± 0.0 ^{ij} | 42.2 ± 0.9 ^{op} | 304.0 ± 3.6 ^b | | | | |
| <i>Polyporus arcularius</i> | | | | | | | | | | |
| | | 207.6 ± 98.0^{ab} | | 820.2 ± 56.9^d | | 34.6 ± 0.2^{bc} | | 66.1 ± 18.6^d | | 227.2 ± 18.9^s |
| 11. | APBN4 | 94.5 ± 2.7 ^{ijk} | 882.3 ± 13.5 ^{op} | 34.5 ± 0.0 ^{ijk} | 87.3 ± 1.1 ^d | 205.6 ± 4.2 ^{jk} | | | | |
| | DH2 | 320.3 ± 7.4 ^b | 755.7 ± 26.6 ^f | 34.66 ± 0.06 ^{hij} | 44.7 ± 0.2 ⁿ | 248.2 ± 6.7 ^e | | | | |
| | MLS6 | 207.9 ± 9.5 ^{de} | 822.6 ± 2.4 ^q | 34.73 ± 0.42 ^{ghi} | 66.4 ± 3.6 ^g | 227.8 ± 1.9 ^g | | | | |

All the sample-wise data are represented as average ± standard deviations (SD) of three independent replications, while species-wise data are represented as average ± SD of all the triplicate data belonging to a single species. The different lowercase letters (a, b, c, d, and so on) after each data in columns indicate the significant difference among the samples/species ($p < 0.05$).

TABLE 4. Microelement contents (mg/100 g dry weight) of the dried mushroom fruiting bodies.

