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Assessment of quantitative phytochemical constituents of wild edible mushrooms collected from Tripura

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For a long time, mushrooms have been playing an important role in several aspects of the human activity. It has been found that edible and medicinal mushrooms contain many biologically active compounds with anti-inflammatory, antitumor, antibacterial, antiviral, cardiovascular, anti-hypercholesterolemia and antioxidant activities. Mushrooms are increasingly being recognized as important food products due to the role they play in human health, nutrition and disease control. Certain species of mushrooms are effectively used as antimicrobial against disease conditions, in the nutraceutical industry and in environmental bioremediation. Notably, *Pleurotus* and *Lentinus* species are particularly abundant in valuable nutrients. This study has an aim to compare the phytochemical analysis of dried methanolic extract of different species of *Lentinus* and *Pleurotus* collected from diverse regions in Tripura, situated in North-East India. The samples were obtained and analysed for phytochemical properties on the dry weight basis using standard methods. They were analysed for phenol, alkaloid, saponin, tannin, terpenoid, ascorbic acid, lycopene, β carotene and flavonoid using standard methods. All the analysis was done in triplicate. Total phenols values obtained ranged between 3.3-6.8 mg gallic acid equivalent (GAE)/g dry weight basis (dwb) and flavonoids 0.53-1.24 mg quercetin equivalent (QE)/g dwb. Total phenols and flavonoids values showed a positive correlation with the saponin, tannin, terpenoid, lycopene, β carotene and ascorbic acid content. The results show that the collected wild edible mushrooms are rich in health-promoting phytochemical compounds.

Keywords: Methanolic extract, mushrooms, phenol, phytochemical, health-promoting

INTRODUCTION

A rising number of individuals are opting for natural medicinal ingredients due to the perception that they are safer and have fewer side effects compared to synthetic drugs (Hamda *et al*. 2008). One natural remedy attracting attention for its healing properties is mushrooms. Concerns about the diminishing effectiveness of synthetic antibiotics, driven by the proliferation of drug-resistant microorganisms, have led to an increasing interest in natural remedies (Yamac and Bilgili, 2006). “Green medicine” is increasingly viewed as a preferable option, given that synthetic drugs often lead to undesirable side effects (Nair and Chanda, 2007). Consequently, researchers are actively searching for new compounds capable of combating infections, with mushrooms being a key focus in this exploration (Cordell, 2000; Thakur and Singh, 2000).

Mushrooms, a subset of macro-fungi characterized by visible fruiting bodies, belong to the Basidiomycota phylum and are distinguishable without the need for a microscope. They exhibit rapid growth and an affinity for colonizing wood, with some mushrooms thriving as saprophytes, while others engage in symbiotic relationships with plants. Many macro-fungi are not only edible but also possess medicinal properties, contributing to their utilization in treating various ailments such as bronchial inflammation, hepatitis, cancer, hypertension, allergies, and more. These mushrooms are abundant sources of biologically active

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compounds, and contemporary research is centered on the exploration of natural pharmaceuticals from diverse sources, including wild edible mushrooms (Iwalokun *et al*. 2007).

