Title: Comparison of Nutritional Composition, Bioactivities, and FTIR- ATR microstructural properties of Commercially Grown Four Mushroom Species in Sri Lanka; *Agaricus bisporus*, *Pleurotus ostreatus*, *Calocybe* sp. (MK-white), *Ganoderma lucidum*

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ABSTRACT

Mushrooms have been consumed as delicacies since ancient times; however, little knowledge is available on the nutritional and bioactive properties of commercially grown mushroom species in Sri Lanka; button (*Agaricus bisporus*), oyster (*Pleurotus ostreatus*), Makandura white (*Calocybe* sp.), and Reishi (*Ganoderma lucidum*). Samples from four mushroom species were analysed for proximate composition, mineral and fatty acid content, and antioxidant, antidiabetic, and microstructural properties. Carbohydrate, protein, fat, ash, and dietary fibre content in mushroom species ranged from 64.83-79.97 %, 10.53-23.29 %, 0.57-4.37 %, 2.80-11.00 %, and 33.04 to 75.33 %, respectively. The highest ($P \leq 0.05$) protein and ash content were observed in *A. bisporus*, and *G. lucidum* had the highest ($P \leq 0.05$) fat and dietary fibre content. When considering the micronutrients, *G. lucidum* comprised higher ($P \leq 0.05$) Ca, Mg, Mn, and Cu, while *A. bisporus* had higher ($P \leq 0.05$) Fe and Zn contents than other species. Essential omega-6 fatty acid, linoleic (18:2n-6) content was in the range of 37- 81 % in studied mushroom samples. Results obtained from FTIR (Fourier transform infrared spectroscopy) in conjunction with ATR (Attenuated total reflectance) revealed the presence of functional groups associated with fat (~1740 cm^{-1}), protein (~1560 cm^{-1}), polysaccharides (1500–750 cm^{-1}) and moisture (~3300 cm^{-1}) in mushroom samples. According to the results, *P. ostreatus* showed the highest ($P \leq 0.05$) polysaccharide content, while *G. lucidum* showed the lowest ($P \leq 0.05$). The highest ($P \leq 0.05$) total phenolic content (TPC) (3.95±0.05 mg GAE/g DW) and total flavonoid content (TFC) (2.17±0.06 mg CE/g DW) were observed in *P. ostreatus*. Antioxidant activity measured by DPPH, ABTS, and FRAP methods was higher ($P \leq 0.05$) in *P. ostreatus* and *A. bisporus* compared to the other two species. Among all the studied mushroom species, *G. lucidum* showed the highest ($P \leq 0.05$) α-amylase (IC_{50} =77.51±6.80 μg/mL) and α-glucosidase (IC_{50} =0.4113± 0.08 μg/mL) inhibition activities. This study reveals the potential of using *A. bisporus*, *G. lucidum*, and *P. ostreatus* for nutritional, functional, and therapeutic uses.

Keywords: antioxidant, bioactive, dietary fibre, flavonoid, microstructure, phenolic

1 INTRODUCTION/ BACKGROUND

Mushrooms are known as functional foods with high nutritional, culinary, and bioactive properties and have been consumed worldwide since ancient times (Jahan & Singh, 2019). Nearly 14,000 identified species of 1.5 million fungi have been estimated to produce fruiting bodies that are sizeable to be recognised as mushrooms (Chang, 2006). Approximately 300 mushroom species are edible, nearly 30 have been domesticated, and ten are commercially cultivated (Barney, 2000).

Mushrooms have been used in traditional medicine, especially in Asian, African, and Middle East countries, for ages (Gupta et al., 2018). They have been used for the treatments of cancers, asthma, gastric ulcers, bronchitis, arthritis, hepatitis, diabetes, and hyperlipidemia, among others, due to antioxidant, anti-inflammatory, immune-enhancing, antimicrobial, tumour attenuating, and other therapeutic properties (Gunawardena et al., 2014; Chowdhury et al., 2015; Bulam et al., 2018; Muszynska et al., 2018). Besides their bioactive potential, mushrooms are recognised for their high nutritional properties. Mushrooms are rich in proteins, carbohydrates, dietary fibre, vitamins, important fatty acids, minerals, essential amino acids, and various bioactive compounds (Dimopoulou et al., 2022). Nutritional inadequacy is a severe health issue in low-income countries, including Sri Lanka, and mushrooms could be a good alternative with multiple nutritional benefits.

The cultivation of mushrooms for commercialisation was introduced by the United Nations Development Program (UNDP) in Sri Lanka (Karunarathna et al.,2017). As a result, several commercial species are currently cultivated in Sri Lanka. Among them, button (*Agaricus bisporus*) and oyster (*Pleurotus ostreatus*) mushrooms are the main species, and *Makandura* white (MK-white; *Calocybe* sp.) is a newly developed commercial species. Reishi mushroom [*Ganoderma lucidum* (Curtis) P. Karst] is a medicinal mushroom, and its cultivation

was refined and established in Sri Lanka by Rajapakse et al. (2010) and Bandaranayake et al. (2012).

There are no reported data on the nutritional and bioactive properties of these four mushroom species grown in Sri Lanka. The nutritional and bioactive properties of mushrooms may vary significantly depending on strain, substrate, cultivation, etc. Therefore, the main objective of this study was to evaluate and compare the nutritional and bioactive properties of *Agaricus bisporus, Pleurotus ostreatus, Calocybe* sp., and *Ganoderma lucidum* (Curtis) P. Karst grown in Sri Lanka. The findings of this study would ameliorate the use of these mushroom species in functional food preparations and therapeutic and medicinal purposes and for overcoming protein and micronutrient malnutrition among the population in developing countries.

2 MATERIALS AND METHODS

2.1 Chemicals and reagents

Ethanol, Folin–Ciocalteu reagent, α-amylase, α-glucosidase, protease, amyloglucosidase, glucose oxidase (GOD), *p*-Nitrophenyl β-D-glucopyranoside (PNPG), acarbose, sodium carbonate, sodium hydroxide, aluminium chloride, sodium nitrite, hexane, sulphuric acid, nitric acid, boric acid, potassium sulfate, copper sulfate, bromocresol green, and methyl red mixed indicator, hydrochloric acid, hydrogen peroxide, Devarda's alloy, sodium methoxide, glycial acetic acid, ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)), DPPH (2,2-Diphenyl-1-picrylhydrazyl), Rhodium and Rhenium internal standards and multi-elemental standard solutions for ICP-OES analysis were purchased from Sigma-Aldrich™, USA.