| No. | Species name & sample code | Cu | | Fe | | Mn | | Zn | |
|------|-----------------------------------|---------------------------------|----------------------------|----------------------------------|---------------------------|---------------------------------|---------------------------|----------------------------------|--------------------|
| | | Average of samples | Average of species | Average of samples | Average of species | Average of samples | Average of species | Average of samples | Average of species |
| 1. | <i>Agaricus bisporus</i> | 3.46 ± 0.03^a | | 24.73 ± 1.15^{bc} | | 3.48 ± 0.02^b | | 8.34 ± 0.19^a | |
| | MLS2 | 3.46 ± 0.03 ^a | | 24.73 ± 1.15 ^{kl} | | 3.48 ± 0.02 ^{fg} | | 8.34 ± 0.19 ^c | |
| 2. | <i>Auricularia auricula-judae</i> | 1.98 ± 0.03^b | | 97.30 ± 4.52^a | | 15.15 ± 0.61^a | | 7.15 ± 0.39^{ab} | |
| | DIM1 | 1.98 ± 0.03 ^d | | 97.30 ± 4.52 ^a | | 15.15 ± 0.61 ^a | | 7.15 ± 0.39 ^e | |
| 3. | <i>Lentinus sajor-caju</i> | 1.12 ± 0.56^c | | 19.01 ± 7.52^c | | 2.54 ± 0.64^c | | 4.60 ± 0.51^{bc} | |
| | APK5 | 1.57 ± 0.01 ^b | | 32.36 ± 0.85 ^{gh} | | 3.50 ± 0.16 ^{fg} | | 5.45 ± 0.07 ^{fg} | |
| | DH3 | 0.32 ± 0.01 ^a | | 7.67 ± 0.16 ^{rs} | | 2.54 ± 0.18 ^l | | 4.28 ± 0.07 ^{klm} | |
| | DIM3 | 0.53 ± 0.00 ^o | | 15.55 ± 0.50 ^p | | 2.79 ± 0.04 ^{jk} | | 4.95 ± 0.07 ^{hi} | |
| | MIS2 | 1.69 ± 0.02 ^s | | 19.49 ± 0.14 ⁿ | | 1.58 ± 0.04 ^o | | 4.01 ± 0.05 ^{mno} | |
| | MIS7 | 1.00 ± 0.04 ^k | | 19.85 ± 0.53 ⁿ | | 2.81 ± 0.02 ^{jk} | | 4.45 ± 0.33 ^k | |
| | MP3 | 1.60 ± 0.04 ^b | | 19.17 ± 0.04 ⁿ | | 1.99 ± 0.01 ⁿ | | 4.49 ± 0.24 ^{jk} | |
| 4. | <i>Lentinus squarrosulus</i> | 1.33 ± 0.51^c | | 35.64 ± 16.98^b | | 3.75 ± 0.94^b | | 5.47 ± 1.94^{abc} | |
| | AAU1 | 1.62 ± 0.03 ^b | | 57.75 ± 1.55 ^c | | 4.70 ± 0.04 ^c | | 8.76 ± 0.17 ^b | |
| | AAU2 | 1.09 ± 0.01 ^l | | 40.77 ± 1.31 ^f | | 2.58 ± 0.05 ^l | | 4.99 ± 0.07 ^h | |
| | AAU3 | 0.70 ± 0.01 ⁿ | | 9.18 ± 0.28 ^{qr} | | 5.00 ± 0.04 ^b | | 3.67 ± 0.12 ^q | |
| | AAU4 | 1.33 ± 0.02 ^l | | 19.87 ± 0.92 ⁿ | | 4.51 ± 0.06 ^{cd} | | 4.34 ± 0.11 ^{kl} | |
| | AAU5 | 0.72 ± 0.02 ⁿ | | 22.74 ± 1.05 ^m | | 4.40 ± 0.08 ^d | | 3.96 ± 0.28 ^{nop} | |
| | AAU6 | 1.87 ± 0.04 ^f | | 49.77 ± 0.18 ^d | | 2.22 ± 0.01 ^m | | 5.52 ± 0.17 ^{fg} | |
| | AAU7 | 1.93 ± 0.05 ^e | | 26.58 ± 0.85 ^{ij} | | 2.99 ± 0.04 ^{ij} | | 5.54 ± 0.13 ^{fg} | |