Mushrooms exhibit an extraordinary capacity to adapt their metabolism to their surroundings, leading to the production of metabolites with notable antimicrobial, antioxidative, and anti-inflammatory properties (Alves *et al*. 2012). They encompass a wide array of bioactive compounds, including terpenoids, flavonoids, tannins, alkaloids, and polysaccharides (Elisashvili, 2012; Vamanu *et al*. 2018). These bioactive components are found in both the fruiting bodies (basidiocarps) and the vegetative part of the fungus (mycelia), collectively contributing to their health-enhancing attributes, which encompass antioxidative, antibacterial, and immune stimulatory effects (Golak-Siwulska *et al*. 2018; Roy *et al.* 2018). This unique combination of properties amplifies the therapeutic potential of mushrooms employed for medicinal purposes. In contemporary times, wild edible mushrooms, such as the genus *Pleurotus*, have gained widespread recognition owing to their nutritional and therapeutic potential (Roy *et al*. 2015a; Roy and Chakraborty, 2018; Roy and Chakraborty, 2023). *Pleurotus ostreatus*, commonly known as the white oyster mushroom, is particularly notable among commercial mushrooms due to its ease of cultivation and significance as a vital food source. These mushrooms are rich in essential nutrients and various secondary metabolites with notable pharmacological effects (Elsayed *et al*. 2014; Singh *et al*. 2015; Roy *et.al.,* 2015b). One of the noteworthy pharmacological attributes associated with *Pleurotus ostreatus* is its antioxidant potential. Prior research has consistently demonstrated the remarkable antioxidant capabilities of these mushrooms. Extracts derived from white oyster mushrooms have shown protective effects on vital organs such as the liver, kidneys, brain, and lungs (Jayakumar *et al*. 2008).

The existing body of literature underscores that *Pleurotus ostreatus* contains a diverse array of bioactive compounds that contribute to its therapeutic properties. These compounds include phenolic components, flavonoids, terpenoids, polysaccharides, lectins, steroids, glycoproteins, as well as several lipid components. Additionally, *Pleurotus ostreatus* is a source of ergothioneine (ET), vitamin C, β-carotene, and selenium. Notably, the ethanol extract of *Pleurotus ostreatus* is believed to function as an exogenous antioxidant, capable of preventing and inhibiting oxidative stress induced by free radicals within the body. Furthermore, it exhibits synergistic effects with the body's endogenous antioxidants (Roumyana *et al*. 2008; Jayakumar *et al*. 2010; Reis *et al*. 2012).

The *Lentinus* genus encompasses diverse fungal species, with *Lentinula edodes*, commonly known as Shiitake mushroom, being highly regarded for its culinary and medicinal properties. It is recognized for its nutrient richness, including proteins and polysaccharides (Sun *et al*. 2021). Shiitake mushrooms have garnered attention for their bioactive compounds, notably polysaccharides (Lee *et al*. 2009; Zheng *et al*. 2015; Tang *et al*. 2020), as well as sterols and nucleosides (Ding *et al*. 2018). Pharmacological studies have unveiled a range of health benefits, such as antitumor and immunoregulatory effects (Carbonero *et al*. 2008; Rungprom *et al*. 2018).

In the same genus, *Lentinus squarrosulus* is valued in traditional medicine for treating various conditions, including ulcers and anemia (Okigbo and Nwatu, 2015). It offers potential for specialized diets due to its high protein and essential mineral content (Anno *et al*. 2016). Phytochemical analyses reveal diverse compounds, including phenolic compounds, saponins, flavonoids, and important primary metabolites (Attarat and Thamisak, 2014; Ademola and Odeniran, 2017), enhancing the medicinal and nutritional significance of *Lentinus* mushrooms.

In this region, information is scarce regarding the specific bioactive components found in these mushrooms, despite numerous reports highlighting their nutritional constituents and various properties such as anti-inflammatory, anticancer, anti-diabetic, anticholestemic, anti-ulcer, and antioxidative potential. Therefore, this study, situated in Tripura, North-East India, aims to explore the phytochemical constituents of wild

edible mushrooms mainly from the *Lentinus* and *Pleurotus* genera.

MATERIALS AND METHODS

Collection of the samples

Five wild edible mushroom species were systematically collected from diverse locations in Tripura, India: *Pleurotus ostreatus* (MCCT P4) and *Lentinus sajor-caju* (MCCT L1) from Garji (South Tripura) – 23°25'22.72"N, 91°30'46.27"E, *Pleurotus giganteus* (MCCT P6) and *Lentinus tuber-regium* (MCCT P7) from Salbagan (West Tripura) – 23°52'53.10"N, 91°17'13.68"E, and *Lentinus tigrinus* (MCCT P2) from Paratia (West Tripura) – 23°26'45.17"N, 91°29'47.77"E. GPS coordinates of each collection site were recorded. Photographs of each sample were taken to document their morphological features, which are provided in Figure 1. The identification of the collected samples was carried out using both morphological and molecular methods. For morphological identification, characteristics such as cap colour, shape, size, stipe features, and overall fruiting body structure were documented during and immediately after collection. For identification, literature on mushroom identification were consulted (Pegler, 1977; Purkayastha and Chandra, 1985; Rogers *et al*. 2005) . In addition to morphological identification, molecular identification was performed by sequencing the ITS region of rDNA, amplified using universal primers ITS1 and ITS4.