2.2 Sample collection and preparation

A total of 750 g of a healthy, fresh button (*Agaricus bisporus*) (Figure 1(a)), oyster (*Pleurotus ostreatus*) (Figure 1(b)), Makandura white (*Calocybe* sp.) (Figure 1(c)), and Reishi (*Ganoderma lucidum*) (Figure 1(d)) mushroom species at correct maturity (3-5 days after forming the first mushrooms) (Figure 1) were collected from the Regional Agriculture Research and Development Center, Makandura, Sri Lanka, packed in polythene bags, labelled, placed in temperature-controlled containers and transported to the laboratory without delay. They were sorted, cleaned, and oven-dried at 40 °C in a forced-air oven (Memmert 854 Schwabach, Germany) for 12 hours and ground into fine particles. Composite oven-dried samples were stored at -20 °C until further analysis.

2.3 Proximate analysis

Moisture, crude protein, fat, and ash contents in triplicates were determined according to the AOAC (2000). Finally, the carbohydrate content was calculated by the difference. Total dietary fibre (TDF) contents were determined in triplicate using sequential enzymatic digestion by α-amylase, protease, and amyloglucosidase enzymes according to AOAC 985.29 (2010) method.

2.4 Mineral analysis

Mineral analysis was done by Inductively coupled plasma-optical emission spectroscopy (ICP-OES; Thermo scientific, iCAP 7000 series, Germany) as prescribed by Nadeeshani et al. (2021) with minor modifications. Mushroom samples were oven dried at 105 ^{o}C for 4 hours until a constant weight was obtained. Dried samples were ground to fine particles, and 0.25 g of each sample was digested with 9 mL of HNO_3 (65%) and 1 mL of H_2O_2 (30%) using a high-pressure laboratory microwave oven (CEM™ Corporation, BR601050, USA). Digested samples were transferred into 50 mL volumetric flasks, made up to volume with de-ionized water, filtered, and stored at 4 °C. As internal standards, 100 μg/L of Rh and

Re were added into test solutions to correct possible matrix effects and instrumental drift. For calibration, multi-elemental standard solutions were prepared with the following concentrations: 10-800 ppb for Na, Mg, K, Ca, and Fe; and 10-80 ppb for Mn, Zn, and Cu. Samples were analysed in triplicates, and the method accuracy was validated by the analysis of TM 25.4 (Environment Canada) as certified reference material.

2.5 Fatty Acid Analysis

Lipids were extracted from mushrooms as described by Nadeeshani et al. (2021) with some modifications. Briefly, 5 g of mushroom powder with 40 mL hexane was shaken on a wrist-action shaker (BURRELL™, USA) for 30 minutes at room temperature and ultra-sonicated (CL-188, USA) for 15 minutes. The supernatant was obtained after centrifugation at 1500 rpm for 10 minutes. Crude oil was obtained after rotary evaporation (40 ^{o}C under vacuum conditions), and the total crude oil content of the mushroom samples was weighed and calculated.

Fatty acid methyl esters (FAME) were prepared according to Nadeeshani et al. (2021). Briefly, an oil sample (60 mg) was weighed into a 15 mL screw-capped methylation tube. Then, 0.3 mL dichloromethane and 2 mL of 0.5 M sodium methoxide were added to the oil and mixed well. Subsequently, the prepared mixture was put in the hot water bath at 50 ^{o}C for 30 minutes until it reached room temperature. After cooling, 5 mL of distilled water was added drop by drop, and glacial acetic acid (0.1 mL) and 0.5 mL of hexane were added and mixed well.

The contents were kept at room temperature for 30 minutes, and the top hexane layer was separated into a 2 mL GC vial. Finally, the vials were capped and sealed further with Parafilm™ and kept immediately at – 20 ^{o}C until analysis by GC (GC system, US 16443037, USA). The column used for analysis was Agilent J&W CP-Sil 88 for FAME (100 m, 250 μm, 0.2 μm), and running conditions in GC were: injection volume (1 μL), carrier gas (hydrogen), pressure mode (constant); inlet: split/spitless 260 °C, split ration 50:1; oven conditions: 100 °C

(5 minutes), 8 °C /minute to 180 °C (9 minutes), 1 °C /minute (15 minutes). FID was adjusted for 260 °C, and airflow was: hydrogen 40 L/minute, air 400 mL/minute, makeup gas 25 mL/minute.

2.6 FTIR-ATR analysis

Powdered mushroom samples were analysed by Fourier Transform Infrared Spectroscopy with attenuated total reflectance (FTIR-ATR). The FTIR spectra were recorded on a NICOLET iS50 FT-IR analyser, GladiATR diamond ATR module (Thermo Fisher Scientific, Madison, WI, USA) mounted on an FTIR spectrometer. Absorbance spectra were obtained with a spectral resolution of 4 cm^{-1} over the wavenumber range of 4000-400 cm^{-1}. The absorbance was calculated over 24 and 32 scans, respectively. Triplicates of each sample from each data set were analysed for the primary analysis (Rodrigues et al., 2015).

2.7 Determination of water-soluble crude polysaccharide contents and beta-linked polysaccharide contents

Polysaccharides were extracted and purified using the method of (Su et al., 2016; Wu et al., 2013) with some slight modifications. In brief, 5 g of each mushroom powder was extracted into 200 mL of boiling water for 3 hours. Following filtration, a fourfold volume of ethanol (95 %) was gradually added while mixing the filtrate well. The mixture was centrifuged to separate the supernatant from the residue after being maintained at 4 °C overnight. To get the crude content of water-soluble polysaccharides, the residue was washed with ethanol and lipolyzed.

From crude polysaccharides, water-soluble β-linked polysaccharides (WSP) were extracted. In brief, 100 mL of crude polysaccharide solution (1 $mgmL^{-1}$, dissolved in deionised water) was mixed with 0.4 mL of α-amylase (2 $mgmL^{-1}$; 60 UmL^{-1}), and 0.4 mL of α-glucosidase (1 $mgmL^{-1}$; 50 UmL^{-1}). The mixture was put into dialysis bags (molecular weight cutoff of 12-14

kDa) and dialysed for three days at 4 °C with water changes every eight hours. The sevag reagent (chloroform: n-butanol = 4:1, v/v) at a ratio of 3:1 (v/v) was used to deproteinise the dialysed solution. Then the WSP β-linked polysaccharide content was precipitated at 4 °C overnight with the fourfold volume of ethanol (95 %). Centrifugation followed by sequential ethanol washes, resolving in deionised water, and lyophilisation to produce WSP from mushroom species of *Agaricus bisporus, Pleurotus ostreatus, Calocybe* sp., and *Ganoderma lucidum* (Curtis) P. Karst (Su et al., 2016).