| | DH1 | 0.58 ± 0.00 ^o | | 46.76 ± 0.57 ^e | | 3.22 ± 0.09 ^{hi} | | 4.35 ± 0.11 ^{kl} | |
| | DIM2 | 1.58 ± 0.01 ^b | | 23.13 ± 0.20 ^m | | 4.43 ± 0.03 ^d | | 4.14 ± 0.09 ^{lmo} | |
| KB2 | 1.87 ± 0.04 ^f | | 59.88 ± 1.34 ^b | | 3.41 ± 0.05 ^{gh} | | 9.38 ± 0.08 ^a | | |
| 5. | <i>Lentinus velutinus</i> | 0.74 ± 0.02^{cd} | | 7.05 ± 0.40^e | | 4.08 ± 0.20^b | | 2.31 ± 0.02^d | |
| | KM5 | 0.74 ± 0.02 ^{mn} | | 7.05 ± 0.40 ^s | | 4.08 ± 0.20 ^e | | 2.31 ± 0.02 ^r | |
| 6. | <i>Lycoperdon scabrum</i> | 0.88 ± 0.07^c | | 11.26 ± 0.66^d | | 0.72 ± 0.04^d | | 4.35 ± 0.38^c | |
| | BP9 | 0.97 ± 0.02 ^k | | 10.97 ± 0.40 ^p | | 0.76 ± 0.02 ^{qr} | | 4.22 ± 0.09 ^{klmn} | |
| | DIM8 | 0.88 ± 0.02 ^l | | 12.13 ± 0.59 ^p | | 0.75 ± 0.01 ^{qr} | | 3.90 ± 0.08 ^{opq} | |
| | KM7 | 0.78 ± 0.03 ^m | | 10.65 ± 0.12 ^{pq} | | 0.67 ± 0.03 ^{rs} | | 4.91 ± 0.09 ^{hi} | |
| RB6 | 0.88 ± 0.02 ^l | | 11.28 ± 0.07 ^p | | 0.72 ± 0.01 ^{qr} | | 4.35 ± 0.01 ^{kl} | | |
| 7. | <i>Panus lecomtei</i> | 0.25 ± 0.02^d | | 7.12 ± 0.30^e | | 0.75 ± 0.02^d | | 5.35 ± 0.06^{abc} | |
| | APBN3 | 0.25 ± 0.02 ^r | | 7.12 ± 0.30 ^s | | 0.75 ± 0.02 ^{qr} | | 5.35 ± 0.06 ^g | |
| 8. | <i>Pleurotus giganteus</i> | 0.86 ± 0.48^{cd} | | 5.95 ± 2.84^e | | 0.92 ± 0.70^d | | 2.54 ± 1.64^d | |
| | KM1 | 0.42 ± 0.02 ^p | | 3.37 ± 0.21 ^t | | 0.29 ± 0.06 ^t | | 1.04 ± 0.06 ^s | |
| | KM2 | 1.30 ± 0.03 ^l | | 8.53 ± 0.39 ^{rs} | | 1.55 ± 0.06 ^{op} | | 4.03 ± 0.12 ^{mno} | |
| 9. | <i>Pleurotus ostreatus</i> | 0.78 ± 0.03^{cd} | | 33.12 ± 0.09^b | | 0.47 ± 0.04^d | | 3.82 ± 0.03^d | |
| | MP1 | 0.78 ± 0.03 ^m | | 33.12 ± 0.09 ^g | | 0.47 ± 0.04 st | | 3.82 ± 0.03 ^{pq} | |
| 10. | <i>Pleurotus pulmonarius</i> | 1.43 ± 0.64^{bc} | | 30.82 ± 0.66^b | | 3.31 ± 0.48^b | | 6.25 ± 1.67^{abc} | |
| | MLS1 | 2.02 ± 0.02 ^d | | 30.65 ± 0.96 ^h | | 3.69 ± 0.38 ^f | | 7.77 ± 0.12 ^d | |
| | MP2 | 0.85 ± 0.01 ^l | | 30.99 ± 0.27 ^h | | 2.94 ± 0.04 ^j | | 4.72 ± 0.01 ^{ij} | |
| 11. | <i>Polyporus arcularius</i> | 2.10 ± 0.45^b | | 25.68 ± 2.31^b | | 0.96 ± 0.32^d | | 5.65 ± 1.32^{abc} | |
| | APBN4 | 1.58 ± 0.01 ^b | | 23.03 ± 0.22 ^{lm} | | 1.33 ± 0.03 ^p | | 4.13 ± 0.09 ^{lmo} | |
| | DH2 | 2.61 ± 0.02 ^b | | 28.25 ± 0.16 ^l | | 0.59 ± 0.03 ^{rs} | | 7.15 ± 0.07 ^e | |
| MLS6 | 2.10 ± 0.11 ^c | | 25.74 ± 0.87 ^{jk} | | 0.97 ± 0.06 ^q | | 5.66 ± 0.28 ^f | | |