Upon collection, the specimen were transported back to the laboratory within 4–6 hours in sterile, airtight containers to preserve their quality. The fruiting bodies were cleaned, destalked, and air-dried at room temperature with adequate ventilation. After initial drying, they were placed in a hot air oven (Model: ROV/DG) at 40–50°C for 6 hours to ensure complete dryness. Once thoroughly dried, the samples were pulverized using a blender. Dried powdered samples were then stored in sterilized containers at room temperature for further analysis.

Extract preparation from dried wild edible mushrooms for analysis of bioactive compound

The methanolic extract was prepared from the tested mushroom samples followed by a slightly modified method by Mau *et al*. (2004). The fine powder form of mushrooms (10 g) was extracted using methanol for 24 h. By using Whatman filter paper 1, the extracts were filtered and then concentrated under reduced pressure in a rotary evaporator for evaporating the excess solvent. The dry extract was kept in a hot air oven at about 45°C for residual evaporation. Then the dry powder was stored at -20ºC for further use. Ultimately, the dry powder of the samples was dissolved in methanol to make the 10% stock solution and was employed for the quantification of different phytochemicals such as phenol, flavonoid, ascorbic acid, tannins, terpenoids, saponins, β-carotene, and lycopene content.

Phytochemical analysis

Quantitative estimation of total Phenolic compound

The quantification of phenol contents in the methanolic extracts (0.01mg/ml) of the experimental samples was carried out using a spectrophotometric method. In this method, 0.5 ml of Folin and Ciocalteu's reagent was mixed with an equal volume of the tested sample. After approximately 4-5 mins, 0.5 ml of a saturated Na_2CO_3 aqueous solution was added to the mixture, and the volume was adjusted to 5 ml by adding distilled water (Barros *et al*. 2007). The test tubes containing the reaction mixture were then kept in a dark environment for about 1 hr, following which the absorbance was measured at 650 nm using a spectrophotometer (Model - Eppendorf AG 22331 Hamburg). The total phenol content for each sample was determined and expressed in milligrams of gallic acid equivalents (GAEs) per gram of dry weight.

Quantitative estimation of total Flavonoid

The determination of flavonoid contents in the tested extracts was conducted following a slightly modified method inspired by Fatemah *et al*. (2012). The methanolic extract of the mushrooms was mixed with 0.10 ml of $NaNO_2$, and the resulting reaction mixture was allowed to stand at room temperature for approximately 5 minutes. Following this, 0.10 ml of $AlCl_3 \cdot 6H_2O$ was introduced into the mixture and left for an additional 5 minutes at room temperature. Finally,

0.6 ml of a 10% NaOH solution was added to the reaction mixture, and the absorbance was subsequently measured at 510 nm using a spectrophotometer (Model - Eppendorf AG 22331 Hamburg) after allowing it to stand for 10 minutes at room temperature. The total flavonoid content for each sample was determined and expressed in milligrams of quercetin equivalents per gram of dry weight.