2.8 Preparation of water and ethanolic extracts

Powdered samples (2.5 g) were extracted into 50 mL of distilled water or ethanol (65 % and 80 %) by sonicating (CL-188, USA) for 30 minutes at 40 kHz. Then the content was centrifuged (5340R, Germany) for 15 minutes at 7500 rpm, and the supernatant was separated. Then, it was concentrated by a rotary evaporator (Heidolph™, 200003264, Germany) at 37 °C under vacuum conditions. Finally, concentrated sample extracts were freeze-dried (CHRIST™, ALPHA 1-4LD Plus, Germany) and stored at -20 °C until analysis (Bakir et al., 2018; Chowdhury et al., 2015).

2.9 Total phenolic content (TPC)

TPC of the extracts was determined as described by Samatha et al. (2012) with minor modifications. The sample extract (20-50 μL), 105 μL of 10 % Folin-Ciocalteu's reagent dissolved in deionised water, and 15 μL of deionised water were mixed to prepare the reaction mixture. After 3 minutes, 80 μL of Na_2CO_3 (7.5 %, w/v) was added and incubated for 30 minutes at room temperature (RT). The absorbance was taken by a UV-visible microplate spectrophotometer (Omega 415-3441, Germany) at 760 nm. Results were expressed in milligrams of gallic acid equivalents (mg of GAE) per gram dry weight (DW). All tests were conducted in triplicate.

2.10 Total flavonoid content (TFC)

TFC was determined according to the procedure of Agbo et al. (2015) with slight modifications. The mixture was incubated for 6 minutes after adding the extract (30-150 μL) and 20 μL of $NaNO_2$ (5 %, w/v). Again, it was incubated for 6 minutes after adding 20 μL of $AlCl_3$ (10%, w/v), followed by adding 200 μL of NaOH (4 %, w/v). After 15 minutes of incubation, absorbance was measured in triplicate using a UV-visible microplate spectrophotometer at 510 nm. TFC was expressed in milligrams of catechin equivalents (CE) per gram dry weight (DW).

2.11 DPPH radical-scavenging capacity

The procedure described by Sanjeevkumar et al. (2016) was used with minor modifications. DPPH solution (100 μL) was added to different extract volumes (0-150 μL). The reaction mixture was allowed to stand for 30 minutes at the dark room temperature, and the absorbance was measured at 517 nm. The sample concentration with 50 % inhibition (IC_{50}) was calculated.

2.12 ABTS radical-scavenging capacity

ABTS (2.5 mM) and $K_2S_2O_8$ (5.0 mM) were mixed and kept in the dark for 12 hours at room temperature to generate ABTS radicals. ABTS radical solution (150 μL) was added to 50 μL of the extract, and the absorbance was read at 734 nm in triplicate after keeping 10 minutes in the dark (Liyanage et al., 2016). Results were expressed in μmol Trolox equivalents (TE) per gram dry weight (DW).

2.13 Ferric reducing antioxidant power (FRAP) Assay

FRAP reagent (150 μL) containing TPTZ (10 mM in 10 mM HCl), $FeCl_3$ (10 mM), and 30 μL of pH 3.6 acetate buffer (300 mM) with the ratio of 1:1:10 (v/v/v) was pre-incubated at

37 °C for 8 minutes (Shukla et al., 2016). The sample extract (50 μL) was added to 150 μL of FRAP reagent and incubated for 30 minutes at RT. The absorbance was measured at 593 nm, and results were expressed in μmol Fe^{2+} equivalents per gram dry weight (DW).

2.14 Alpha-amylase inhibition assay

α-Amylase inhibitory activity was determined using a glucose assay kit (Visvanathan et al., 2016). α-Amylase (4U/mL) and 4 g/mL of starch were prepared in 0.02 M (pH 6.9) phosphate buffer Saline (PBS). PBS (40 μL), sample extract (40 μL), and the enzyme (40 μL) were added to a microplate and kept at 37 °C for 15 minutes. After the incubation, 100 μL of glucose oxidase (GOD) was added, and the absorbance was measured at 505 nm using a UV-visible microplate spectrophotometer. The inhibition activity was expressed as IC_{50} values.

2.15 Alpha-glucosidase inhibition assay

PNPG (0.7 mM) and α-glucosidase enzyme (2U/mL) were prepared by using 0.1 M (pH 6.9) PBS. PBS (100 μL), sample extract (20 μL), and the enzyme (25 μL) were added and kept at 37 °C for 15 minutes. Afterwards, 50 μL of PNPG was added and kept for 30 minutes at room temperature, and absorbance was measured at 400 nm using a UV-visible microplate spectrophotometer. The inhibition activity was expressed as an IC_{50} value (Phan et al., 2013).

2.16 Statistical analysis

Statistical analyses were conducted using the SAS Statistical Analysis System SAS/IML 14.1 (SAS Institute Inc., Cary, NC). Statistical analysis was done by one-way analysis of variance (ANOVA) with Tukey comparison to obtain the difference among means of triplicate experimental data ($P \leq 0.05$). Pearson's correlation coefficient ($P \leq 0.05$) was used to find the relationship between TPC/TFC and antioxidant capacities and TPC/TFC and antidiabetic activities.

3. RESULTS AND DISCUSSION

3.1 Proximate composition

Among the four mushroom species, moisture, ash, and protein contents were higher ($P \leq 0.05$) in *A. bisporus*, while fat and total dietary fiber contents were higher ($P \leq 0.05$) in *G. lucidum* compared to other studied mushroom species. *Calocybe* sp. contained higher ($P \leq 0.05$) carbohydrate content than other species (Table 1). Protein content in the mushrooms was in the range of 10.53-23.29 % showing the potential of promoting them for protein malnutrition in developing countries. As rich sources of dietary fibre, 100 g intake of studied mushroom species can fulfil 6.29-65.83 % of recommended daily intake of dietary fibre (Trumbo et al. 2002).

Table 1 Proximate composition and TDF contents of four mushroom species on a DW basis (%)

Mushroom species	*G. lucidum*	*A. bisporus*	*P. ostreatus*	*Calocybe* sp.
Moisture content*	73.78±0.21^{d}	92.66±0.16^{a}	89.23±0.82^{b}	82.05±0.81^{c}
Fat content	4.37±0.31^{a}	0.57±0.01^{c}	3.99±0.24^{a}	1.16±0.11^{b}
Ash content	2.80±0.20^{d}	11.00±0.16^{a}	7.89±0.06^{c}	8.40±0.02^{b}
Protein	15.45±0.15^{c}	31.30±0.10^{a}	23.29±0.51^{b}	10.53±0.15^{d}
Carbohydrate content	77.38±0.91^{a}	56.78±0.64^{c}	64.83±0.54^{b}	79.97±0.78^{a}
TDF	75.33±0.83^{a}	32.59±0.02^{c}	40.56±0.12^{b}	33.04±3.32^{c}

Data represent the mean values±SD (n=3) of three independent experiments. Means followed by the same letters in a row are not significantly different (P<0.05). Moisture content* - Fresh weigh basis

Values for the proximate composition (Table 1) of *A. bisporus* fell in line with the findings of Mattila et al. (2001), Nhi & Hung (2012), and Ahlawat et al. (2012), nevertheless values reported by Kakon et al. (2012) and Enas et al. (2016) were relatively higher than the findings of this study. Similar values to the proximate composition of *P. ostreatus* have been reported by Manzi et al. (1999), Nhi & Hung (2012), and Reis et al. (2012). The proximate composition of *G. lucidum* observed in this study aligned with what Shamaki et al. (2012) and Garuba *et al*. (2020) reported. In contrast, total dietary fiber and fat contents were higher than the values reported by Ogbe & Obeka (2013).