All the sample-wise data are represented as average ± standard deviations (SD) of three independent replications, while species-wise data are represented as average ± SD of all the triplicate data belonging to a single species. The different lowercase letters (a, b, c, d, and so on) after each data in columns indicate the significant difference among the samples/species ($p < 0.05$).

TABLE 5. Total phenolic content, DPPH radical scavenging activity and antihemolytic activity of the mushroom samples.

| No. | Species name & sample code | Total phenolic content (mg GAE/100 g dry weight) | | DPPH radical scavenging activity | | | | Antihemolytic activity; IC ₅₀ (μg/mL of extract) | |
|-----|-----------------------------------|--|----------------------------|-------------------------------------|---------------------------|---------------------------------|---------------------------|---|--------------------|
| | | Average of samples | Average of species | IC ₅₀ (μg/mL of extract) | | TEAA (mg/100 g dry weight) | | Average of samples | Average of species |
| | | | | Average of samples | Average of species | Average of samples | Average of species | | |
| 1. | <i>Agaricus bisporus</i> | 299.2 ± 21.0^a | | 979.3 ± 51.9^a | | 120.2 ± 6.5^{de} | | 462.9 ± 2.6^{cd} | |
| | MLS2 | 299.2 ± 21.0 ^{pa} | | 979.3 ± 51.9 ^a | | 120.2 ± 6.5 ^{op} | | 462.9 ± 2.6 ^j | |
| 2. | <i>Auricularia auricula-judae</i> | 172.5 ± 3.6^d | | 828.7 ± 39.8^b | | 93.9 ± 4.6^e | | Not determined | |
| | DIM1 | 172.5 ± 3.6 ^{ts} | | 828.7 ± 39.8 ^c | | 93.9 ± 4.6 ^q | | Not determined | |
| 3. | <i>Lentinus sajor-caju</i> | 831.3 ± 156.9^a | | 330.6 ± 52.4^f | | 345.9 ± 38.0^a | | 340.9 ± 58.5^d | |
| | APK5 | 622.5 ± 60.7 ^{sh} | | 277.5 ± 3.3 ^m | | 396.1 ± 4.7 ^a | | 283.8 ± 36.1 ^{lmn} | |
| | DH3 | 677.1 ± 45.8 ^{fg} | | 322.0 ± 7.6 ^{ijkl} | | 341.4 ± 8.1 ^e | | 299.3 ± 20.9 ^m | |
| | DIM3 | 923.7 ± 67.7 ^b | | 434.8 ± 8.6 ^h | | 296.4 ± 5.9 ^{sh} | | 316.8 ± 24.1 ^{lm} | |
| | MIS2 | 836.1 ± 26.4 ^{cd} | | 292.0 ± 11.7 ^{lm} | | 389.7 ± 15.8 ^a | | 342.2 ± 22.6 ^f | |
| | MIS7 | 876.6 ± 82.4 ^{bc} | | 333.8 ± 10.9 ^{ijk} | | 318.0 ± 10.4 ^e | | 438.9 ± 28.1 ^{jk} | |
| | MP3 | 1051.5 ± 41.4 ^a | | 323.7 ± 3.1 ^{ijk} | | 333.6 ± 3.2 ^{cd} | | 364.1 ± 43.3 ^{kl} | |
| 4. | <i>Lentinus squarrosulus</i> | 418.6 ± 158.6^c | | 361.8 ± 73.9^{def} | | 328.5 ± 46.7^a | | 481.1 ± 142.3^c | |
| | AAU1 | 254.8 ± 7.3 ^{qr} | | 249.6 ± 4.5 ⁿ | | 394.9 ± 7.1 ^a | | 325.0 ± 59.0 ^{klm} | |
| | AAU2 | 175.4 ± 49.8 ^{ts} | | 339.0 ± 15.3 ^{ij} | | 324.6 ± 15.0 ^{de} | | 409.4 ± 5.5 ^k | |
| | AAU3 | 247.7 ± 30.3 ^{qr} | | 352.7 ± 6.6 ^f | | 317.1 ± 5.9 ^e | | 281.8 ± 1.2 ⁿ | |
| | AAU4 | 561.6 ± 45.6 ^{hi} | | 466.4 ± 35.2 ^g | | 269.1 ± 20.2 ^{jk} | | 671.2 ± 1.9 ^{cd} | |