Estimation of β-carotene and lycopene content

The total â-carotene and lycopene contents in the tested extracts were quantified using a modified method based on Nagata and Yamashita (1992). Approximately 200 mg of dried methanolic extract from each sample was vigorously shaken with a 20 ml solution mixture of hexane and acetone (in a ratio of 6:4) for about 2-3 minutes. The entire reaction mixture was then filtered through Whatman filter paper. Finally, optical density (OD) measurements were taken at three distinct wavelengths: 453 nm, 505 nm, and 663 nm. The total contents of β-carotene and Lycopene were determined by:

Lycopene= " 0.0458 × OD at 663nm + 0.372 × OD at 505nm " 0.0806 × OD at 453nm

β-Carotene = 0.216 × OD at 663nm " 0.304 × OD at 505nm + 0.452 × OD at 453nm

Determination of Alkaloids content

The concentrations of alkaloid contents in the tested extracts were determined using the gravimetric method outlined by Haborne (1973). Initially, 5 g of each powdered sample was blended with 10 ml of a 10% ethanolic acetic acid solution. Subsequently, the reaction mixture was vigorously shaken and allowed to sit for approximately 3 hrs before being filtered through Whatman filter paper. After evaporating the sample, concentrated NH_4OH was added drop by drop to precipitate the alkaloids. To separate the precipitate, a pre-weighed filter paper was employed and subsequently washed with a 1% NH_4OH solution. Following this, the filter paper containing the precipitate was air-dried in a hot air oven at 55°C for 45 minutes, placed in desiccators, and then re-weighed to achieve a constant weight. The total alkaloid content was determined by the weight difference of the used filter papers and expressed as a percentage of the weight of the experimental sample analyzed.

Estimation of total tannin content

The determination of tannin contents in the methanolic extracts (0.01mg/ml) of the experimental samples followed a spectrophotometric method by Broadhurst and Jones (1978). In this method, 0.5 ml of Folin and Ciocalteu's reagent was combined with 1 ml of the tested sample. After approximately 4-5 mins, 1 ml of saturated Na_2CO_3 aqueous solution was added to the mixture, and the volume was adjusted to 8 ml by adding distilled water. The test tubes containing the reaction mixture were then placed in a dark environment for approximately 30 minutes. The supernatant was obtained through centrifugation, and subsequently, the absorbance was measured at 725 nm using a spectrophotometer (Model - Eppendorf AG 22331 Hamburg). Different concentrations of tannic acid were utilized to create a standard graph for reference. The total tannin content for each sample was determined and expressed in milligrams of tannic acid equivalents (TAEs) per gram of dry weight.

Quantitative test for terpenoid content

To prepare the dried mushroom extract, 100 mg (W1) of the extract was dissolved in 10 ml of ethanol for a period of 28 hrs, following the method by Indumathi *et al*. (2014). After filtration, the ethanolic solution was subjected to extraction with 15 ml of petroleum ether using a separating funnel. The ether extract was then separated into pre-weighed glass vials and left to completely dry (W2). Following the evaporation of the ether, the total terpenoid content was calculated using the following formula: (W1-W2/W1×100).

Estimation of ascorbic acid

To estimate the ascorbic acid contents, a volumetric method as outlined by Sadasivam and Manickam (1992) was employed. This method involves redox titration with 2,6-dichlorophenol indophenol (DCIP) to determine the vitamin C content. During this process, the oxidation of

ascorbic acid results in the formation of dehydroascorbic acid. This is a redox reaction where vitamin C (ascorbic acid) is oxidized to dehydroascorbic acid, and DCIP is reduced to a colourless compound known as $DCIPH_2$ (Dichlorophenolindophenol hydride). DCIP exhibits a blue color in neutral solution and turns pink in acidic conditions. The reduced form of DCIP is colorless, so the titration's endpoint is indicated by the appearance of a faint pink colour. All reagents used in the analysis were of analytical grade, and the titrations were conducted in triplicate for accuracy.