Studies on the proximate composition of Makandura white (*Calocybe* sp.) are limited in published literature. However, reported data from a few studies on the proximate composition of *Calocybe indica* confirmed the findings of the present study (Chelladurai et al., 2014; Chelladurai et al., 2021). According to Bernas et al. (2006), carbohydrate content in mushrooms was in the range of 16-85 %, which agreed with the findings of the present study. In addition, Das et al. (2014) stated that the fat content of mushrooms varied from 1.6 % to 5 %, which aligned with the present findings.

According to the results, *A. bisporus* could be considered a food source rich in proteins and micronutrients for vegetarians and vegans to satisfy their nutritional needs. *G. lucidum* and *P. ostreatus* contain healthy fats, which are very important to cardiac health in the human body.

3.2 Mineral contents

Table 2 Mineral contents of four mushroom species on the DW basis (mg/kg)

	G. lucidum	*A. bisporus*	*P. ostreatus*	*Calocybe* sp.
Zn	422.58±2.67^{c}	885.27±2.42^{a}	700.98± 5.03^{b}	418.56±19.80^{c}
Mn	99.60±0.38^{a}	56.31±0.61^{c}	90.62±0.47^{b}	34.41±0.40^{d}
Fe	116.22± 2.64^{b}	32.59± 4.12^{c}	148.27± 10.37^{a}	31.34± 0.24^{c}

Cu	647.14±34.70[a]	497.09±29.41[b]	254.04±29.79[c]	131.67±12.96[d]
K	6120.32±16.80[d]	18231.19±131.08[c]	22013.75±798.22[b]	25114.77±174.69[a]
Na	91.02±4.92[d]	576.61±36.78[a]	156.41±11.78[c]	360.93±2.88[b]
Ca	35275.85±34.95[a]	21401.57±167.08[c]	6138.73±97.88[d]	25902.24±153.77[b]
Mg	11143.96±2.32[a]	10909.28±6.63[b]	11344.73±41.66[a]	9599.24±245.84[c]

Data represent the mean values±SD (n=3) of three independent experiments. Means followed by the same letters in a row are not significantly different (P<0.05).

Among studied mushroom species, *G. lucidum* contained higher (P≤0.05) Ca, Cu, and Mn contents (Table 2), whereas *A. bisporus* comprised higher (P≤0.05) Na and Zn content. The highest (P≤0.05) Mg and Fe contents were observed (P≤0.05) in *P. ostreatus*, while *Calocybe* sp. contained the highest (P≤0.05) K content. Consuming *P. ostreatus* (100 g) may fulfil 8.9-20.0 % and 29.1-38.2 % of daily dietary reference intakes of Fe and Mg, respectively. 100 g of *G. lucidum* may fulfil 71.1-92.5 % of daily dietary reference intakes Ca (Table & Table, 2001). These observed values were strongly in accordance with the findings of Uzun et al. (2011) and Woldegiorgis (2015) and somewhat in agreement with the studies done by Mallikarjuna et al. (2015) and Nnorom et al. (2020). Routine consumption of these mushroom species rich in essential minerals would adequately protect bones, muscles, heart, and brain (Gupta & Gupta, 2014).

3.3 Fatty acid profile

The fatty acid composition of four mushroom species (*G.lucidum*, *P. ostreatus*, *A. bisporus*, *Calocybe* sp.) was investigated by using the GC method. According to the results, the carbon chain lengths of fatty acids were from 4 to 22. Values obtained for fatty acid composition in Table 3 differed among all four species (Table 03). Total fatty acids result in ratios of unsaturated: saturated fatty acids (U: S) ranging from 1.8 (*P. ostreatus*) to 4.5 (*A. bisporus*). Findings were consistent with previous studies showing that many species of higher

Basidiomycetes in the family *Agaricaceae* showed large proportions of unsaturated FAs (Pedneault et al., 2006). According to the values in Table 3, mushrooms were greatly rich in unsaturated fatty acids (57.76-81.90 %) but fairly rich in saturated fatty acids (18.09-33.13 %), which is consistent with the findings that unsaturated fatty acids predominate over saturated fatty acids in mushrooms (Pelin et al., 2013: Saini et al., 2021). Further, all studied mushroom species were superior in polyunsaturated fatty acids (37.51-81.28 %).

Analysis of FA profiles shows that polyunsaturated fatty acid (PUFA) of linoleic (18:2n-6) existed in all four mushroom species, and values in Table 3 have expressed it as the most available fatty acid (37.51-81.28 %) type, which was similar to the results of Suqin et al. (2010). Palmitic (16:0) acid also was in the 8-18% range. Among saturated fatty acid (SFA) types, palmitic acid was the most available type, followed by stearic acid (18:0), which agrees with the results of Suqin et al. (2010). Oleic acid (9c-18:1) was the major monounsaturated fatty acid (MUFA), existing in the range of 0.42-29.35 % of total fatty acids. *Calocybe* sp. and *A. bisporus* consisted wide variety of fatty acids than the other two species.

According to the previous findings, mushrooms are not significant dietary sources of fatty acids due to their less availability. But nearly 75 % of these fatty acids are health-beneficial PUFA components. A maximum of 0.1 g of total fatty acids can be obtained from a serving size of 1 cup of mushrooms (70 g fresh or 5.6 g dehydrated, an average of 92.0 % of water content). Thus, despite the low levels of total fatty acids, consuming mushrooms would supply health-beneficial fatty acids and prevent cardiovascular diseases and other oxidative stress-related chronic diseases (Sande et al., 2019).

3.4 Polysaccharide composition

Table 4 Yields of crude water-soluble polysaccharides (WSP) content and *β*-linked WSP

Sample	Crude polysaccharide content (%) DW	β-linked polysaccharide content (%) DW
G. lucidum	4.43±0.02[d]	1.12±0.00[d]
A. bisporus	9.16±0.03[c]	6.34±0.01[c]
P. ostreatus	17.20±0.13[a]	11.12±0.12[b]
Calocybe sp.	14.43±0.11[b]	12.45±0.02[a]

Data represent the mean values±SD (n=3) of three independent experiments. Means followed by the same letters in a row are not significantly different (P<0.05).