| | AAU5 | 297.4 ± 6.3 ^{pa} | | 431.5 ± 14.0 ^h | | 294.4 ± 9.4 ^{sh} | | 663.2 ± 5.0 ^{cd} | |
| | AAU6 | 398.7 ± 30.9 ^{lmno} | | 317.8 ± 15.2 ^{kl} | | 394.0 ± 19.0 ^a | | 589.15 ± 4.9 ^e | |
| | AAU7 | 520.8 ± 17.5 ^{ijk} | | 328.8 ± 9.4 ^{ijk} | | 357.4 ± 10.3 ^b | | 557.7 ± 3.9 ^g | |
| | DH1 | 568.0 ± 14.5 ^{hi} | | 339.7 ± 8.7 ^{ij} | | 312.5 ± 7.9 ^{ef} | | 457.3 ± 7.2 ^j | |
| | DIM2 | 612.5 ± 15.0 ^{sh} | | 486.7 ± 8.7 ^g | | 260.9 ± 4.6 ^t | | 552.8 ± 2.4 ^g | |
| KB2 | 549.4 ± 15.8 ^{hij} | | 305.5 ± 4.8 ^{klm} | | 359.7 ± 5.6 ^b | | 303.9 ± 1.2 ^m | | |
| 5. | <i>Lentinus velutinus</i> | 248.0 ± 31.6^c | | 833.1 ± 12.9^b | | 120.5 ± 1.9^{de} | | 579.3 ± 1.3^{bc} | |
| | KM5 | 248.0 ± 31.6 ^{qr} | | 833.1 ± 12.9 ^c | | 120.5 ± 1.9 ^{op} | | 579.3 ± 1.3 ^f | |
| 6. | <i>Lentinus scabrum</i> | 396.6 ± 57.5^c | | 711.4 ± 32.3^c | | 141.5 ± 14.9^c | | 630.7 ± 64.9^b | |
| | BP9 | 382.5 ± 12.3 ^{mno} | | 731.4 ± 17.0 ^d | | 139.3 ± 3.2 ⁿ | | 727.2 ± 17.3 ^c | |
| | DIM8 | 321.1 ± 2.9 ^{opq} | | 677.8 ± 8.3 ^e | | 164.9 ± 2.0 ^m | | 639.7 ± 15.0 ^d | |
| | KM7 | 411.2 ± 13.9 ^{lmn} | | 688.7 ± 14.2 ^e | | 129.3 ± 2.7 ^{no} | | 579.3 ± 1.3 ^f | |
| RB6 | 471.4 ± 16.2 ^{kl} | | 747.7 ± 10.7 ^d | | 131.8 ± 1.9 ^{no} | | 576.7 ± 13.7 ^f | | |
| 7. | <i>Panus lecomtei</i> | 780.9 ± 14.6^{ab} | | 417.6 ± 6.0^{def} | | 276.8 ± 4.0^b | | 550.9 ± 2.2^e | |
| | APBN3 | 780.9 ± 14.6 ^{de} | | 417.6 ± 6.0 ^h | | 276.8 ± 4.0 ^{ij} | | 550.9 ± 2.2 ^g | |
| 8. | <i>Pleurotus giganteus</i> | 330.6 ± 18.7^c | | 444.1 ± 109.2^{de} | | 266.3 ± 39.9^b | | 1037.5 ± 114.1^a | |
| | KM1 | 330.2 ± 18.8 ^{no} | | 344.6 ± 6.0 ^{ij} | | 302.5 ± 5.24 ^{fg} | | 937.7 ± 10.3 ^b | |
| | KM2 | 331.0 ± 22.8 ^{no} | | 543.6 ± 7.0 ^f | | 230.1 ± 3.0 ^l | | 1137.2 ± 50.8 ^a | |
| 9. | <i>Pleurotus ostreatus</i> | 461.3 ± 38.0^{bc} | | 426.9 ± 13.5^d | | 284.3 ± 9.2^{ab} | | 350.4 ± 7.6^{cd} | |
| | MP1 | 461.3 ± 38.0 ^{klm} | | 426.9 ± 13.5 ^h | | 284.3 ± 9.2 ^{hi} | | 350.4 ± 7.6 ^l | |
| 10. | <i>Pleurotus pulmonarius</i> | 662.1 ± 139.6^{ab} | | 332.4 ± 10.0^{ef} | | 342.4 ± 21.3^a | | 357.5 ± 154.6^{cd} | |
| | MLS1 | 608.9 ± 199.4 ^{gh} | | 339.0 ± 6.2 ^{ij} | | 324.2 ± 5.9 ^{de} | | 498.4 ± 1.6 ^h | |
| | MP2 | 715.3 ± 22.6 ^{ef} | | 325.8 ± 9.0 ^{ijk} | | 360.7 ± 10.0 ^l | | 216.6 ± 13.8 ^o | |
| 11. | <i>Polyporus arcularius</i> | 109.1 ± 46.9^c | | 914.6 ± 17.7^a | | 124.4 ± 7.3^d | | 487.7 ± 7.3^c | |
| | APBN4 | 156.7 ± 5.9 ^s | | 921.8 ± 13.6 ^b | | 115.1 ± 1.7 ^p | | 485.9 ± 1.5 ⁱ | |
| | DH2 | 59.2 ± 27.7 ^t | | 917.6 ± 18.6 ^b | | 130.1 ± 2.6 ^{no} | | 496.9 ± 0.8 ^h | |
| | MLS6 | 111.4 ± 29.4 st | | 904.5 ± 21.8 ^b | | 127.8 ± 3.1 ^{no} | | 480.4 ± 1.5 ⁱ | |
| 12. | Reference standard (Tocopherol) | | | 18.9 ± 2.3 ^o | | | | 286.5 ± 2.1 ⁿ | |