Determination of total saponin content

The determination of total saponin contents in the tested extracts followed a slightly modified vanillin sulfuric acid colorimetric reaction method as described by Makkar *et al*. (2007). Approximately 50 µl of the mushroom methanolic extract was mixed with 250 µl of vanillin reagent, and the reaction mixture was allowed to sit at room temperature for about 5 minutes. Following this, 2.5 ml of 72% sulfuric acid was introduced into the mixture, and it was left to stand for an additional 5 mins at room temperature. Subsequently, this reaction mixture was placed in a water bath at 70°C for 15 mins. After 10 mins, it was cooled in ice-cold water, and finally, the absorbance was measured at 544 nm using a spectrophotometer (Model - Eppendorf AG 22331 Hamburg). The total saponin contents for each sample were quantified and expressed as diosgenin equivalents (mg DE/g extract), obtained from the standard graph.

Data analysis

All the experiments were conducted in triplicate, and the results were presented as Mean±SE (Standard Error) values. The calculation of mean values and standard errors was performed using MS Excel and Origin version 7.0 (Edwards, 2002). To assess the significance of the various antioxidant properties of the mushroom samples, we conducted One-way analysis of variance (ANOVA) and utilized the Tukey test for post-hoc analysis. This statistical analysis was carried out using Past Software (Hammer *et al*. 2001)

RESULTS AND DISCUSSION

In Table 1 **,** the results for various phytochemical constituents like crude phenol, flavonoid, tannin, terpenoid, saponin, total alkaloid, ascorbic acid, β-carotene, and lycopene contents of selected five wild mushrooms from Tripura were reported. To assess the significant impact of these five wild edible mushroom samples on these phytochemical constituents, an analysis of variance (ANOVA) was performed initially. If a significant impact was identified at a 5% significance level (signified by a *p*-value < 0.05) for at least one of the mushroom samples, the Tukey test was subsequently employed to determine which mushroom sample exhibited the most significant and potent effect. As given in the final column of Table 1, the reported *p*-values for the corresponding One-way ANOVA and Levene's test for homogeneity of variance were all below 0.05. This signifies the existence of significant differences among the selected mushroom samples for each phytochemical property. To determine which mushroom samples demonstrated the greatest significance across the various phytochemical constituents, an analysis using the Tukey test for post-hoc examination was carried out, and the results are elaborated below.

The analysis of phenol content in different mushroom samples revealed significant variations. *Lentinus tigrinus* (MCCT P2) exhibited the highest phenol content at 6.8 mg GAE/g, while *Pleurotus giganteus* (MCCT P6) revealed the lowest at 3.4 mg GAE/g. The small *p*-value from the One-way ANOVA (6.47E-07) indicates highly significant differences in phenol content among the samples. Although Levene's test (0.07386) suggests some similarity in variance, the ANOVA results confirm that these differences are statistically significant, demonstrating that phenol content varies significantly across these mushroom samples.

Flavonoid content among the mushroom samples significantly varies. *Lentinus tigrinus* (MCCT P2) had the highest at 0.67 mg QE/g, while *Pleurotus giganteus* (MCCT P6) has the lowest at 0.7 mg QE/g. The small *p*-value from One Way ANOVA (1.90E-07) confirms significant

Table 1: Quantitative Phytochemical constituents (Phenol, Flavonoid, Alkaloid, Tannin, Terpenoid, Saponin, Lycopene, β-carotene, and Ascorbic acid Content) of five wild edible mushrooms