Table 4 reviews the yields of crude WSP content and β-linked WSP. The results showed that the yields of WSP of these four species ranged from 4.43 to 17.20 %. *G. lucidum* contained a considerably higher (P≤0.05) amount of WSP than other species, while the lowest (P≤0.05) content was noticed in *G. lucidum* (4.43 %). When considering the β-linked polysaccharide content, *Calocybe sp.* reported (12.45 %) as the highest (P≤0.05), while *G. lucidum* reported (1.12 %) as the lowest (P≤0.05). The low yields of *G. lucidum* may be described by the hard and woody nature of its spores. The same phenomenon has been discussed in the study by Kozarski et al. (2011b).

Glucan is the most prominent water-soluble polysaccharide in mushrooms. The majority of edible mushrooms contain large amounts of glucans. The β-glucans are more challenging to extract since they are the structural elements of the fungal cell wall than α-glucans (Gong et al., 2020). It is the most available glucan type in most mushrooms. Typically, mushrooms contain larger amounts of β (1-4), (1-6) mixed links, and vary single linkages (Mullins, 1990). Further, β-glucans, long- or short-chain polymers of glucose, are abundant in the cell walls of mushrooms (Manzi & Pizzoferrato, 2000; Cerletti et al., 2021). Different types of β- linked polysaccharides, including β-glucans, are available in mushroom species. For example, pleuran and singer in *Pleurotus* sp, and APK2 in *Calocybe s*p. (Rop et al. 2009) are reported to be good

bioactive agents that aid in antioxidant, antidiabetic, anticarcinogenic, immunomodulating activity, reduce the body weight through decreasing blood cholesterol content (Dissanayake et al. 2018; Nandi et al. 2014). Since mushroom polysaccharides have a wide range of structural characteristics, their biological activities may differ (Maity et al. 2021).

Previous researchers have shown that WSP in *Agaricus* sp., *Pleurotus* sp., and *Ganoderma* sp. have strong antioxidant activities, which are important as natural antioxidants (Yan et al. 2019; Seweryn et al. 2021). Some studies have revealed that most of the polysaccharide fractions in mushrooms have antidiabetic potential, and β-glucans have an excellent impact among them (Wang et al., 2013; Kim et al., 2005). In this study also, there may be a contribution of WSP to antioxidant and antidiabetic activities other than the phenolic and flavonoids. According to Table 9, there is a weak correlation ($P \leq 0.05$) between TPC and TFC with the antidiabetic activities in water extracts. Therefore, WSP can be assumed as one of the impactable components for antidiabetic activities, mostly for α- amylase inhibition activity.

3.5 FTIR-ATR analysis

Typically, mushrooms contain high polysaccharides followed by high protein and low-fat content. By using FTIR-ATR, more specific characterisation of their microstructures at the molecular level can be further determined to establish structure-function relationships in mushroom species. According to the researchers,

i. The prominent broad band centred around 3300 cm^{-1} could be due to O-H and C-H stretching vibrations representing moisture availability. Bands around 2900–2880 cm^{-1} addressed to CH_2 and CH_3 stretching of fatty acids in cell walls.

ii. The bands around 1650 and 1560 cm^{-1} were determined to be amides of proteins, a band at 1740 cm^{-1} that might be caused by the carbonyl stretching vibration of alkyl-esters, which would indicate the presence of oil.

iii. Polysaccharide C-O stretching has been attributed to the 1500-750 cm^{-1} region linked with protein, lipid, and polysaccharide vibrations

iv. A zone between 950 and 750 cm^{-1} has been attributed to the identification of polysaccharide anomeric configuration; a band of 890 cm^{-1} may be linked to β-glycosides and 860–810 cm^{-1} for α-glycosides.

As shown in Figure 3, all four mushroom species have shown four specific regions. But there are some little qualitative differences between the spectra of the four species. All species have shown bands relevant to the major mushroom components of fat, protein, polysaccharides, and moisture by confirming the results of proximate analysis and dietary fibre analysis.

The bands around 1560 cm^{-1} are evidence for the presence of proteins in *A. bisporus*, *P. ostreatus*, and *Calocybe* sp. All four species have shown bands relevant to fatty acids (around 2900–2880 cm^{-1}), polysaccharides (around 1500–750 cm^{-1}), and moisture (around 3300 cm^{-1}), respectively (Zhao et al., 2006; Rodrigues et al., 2015). Furthermore, (Liu et al., 2006) demonstrated that the spectral area between 1200 and 1000 cm^{-1} might serve as an indicator of the mushroom genus. Rodrigues et al. (2015) also indicated that 750 and 1200 cm^{-1} could serve as fingerprints in differentiating mushrooms. The 1600-1200 cm^{-1} patterns found in all four mushroom species provided evidence for glucan-protein complexes (Gonzaga et al., 2005). Bands between 950-750 cm^{-1} show the anomeric configuration of polysaccharides (Barbosa et al., 2003), which are highly complied with all mushroom species. Normally, mushroom polysaccharides contain glucans with diverse types of glycosidic linkages; (1→3)-α-glucans, (1→3), (1→6)-β-glucans and hetero glucans. As well as their side chains consisting of glucuronic acid, mannose, galactose, xylose, arabinose, or ribose in different combinations (Wasser, 2002). *G. lucidum* and *A. bisporus* indicate the bands at 1375 cm^{-1} suggesting the existence of β-glucans. *G. lucidum*, *A. bisporus*, and *Calocybe* sp. represented carbonyl stretching vibration of alkyl-esters by showing bands around 1560 cm^{-1}. Those would indicate

the presence of oil in mushroom bodies. Further, all mushroom species have shown bands around 1000-900 cm^{-1}, indicating C-C vibration for alkanes and C-H bending for alkenes present in major mushroom components (Rasika et al., 2022).

3.6 Total phenolic content (TPC)

It has been reported that mushrooms contain different phenolic compounds, mainly phenolic acids. In general, the results (Figure 2 (a)) demonstrated that the total phenolic contents of *P. ostreatus* and *A. bisporus* were higher ($P \leq 0.05$) in all three extracts, while *Calocybe* sp. and *G. lucidum* showed lower ($P \leq 0.05$) contents compared to other species. The TPC of *A. bisporus* was lower than the reported data by Abugri and McElhenney (2013). Similar research studies on newly developed *Calocybe* sp.; MK-white species are pretty limited. This study aligned with Alispahic et al. (2015), who mentioned *P. ostreatus* and *A. bisporus* as good sources of natural antioxidants. In particular, these high TPC values could be influenced by phenolic compounds, such as myricetin, pyrogallol, homogentisic acid, gentisic acid, ferulic, gallic, 4-hydroxybenzoic acid, protocatechuic, salicylic, syringic, t-cinnamic, and vanillic acid (Gasecka et al., 2018). Consuming antioxidants containing food may assist in preventing the oxidative stress-induced number of non-communicable diseases. These antioxidants may inhibit the growth of cancer cells and also regulate serum lipid oxidation in the human body (Akbarirad et al., 2016).