All the sample-wise data are represented as average ± standard deviations (SD) of three independent replications, while species-wise data are represented as average ± SD of all the triplicate data belonging to a single species. The different lowercase letters (a, b, c, d, and so on) after each data in columns indicate the significant difference among the samples/species ($p < 0.05$). GAE: gallic acid equivalents; TEAA: tocopherol equivalent antioxidant activity.

TABLE 6. Contents of the organic acids, phenolic acids riboflavin and ascorbic acid in dried mushroom fruiting bodies (mg/100 g d.w.) determined using HPLC.

| Sample ID | Ascorbic acid ($t_r = 3.8$ min) | Lactic acid ($t_r = 4.8$ min) | Citric acid ($t_r = 6.0$ min) | Gallic acid ($t_r = 8.5$ min) | Caffeic acid ($t_r = 9.7$ min) | 3,4-Dihydroxybenzoic acid ($t_r = 10.2$ min) | Riboflavin ($t_r = 10.6$ min) | Vanillic acid ($t_r = 10.8$ min) | Pyruvic acid ($t_r = 14.4$ min) | <i>p</i> -Coumaric acid ($t_r = 14.6$ min) | <i>trans</i> -Cinnamic acid ($t_r = 15.4$ min) |
|-----------|-------------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|------------------------------------|--|-----------------------------------|--------------------------------------|-------------------------------------|--|--|
| MLS2 | 114.2±5.6 ^s | 109.5±9.2 ⁱ | 116.5±14.2 ^d | 13.6±0.3 ^s | ND | 19.6±1.9 ^m | 9.2±2.7 ^e | 26.7±2.6 ^a | 2.4±1.0 ^g | ND | 30.4±1.3 ^e |
| DIM1 | 63.5±4.2 ^j | 52.4±5.0 ^k | ND | 2.3±0.0 ^k | 2.7±0.9 ^s | 36.6±0.3 ^k | 42.7±3.1 ^b | 13.2±3.7 ^{bc} | 2.3±0.7 ^g | 1.1±0.3 ^e | 14.5±4.1 ^{hi} |
| APK5 | 31.2±0.8 ⁱ | 140.5±2.9 ^{gh} | 73.7±7.4 ^c | 2.6±0.7 ^h | 8.0±0.3 ^d | 14.5±0.7 ^h | ND | 4.2±4.2 ^{def} | ND | 1.4±0.0 ^e | 9.2±1.0 ⁱ |
| DIM3 | 79.2±1.3 ^{hi} | 120.9±11.2 ^h | 18.6±2.0 ^h | 104.3±7.6 ^{cd} | ND | 61.1±1.3 ⁱ | 65.1±7.6 ^a | 4.9±2.3 ^{cd} | 21.7±1.0 ^e | 2.1±0.1 ^d | 13.4±1.5 ^{hi} |
| DH3 | 55.2±2.1 ^j | 192.7±8.9 ^f | ND | 6.9±0.7 ⁱ | ND | 9.3±0.1 ^o | ND | ND | 2.3±0.3 ^s | ND | 11.3±1.3 ^{ji} |
| MIS2 | 18.3±2.7 ^{mm} | 74.4±7.2 ⁱ | ND | 10.4±1.5 ^h | 4.4±0.0 ^f | 17.1±0.9 ^m | ND | ND | ND | ND | 14.5±2.1 ^{hi} |
| MIS7 | 86.5±6.7 ^h | 150.3±12.1 ^s | 25.1±3.9 ^f | ND | 6.0±0.7 ^e | 11.0±1.2 ^{no} | ND | ND | ND | ND | 12.3±0.7 ⁱ |
| MP3 | 77.3±4.3 ^{hi} | 89.9±9.8 ^{ji} | ND | 7.0±0.9 ^j | ND | 21.4±2.1 ^{lm} | 11.5±1.3 ^c | ND | 10.8±1.0 ^f | ND | 39.0±1.6 ^e |
| AAU1 | 36.8±2.1 ^k | 68.7±10.1 ^l | 3.7±1.9 ⁱ | 2.3±0.3 ^k | ND | 30.4±2.7 ^{hi} | ND | ND | ND | ND | 12.3±0.7 ⁱ |
| AAU2 | 170.6±2.3 ^e | 150.9±2.1 ^s | ND | 2.3±0.3 ^k | ND | 25.2±2.9 ⁱ | ND | ND | ND | ND | 10.2±0.3 |
| AAU3 | 398.1±31.2 ⁿ | 643.2±4.7 ^a | ND | 99.9 ^d | ND | 282.3±2.3 ^c | ND | ND | ND | ND | 19.8±1.2 ^s |
| AAU4 | 85.4±8.4 ^{hi} | 145.1±12.5 ^s | 3.00±0.7 ⁱ | 17.6±3.1 ^{ls} | ND | 51.4±2.6 ⁱ | ND | ND | ND | ND | 11.5±2.1 ⁱ |
| AAU5 | 125.2±3.8 ^f | 148.6±23.8 ^g | ND | 18.5±2.6 ^g | ND | 36.6±6.3 ^k | ND | ND | 1.5±0.7 ^{gh} | ND | 11.7±0.3 ⁱ |
| AAU6 | 347.9±3.9 ^b | 469.8±28.5 ^c | ND | 14.5±0.5 ^s | ND | 73.6±4.6 ^h | ND | ND | ND | ND | 12.1±0.7 ⁱ |
| AAU7 | 6.9±4.1 ^{op} | 62.04±8.7 ^{kl} | 8.6±1.3 ⁱ | 4.7±2.3 ^{kl} | ND | 17.7±0.6 ^m | ND | ND | ND | ND | 11.8±0.3 |
| DH1 | 19.8±0.9 ^m | 32.8±8.2 ⁱ | 20.18±1.8 ^{gh} | 5.2±0.5 ⁱ | ND | 20.1±0.3 ^{lm} | ND | ND | ND | ND | 35.8±1.8 ^{cd} |
| DIM2 | 167.6±10.5 ^e | 174.8±7.3 ^f | 45.6±7.2 ^f | 9.6±0.7 ^h | ND | 61.1±4.5 ⁱ | 29.1±1.7 ^c | 9.4±0.7 ^d | 2.0±0.3 ^{gh} | ND | 15.1±0.7 ^h |
| KB2 | 9.12±3.7 ^o | 95.3±11.8 ^f | ND | 9.8±0.5 ^h | ND | 34.0±1.7 ^h | ND | ND | ND | ND | 14.3±0.3 ^h |
| KM5 | 17.42±2.8 ^{mm} | 38.5±13.8 ^l | ND | 13.6±0.3 ^s | 2.8±0.3 ^s | 1.5±0.8 ^p | 12.5±2.3 ^{de} | ND | ND | ND | 14.5±1.3 ^h |
| BP9 | 165.81±3.7 ^e | 269.4±14.6 ^c | 141.0±3.1 ^c | 66.7±1.3 ^c | 25.6±2.7 ^c | 351.5±10.3 ^a | ND | 7.9±1.6 ^d | 31.6±3.5 ^d | 1.4±0.0 ^e | 41.1±2.3 ^c |
| DIM8 | 36.77±4.3 ^k | 123.9±12.1 | 18.7±3.3 ^h | 15.4±1.0 ^s | 25.6±0.3 ^c | 140.6±6.1 ^f | ND | 3.8±0.7 ⁱ | 80.8±2.7 ^a | 50.8±2.1 ^b | 77.3±1.4 ^a |
| KM7 | 55.2±2.1 ^j | 155.2±8.2 ^g | 18.7±4.1 ^h | 18.0±5.7 ^g | 25.9±1.6 ^c | 164.8±5.9 ^e | ND | 3.8±1.0 ^f | 80.8±4.6 ^a | 53.8±4.6 ^b | 66.7±3.2 ^b |
| RB6 | 92.1±4.7 ^h | 202.2±11.4 ^f | 141.0±12.1 ^c | 24.2±3.0 ^f | 25.6±0.7 ^c | 199.4±13.6 ^d | ND | 3.8±1.3 ^f | 36.51±2.0 ^e | 39.2±0.7 ^c | 20.7±1.7 ^s |
| APBN3 | 81.0±10.5 ^{hi} | 184.6±16.1 ^f | 165.5±10.0 ^b | 26.9±0.8 ^f | 25.6±0.0 ^f | 178.6±4.8 ^d | ND | 3.8±1.3 ^f | 41.43±2.7 ^c | 45.1±3.9 ^b | 30.4±1.3 ^e |
| KM1 | 20.18±4.1 ^m | 166.6±14.2 ^g | ND | 64.5±0.6 ^c | 12.5±2.8 ^d | 49.0±1.9 ⁱ | 16.3±0.7 ^d | 1.9±0.3 ^s | 1.0±0.3 ^{hi} | ND | 14.5±1.6 ^h |
| KM2 | 283.78±21.3 ^c | 178.1±7.8 ^f | ND | 164.1±2.7 ^b | 38.7±1.3 ^b | 175.2±3.5 ^d | 38.9±4.5 ^b | 12.1±0.0 ^c | 2.0±0.3 ^s | ND | 15.6±1.7 ^h |
| MP1 | 352.0±5.8 ^b | 463.6±12.4 ^c | 190.0±8.6 ^a | 197.3±3.5 ^a | 48.5±1.3 ^a | 322.1±4.9 ^b | ND | 16.3±1.4 ^b | 64.83 ^b | 71.2±3.1 ^a | 68.8±2.1 ^b |
| MLS1 | 407.3±9.2 ^a | 537.1±21.6 ^b | ND | 119.8±10.7 ^c | ND | 88.7±2.1 ^h | 71.5±3.1 ^a | 16.3±0.7 ^b | 3.3±1.0 ^s | ND | 26.1±0.3 ^f |
| MP2 | 213.7±8.5 ^d | 351.0±18.4 ^d | 92.1±4.8 ^d | 146.3±13.6 ^{bc} | ND | 109.5±2.8 ^s | ND | 7.9±1.3 ^d | 2.0±0.2 ^s | ND | 32.6±3.2 ^{de} |
| APBN4 | 13.73±3.1 ^{no} | 39.6±5.6 ^f | ND | 2.4±0.5 ^h | ND | 11.8±1.4 ^{no} | ND | ND | 1.8±0.0 ^h | 1.4±0.6 ^c | 35.8±3.8 ^{cd} |
| DH2 | 52.44±5.7 ⁱ | 59.76±5.7 ^{kl} | 86.0±7.1 ^e | 11.4±2.5 ^{gh} | 2.8±0.3 ^s | 67.1±6.9 ⁱ | 70.3±2.3 ^a | ND | 1.3±0.4 ^h | 1.1±0.3 ^e | 32.6±1.3 ^{de} |
| MLS6 | 11.75±3.4 ^o | 91.58±2.1 ⁱ | 3.0±0.7 ⁱ | 2.6±3.8 ^h | 2.6±0.0 ^g | 9.3±2.3 ^{no} | 11.3±1.9 ^e | ND | 1.3±0.3 ^h | 1.1±0.0 ^e | 12.3±0.7 ^{hi} |