3.7 Total flavonoid content (TFC)

Flavonoids are a type of phenolic compound with multiple health benefits. TFC results (Figure 2 (b)) revealed that ethanolic extracts of *P. ostreatus* had the highest ($P \leq 0.05$) total flavonoid content (2.17 mg CE/g DW) while *G. lucidum* (0.02 mg CE/g DW) showed the lowest ($P \leq 0.05$) content. Moreover, TFC in the water extract of *A. bisporus* was lower ($P \leq 0.05$) than that in ethanolic extracts and agreed with Gan et al. (2013). It was further

411 confirmed by the observations for flavonoids in *G. frondosa* mushrooms by Yeh et al. (2011).
412 Flavonoids possess a wide range of medicinal benefits, including anticancer, antioxidant, anti-
413 inflammatory, antiviral, neuroprotective, and cardio-protective (Ullah et al., 2020). Thus,
414 incorporating flavonoid-rich mushrooms into daily diets may help stay healthy by reducing the
415 risk of serval life-threatening diseases.
416 .

417 **3.8 Antioxidant activities**

418 **3.8.1 DPPH activity**

419 **Table 5** DPPH activities of different extracts of mushroom species (IC_{50} = mg/mL)

Sample	DPPH in water	DPPH in 65 % ethanol	DPPH in 80 % ethanol
G. lucidum	1.15± 0.04^{c}	0.28± 0.07^{c}	0.45± 0.04^{c}
A. bisporus	1.21±0.12^{c}	0.45±0.04^{c}	1.20±0.06^{b}
P. ostreatus	2.21±0.12^{b}	3.17±0.08^{b}	4.34±0.18^{a}
Calocybe sp.	8.18±0.08^{a}	4.28±0.15^{a}	4.21±0.11^{a}

420 Values are expressed as mean values± SD (n=3). Means with different superscript letters according to mushroom
421 species within a column are significantly different ($p<0.05$).

422 This assay measures the hydrogen-donating abilities of antioxidant compounds in
423 mushroom extracts. These compounds reduce stable DPPH radical into non-radical form by
424 changing purple to yellow in the reaction mixture. As shown in Table 5, ethanolic extracts of
425 *G. lucidum* showed the highest ($P \leq 0.05$) radical scavenging activities with the lowest ($P \leq 0.05$)
426 IC_{50} values (0.28± 0.07 mg/mL in 65 % and 0.45±0.04 mg/mL in 80% ethanolic extracts). The
427 DPPH activity of *G. lucidum* was reported by Tamrakar et al. (2016) and Uddin Pk et al. (2019)
428 as 0.47±0.011 mg/mL and 867.81±3.01 μg/mL, respectively, which were by the findings of the
429 current study.

Calocybe sp. showed the lowest ($P \leq 0.05$) radical scavenging activities (8.18, 4.28, and 4.21 mg/mL) among studied mushroom species. The DPPH radical scavenging activity of *A. bisporus* was similar to the previously published literature that ranged from, ranging mg/mL (Gan et al., 2013). Masalu et al. (2012) described that varied scavenging activities observed in different solvent extracts could be due to their diverse light sensitivities.

3.8.2 ABTS activity

Table 6 ABTS activities of different extracts of mushroom species on DW basis (μmol TE/g)

Sample	ABTS in water	ABTS in 65 % ethanol	ABTS in 80 % ethanol
G. lucidum	59.54± 1.03^{d}	168.84± 3.09^{b}	270.98± 2.86^{b}
A. bisporus	422.48±7.56^{a}	289.46±14.10^{a}	350.49±7.73^{a}
P. ostreatus	290.68±2.73^{b}	271.62±4.47^{a}	189.58±2.94^{c}
Calocybe sp.	89.16±2.76^{c}	85.98±1.31^{c}	105.07±1.29^{d}

Values are expressed as mean values± SD (n=3). Means with different superscript letters according to mushroom species within a column are significantly different ($p<0.05$).

Compared to the Trolox standard, the ABTS assay evaluates the relative capacity of antioxidants to scavenge the ABTS produced in the aqueous phase. According to the results, *A. bisporus* displayed (Table 6) the highest ($P \leq 0.05$) ABTS radical scavenging activity (422.48 μmol TE/g DW) among all extracts, followed by *P. ostreatus* and *G. lucidum* (Table 6). Wang et al. (2021) and Lam & Okello (2015) have observed the ABTS activity of whole *P. ostreatus* as 2.73 μmol TE/g DW and 48.12 μmol TEAC/g in water extracts were lower than the results of the present study.

As mentioned by Alispahic et al. (2015), Chye et al. (2008), and Kozarski et al. (2015a), *A. bisporus* and *P. ostreatus* contain β-carotene, lycopene, ascorbic acid, lycopene, gallic acid, protocatechuic acids, kaempferol, naringin, and resveratrol which are responsible for these

antioxidant activities. The lowest ($P \leq 0.05$) ABTS antioxidant activity was observed for the water extract of *G. lucidum* (59.54 μmol TE/g DW). However, two extracts of *Calocybe* sp. had a lower ($P \leq 0.05$) antioxidant activity than the other three mushroom species.

3.8.3 FRAP activity

Table 7 FRAP radical scavenging activities of different extracts of mushroom species on DW basis (mmol Fe^{2+} Eq/g)

Sample	FRAP in water	FRAP in 65 % ethanol	FRAP in 80 % ethanol
G. lucidum	14.41 ± 1.04^{c}	27.27 ± 0.74^{c}	10.94 ± 0.71^{a}
A. bisporus	17.81 ± 1.51^{b}	36.19 ± 1.96^{b}	7.26 ± 0.72^{b}
P. ostreatus	30.32 ± 0.76^{a}	53.17 ± 0.63^{a}	2.83 ± 0.22^{c}
Calocybe sp.	0.75 ± 0.08^{d}	4.07 ± 0.23^{d}	3.27 ± 0.23^{c}

Values are expressed mean values± SD (n=3). Means with different superscript letters species, simple within a column are significantly different ($p<0.05$).