All the sample-wise data are represented as average ± standard deviations (SD) of three independent replications. The different lowercase letters (a, b, c, d, and so on) after each data in columns indicate the significant difference among the samples/species ($p < 0.05$). ND: not detected; t_r : retention time.

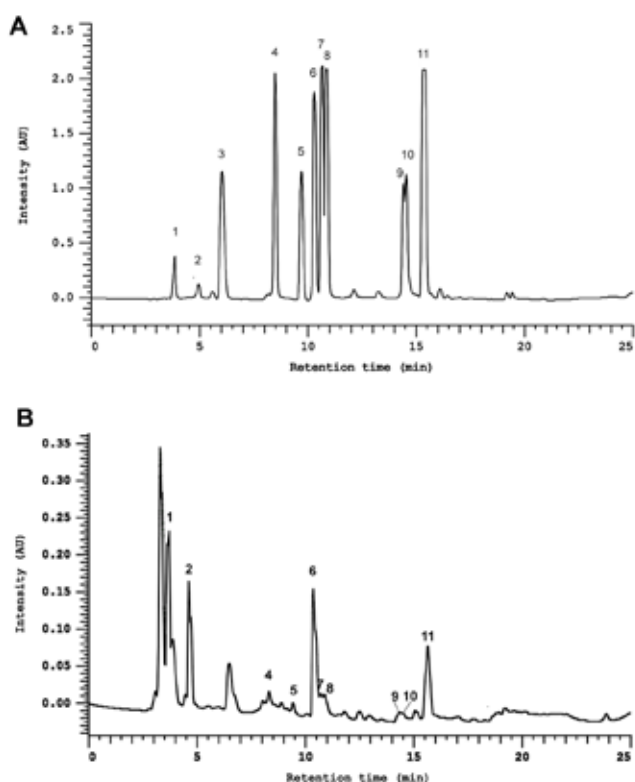


FIGURE 1. High performance liquid chromatography (HPLC) separation of the reference standards (A) and mushroom extract of DIM1 sample (B). Peaks for different compounds are designated with numbers: 1. ascorbic acid, 2. lactic acid, 3. citric acid, 4. gallic acid, 5. caffeic acid, 6. 3,4-dihydroxybenzoic acid, 7. riboflavin, 8. vanillic acid, 9. pyruvic acid, 10. *p*-coumaric acid, and 11. *trans*-cinnamic acid.

samples [Gąsecka *et al.*, 2018; Valentão *et al.*, 2005; Yahia *et al.*, 2017]. It is noteworthy that apart from the selected peaks, there were other peaks in the HPLC chromatograms which were not identified using standards. Therefore, we do not deny the presence of other compounds in the mushroom extracts as they were not targeted for evaluation in this study. It was previously reported that the organic acid profile varied among different wild growing species of *Agaricus*, where lactic acid and succinic acid were found most abundantly in the tested mushrooms [Gąsecka *et al.*, 2018]. In the present study, similar findings were recorded with heterogeneous distribution of organic acids among different mushroom species with intra-specific variations in the relative abundance of few organic acids. The presence of important phenolic acids in the fruiting bodies of edible mushrooms make them functional foods as they can protect human body from different diseases due to their strong antioxidant properties [Ribeiro *et al.*, 2015; Valentão *et al.*, 2005].

CONCLUSIONS

Based on the findings of our study, the dried edible mushrooms, especially *L. sajor-caju* and *L. squarrosulus* had a high nutritive value and a potent antioxidant activity. The samples of other mushroom species including, *P. ostreatus*, *P. pulmonarius*, *A. bisporus* and *A. auricle-judae*, also featured considerable nutritional and antioxidant properties. The high

nutritional value of these wild edible mushrooms may enlighten their scope for domestication and cultivation, thereby contributing towards the complementation of food security and nutritional demands among the indigenous communities of North-east India, especially, the people preferring the vegetarian diet. Moreover, mushroom extracts could be an emerging source of natural antioxidants, like phenolic acids (3,4-dihydroxybenzoic and *trans*-cinnamic acids) and ascorbic acid. Our study has demonstrated the antihaemolytic activity of several wild edible mushrooms from Northeast India, which contained several important organic acids and mentioned antioxidant metabolites. Consumption of such wild edible mushrooms with radical scavenging activity and antihaemolytic potential might be beneficial to combat oxidative damages in the human body.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ORCID IDs

M. Barooah <https://orcid.org/0000-0002-3768-5336>
 R.C. Boro <https://orcid.org/0000-0002-0866-3357>
 S. Dullah <https://orcid.org/0000-0002-6433-2149>
 A. Ghosh <https://orcid.org/0000-0001-8576-105X>
 D.J. Hazarika <https://orcid.org/0000-0001-6370-4230>
 M. Kakoti <https://orcid.org/0000-0002-5021-7692>
 A. Parveen <https://orcid.org/0000-0002-9745-4118>
 D. Saha <https://orcid.org/0000-0001-7258-9801>

SUPPLEMENTARY MATERIALS

The following are available online at
<http://journal.pan.olsztyn.pl/Nutritional-Properties-Antioxidant-and-Antihaemolytic-Activities-of-the-Dry-Fruiting,144044,0,2.html>

Supplementary Table S1: Retention times and absorption maxima of the 11 standards used for the identification of the compounds in the mushrooms.

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