This method is based on the reducing ability of the colourless Fe^{3+}-TPTZ complex to the blue Fe^{2+}-tripyridyltriazine complex, with the electron-donating capacity of antioxidants in mushroom samples. According to the results in Table 7, extracts of *P. ostreatus* showed the highest ($P \leq 0.05$) reducing effects (30.32 and 53.17 mmol Fe^{2+} Eq/g DW in water and 65% ethanol extracts, respectively). The previous study by Gan et al. (2013) reported the FRAP activities of *A. bisporus* as 84.69 μmol Fe^{2+} Eq/ g DW in 60 % ethanol and 186.72 μmol Fe^{2+} Eq/ g DW in water. FRAP values reported by Witkowska et al. (2011) (1.54-18.83 mmol Fe^{2+} Eq/100 g in methanol/acetone mixture) and Islam et al. (2016) (0.27-39.98 mmol Fe^{2+} Eq/100 g in acetone/water/acidic acid mixture) were lower than our findings. The differences in extraction methods could cause these variations.

Antioxidants found in certain foods may protect body cells against free radicals by neutralising them. Incorporating antioxidants-rich *G. lucidum* and *P. ostreatus* mushroom

species into the diet may help the body to fight against oxidative stress-induced diseases, including heart diseases, diabetes mellitus, cancers, and other infectious diseases.

3.9 Antidiabetic activities

Table 8 α-Amylase and α-glucosidase inhibitory activities in four mushroom species

Sample	*G. lucidum*	*A. bisporus*	*P. ostreatus*	*Calocybe* sp.
	α-glucosidase inhibitory activities (IC_{50} μg/mL)			
Water	>1000	>1000	>1000	>1000
65% ethanol	131.17±2.67^{a}	7.97±0.83^{c}	14.69±3.54^{c}	31.53±8.92^{b}
80% ethanol	0.41± 0.08^{c}	15.64±2.00^{b}	41.45±0.399^{a}	21.12±7.84^{b}
	α-amylase inhibitory activities (IC_{50} μg/mL)			
Water	646.7±82.8^{ab}	755.2±129.6^{a}	394.5±35.5^{c}	444.9±83.6^{bc}
65% ethanol	672.6± 25.9^{c}	2128.1±170^{a}	383.8±48.8^{c}	1695.5±107.3^{b}
80% ethanol	77.51± 6.8^{c}	1362.0±85.4^{a}	132.0±10.3^{d}	929.3±26.4^{b}

Values are expressed values± SD (n=3). Means with different superscript letters according to mushroom species within a column are significantly different ($p<0.05$).

3.9.1 Alpha-amylase inhibition activity

Alpha-amylase inhibitors are copious in mushrooms that produce a large number of diverse protein inhibitors of α-amylases to regulate the activity of the enzyme (Prabu & Kumuthakalavalli, 2017). It can be seen that (Table 8), the α-amylase inhibitory activity of *P. ostreatus* was the lowest ($P\leq0.05$) among all extracts. However, the activity of 80 % ethanolic extract of *G. lucidum* (77.51 μg/mL) showed the highest ($P\leq0.05$), and it was almost similar to the activity of the acarbose. In contrast, others expressed lower ($P\leq0.05$) activities than acarbose. These observations were supported by findings from an *in-vivo* study by Molz et al.

(2014), where the polysaccharide fractions of *G. lucidum* showed potential hypoglycemic activities. According to previous findings, *P. ostreatus* has been identified as a rich source of tocopherol and thiamin compared to *A. bisporous*, and these compounds may be responsible for the antidiabetic activities (Mattila et al., 2002), which could be a possible reason for the high α-amylase inhibitory activity shown by *P. ostreatus* in most of the extracts. An *In-vitro* study done by Prabu (2014) using *C. indica* has recorded an IC_{50} value of 38.06±0.82 μg/mL for α-amylase inhibitory activity, which was lower ($P \leq 0.05$) than the observed value for *Calocybe* sp. of MK-white in the present study.

3.9.2 Alpha-glucosidase inhibition activity

The presence of α-glucosidase enzyme inhibitors in mushroom extracts is helpful in reducing the digestion rate of carbohydrates and preventing type 2 diabetes mellitus (Prabu & Kumuthakalavalli, 2017). According to our study, the IC_{50} values obtained for the α-glucosidase inhibition activity assay were above 1000 μg/mL showing very low enzyme inhibition activities. The highest ($P \leq 0.05$) α-glucosidase enzyme inhibiting activity (IC_{50}=0.41 μg/mL) was demonstrated by 80 % ethanol extracts of *G. lucidum* followed by 65 % ethanolic extracts of *A. bisporus* and *P. ostreatus* ($P \leq 0.05$). An *in-vitro* study done by Prabu, (2014) using *C. indica* had shown an IC_{50} value of 281.27±6.69 μg/mL for α-glucosidase inhibition activity, which was higher ($P \leq 0.05$) than that of *Calocyb*e sp. of MK-white in this study. The analysis of antidiabetic activities in *Pleurotus* sp. by Prabu & Kumuthakalavalli (2017) has shown quite opposite results of α-amylase and α-glucosidase inhibition activities to this study. Stojkovic et al. (2019) and Wu and Xu (2015) have indicated that the IC_{50} values of *G. lucidum* and *A. bisporus* were 4.88 mg/mL in 70 % ethanol and 357.23 μg/mL in methanol extracts, which were lower ($P \leq 0.05$) than activities shown for water extracts in this study.

This study with reported results supported using mushrooms in the pharmaceutical industries as natural antidiabetic agents through key enzyme inhibition. More clinical trials are needed to identify their antidiabetic properties for better glycemic control with minimal macrovascular and microvascular complications in diabetic patients. (Lee et al., 2019).

3.10 Correlation

Table 9 Correlation between TPC/TFC with antioxidant/antidiabetic activities

Activities	Water		65 % ethanol		80 % ethanol	
	TPC	TFC	TPC	TFC	TPC	TFC
ABTS	0.892	0.784	0.819	0.503	0.623	0.582
FRAP	0.816	-0.050	0.500	0.765	0.534	0.721
DPPH	-0.445	-0.661	0.794	-0.798	0.364	-0.701
α-Amylase inhibitory activity	-0.053	0.386	0.531	-0.498	0.047	-0.936
α-Glucosidase inhibitory activity	-0.467	0.133	-0.680	-0.526	-0.825	-0.863

Each value is expressed according to the Pearson correlation coefficient (r) significantly different ($p \leq 0.05$).

Given the results in correlation analysis (Table 9), ABTS and FRAP activities displayed a strong positive correlation ($P \leq 0.05$) with TPC (r = 0.892, 0.819, 0.623, r = 0.816). TFC results are similar to the reported results of Gan et al. (2013) in that total flavonoid content in 60 % ethanol extract had a strong positive correlation ($P \leq 0.05$) with the FRAP activities (r = 0.985). DPPH radical scavenging assay expressed a strong negative correlation ($P \leq 0.05$) (r = -0.661, -0.798, -0.701) with TFC in all extracts. Ethanolic extracts had a positive correlation ($P \leq 0.05$) (r = 0.794, 0.364) with total phenolic contents. Tamrakar et al. (2016) have mentioned that the correlation ($P \leq 0.05$) between DPPH and TPC mainly depends on the presence of phenolics, terpenes, ergosterol, lectin, and β-glucans and non-phenolic active constituents; amino, uronic acids, etc. (Jacobo-Velazquez & Cisneros-Zevallos et al., 2009; Prasetyo et al., 2013; Chye et al., 2008).

IC_{50} values of α–amylase and α–glucosidase inhibition activity assays had moderate and strong negative correlations ($P \leq 0.05$) (r = -0.498, -0.936, -0.526, -0.863) with TFC in ethanol extracts, sequentially. It expressed a good relationship between flavonoids to control Diabetes Mellitus through the mechanisms of inhibiting α-glucosidase enzymes (Bharti et al., 2018; Gasecka et al., 2018).

According to the overall results, consumption of *G. lucidum* will be important as a healthy fat source for reducing the risk of heart diseases and a good source of dietary fibre, which helps to keep the health of the digestive system. *A. bisporus* can be considered as a very important protein source mostly for vegetarians and vegans while having the potential to be a good mineral source to consumers because of the presence of the highest ($P \leq 0.05$) mineral content. Further, its PUFA content may facilitate the prevention of some chronic diseases. The good nutritional composition and antioxidant and antidiabetic activities of *P. ostreatus* may deliver multiple health benefits and reduce the risk of Non-Communicable Diseases (NCD). Newly developed *Calocybe* sp. (MK-white) had comparatively lower ($P \leq 0.05$) nutritional properties and bioactivities than other species.

CONCLUSION

In summary, the protein and ash contents were higher ($P \leq 0.05$) in *A. bisporus*, while *G. lucidum* showed the highest fat and dietary fiber content. Most of the studied minerals were abundantly ($P \leq 0.05$) available in *G. lucidum*, *A. bisporus*, and *P. ostreatus* species compared to *Calocybe* species. Mushrooms were rich in PUFA, and *A. bisporus* was superior among them. Different peaks related to microstructural properties (obtained from FTIR-ATR) in all mushroom species showed the bands associated with the fat, protein, polysaccharides, and moisture. The polysaccharide composition was higher ($P \leq 0.05$) in *Calocybe* sp. and *P. ostreatus* than in other species. In addition, *P. ostreatus*, *G. lucidum*, and *A. bisporus* showed

549 higher ($P \leq 0.05$) antioxidant and antidiabetic activities than other species while *Calocybe* sp.
550 (MK-white) demonstrated the lowest ($P \leq 0.05$). Significant correlations ($P \leq 0.05$) existed
551 between TPC, TFC, antioxidant, and antidiabetic activities in studied mushroom species.

552 **SUGGESTIONS FOR FUTURE STUDIES**

553 Identifying and quantifying individual phenolic and flavonoid compounds in studied
554 mushrooms would help distinguish the compounds responsible for the identified bioactivities.
555 Higher protein content and favourable micronutrient composition in these studied mushrooms
556 highlighted the potential of using them as one of the cheapest sources to overcome protein and
557 micronutrient malnutrition among the populations in developing countries. Encouraging the
558 cultivation and consumption of these mushroom species is essential to exploit their properties.

559

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Ethics declarations

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Ruvini Liyanage conceived, designed, and guided this study. Thilini Chathurangi Kananke guided in anaylsis. Malmi Apsara Wickramasinghe carried out the experiments and wrote the manuscript. Suriya Mudiyanselage Sewwandi and Isuri Rathnayake carried out the experiments. Harshani Nadeeshani contributed to the manuscript writing.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon a reasonable request.

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Table 3 Fatty acid composition (percentage (%)) of mushroom species

	Component (methyl esters)	*G.lucidum*	*P. ostreatus*	*A. bisporus*	*Calocybe* sp.
1	Butyric acid C4:0	1.23	ND	0.31	0.29
2	Caproic acid C6:0	ND	ND	0.13	0.12
3	Caprylic acid C8:0	0.54	ND	0.14	0.16
4	Capric acid C10:0	ND	0.54	ND	ND
5	Lauric acid C12:0	0.34	0.49	0.31	0.05
6	Myristic acid C14:0	0.35	0.67	0.49	0.15

7	Pentadecanoic acid C15:0	1.14	ND	1.07	0.05
8	Palmitic acid C16:0	18.75	8.39	10.21	10.97
9	Palmitoleic acid C16:1	ND	ND	0.20	ND
10	Heptadecanoic acid C17:0	ND	2.26	0.52	0.14
11	Stearic acid C18:0	10.78	3.07	3.40	15.93
12	Oleic acid C18:1	29.35	9.17	0.42	24.98
13	Linoleic acid C18:2 cis(n6)	37.51	39.57	80.54	43.21
14	Arachidic acid C20:0	ND	ND	1.02	0.38
15	Behenic acid C22:0	ND	ND	0.49	0.34
16	Cis-8,11,14-Ecosapentrienoic acid C20:3n6	ND	ND	0.74	ND
17	Lignoceric acid C24:0	ND	16.37	ND	0.89
18	Cis-5,8,11,14,17-Ecosapentaenoic acid C20:5n3 (EPA)	ND	ND	ND	1.87
19	Cis-4,7,10,13,16,19-Decosahexaenoic acid C22:6 (n3) (DHA)	ND	9.02	ND	0.48
	SFA (saturated fatty acids)	33.13	31.79	18.09	29.47
	UFA (unsaturated fatty acids)	66.86	57.76	81.90	70.54

MUFA (monounsaturated fatty acids)	29.35	9.17	0.62	24.98
PUFA (polyunsaturated fatty acids)	37.51	48.59	81.28	45.56

ND: not determined.

Figure legends

Figure 1 (a) *A. bisporus*, **(b)** *P. ostreatus*, **(c)** *Calocybe* sp., and **(d)** *G. lucidum* at the correct maturity

Figure 2 (a) Total phenolic contents (TPC) of mushroom species in different extracts on DW (mg GAE/g DW). Data represent the mean values±SD of three independent estimations. Means followed by the same letters in a type of extract are not significantly different ($P<0.05$),

(b) Total flavonoid contents (TFC) of mushroom species in different extracts on DW (mg CE/g DW). Data represent the mean values±SD of three independent estimations. Means followed by the same letters in a type of extract are not significantly different ($P<0.05$).

Figure 3 FTIR-ATR spectra of the four different mushroom species between 4000-400 cm^{-1} wavenumber