Experiments	*Lentinus tigrinus* (MCCT P2)	*Pleurotus ostreatus* (MCCT P4)	*Pleurotus giganteus* (MCCT P6)	*Lentinus tuber-regium* (MCCT P7)	*Lentinus sajor-caju* (MCCT L1)	*p*-value	Levene's test for homogeneity of variance, from means
Phenol (mg GAE/g)	6.8±0.061a	4.07±0.011b	3.4±0.02c	4.67±0.03d	3.33±0.01e	6.47E-07	0.07386
Flavonoid (mg QE/g)	0.67±0.006a	0.53±0.002a	0.7±0.001b	1.24±0.01c	0.65±0.001b	1.90E-07	0.03799
Alkaloid (mg/g)	3±0.1a	1.8±0.2b	1.57±0.057b	1.83±0.208b	3.133±0.057a	1.33E-07	0.2333
Tannin (mg TAE/g)	1.29±0.02a	1.90±0.028b	0.34±0.05c	1.97±0.04b	0.19±0.006d	4.65E-14	0.02738
Terpenoid (mg/g)	0.361±0.025a	0.307±0.06b	0.455±0.02c	0.468±0.01c	0.46±0.02c	9.56E-07	0.2766
Saponin (mg DE/g)	1.72±0.04a	1.25±0.05b	0.93±0.006c	3.49±0.06d	1.86±0.037e	6.97E-14	0.05452
Lycopene (mg/g)	0.167±0.01a	0.024±0.002b	0.106±0.001c	0.231±0.01c	0.133±0.001a	1.39E-08	0.02513
β-Carotene (mg/g)	0.27±0.010a	0.093±0.005b	0.159±0.018c	0.320±0.007a	0.113±0.001c	1.40E-06	0.00312
Ascorbic acid (mg/g)	0.33±0.05a	1.33±0.05a	1.83±0.02b	1.9±0.01b	2.33±0.05b	0.0038	0.0918

The values are presented as the mean ± standard error (SE) of three replicates. Significant differences between different groups, as indicated by distinct alphabets, were observed at a significant level of $p<0.05$ for varying sample concentrations.

differences, indicating distinguishable variations in flavonoid content across the samples.

Alkaloid content displayed significant diversity among the mushroom samples. *Lentinus tuber-regium* (MCCT P7) exhibited the highest content at 1.83 mg/g, whereas *Pleurotus giganteus* (MCCT P6) displayed the lowest at 1.57 mg/g. The One Way ANOVA confirmed significant variations with a *p*-value of 1.33E-07. Levene's test (0.2333) suggested some degree of variance homogeneity, but the ANOVA results emphasized statistically significant differences, highlighting diverse alkaloid profiles.

Tannin content significantly varied among the mushroom samples. *Lentinus tuber-regium* (MCCT P7) had the highest content at 1.97 mg TAE/g, while *Pleurotus giganteus* (MCCT P6) demonstrated the lowest at 0.34 mg TAE/g. The One Way ANOVA emphasized highly significant differences with a *p*-value of 4.65E-14, indicating substantial diversity. Levene's test (0.02738) suggested non-uniform variance, further emphasizing these significant differences.

Terpenoid content significantly differed among the mushroom samples. *Lentinus tuber-regium* (MCCT P7) had the highest content at 0.468 mg/g, whereas *Pleurotus giganteus* (MCCT P6) had the lowest at 0.34 mg/g. The One Way ANOVA revealed highly significant differences with a *p*-value of 9.56E-07, highlighting notable diversity. Levene's test (0.2766) indicated some variance, further emphasizing these significant variations. Saponin content demonstrated significant variation among the mushroom samples. *Lentinus tuber-regium* (MCCT P7) displayed the highest content at 3.49 mg DE/g, while *Pleurotus giganteus* (MCCT P6) had the lowest at 0.93 mg DE/g. The One-way ANOVA confirmed highly significant differences with a *p*-value of 6.97E-14, indicating substantial diversity. Levene's test (0.05452) indicated variance, further emphasizing these significant variations. The present findings revealed differences in lycopene and β-carotene content among all mushroom samples. Specifically, *Lentinus tigrinus* (MCCT P2) exhibited the highest lycopene content at 1.67 mg/g, while *Pleurotus ostreatus* (MCCT P4) had the lowest at 0.024 mg/g. The One Way ANOVA underscored highly significant differences in lycopene content with a *p*-value of 1.39E-08. Levene's test suggested homogeneity in variance (0.02513), meaning that the variance in lycopene content was relatively consistent across the samples. Similarly, the highest β-carotene content (0.320 mg/g) was found in case of *Lentinus tuber-regium* (MCCT P7), while *Pleurotus ostreatus* (MCCT P4) exhibited the lowest amount (0.093 mg/g). The One-way ANOVA confirmed significant differences in β-carotene content with a *p*-value

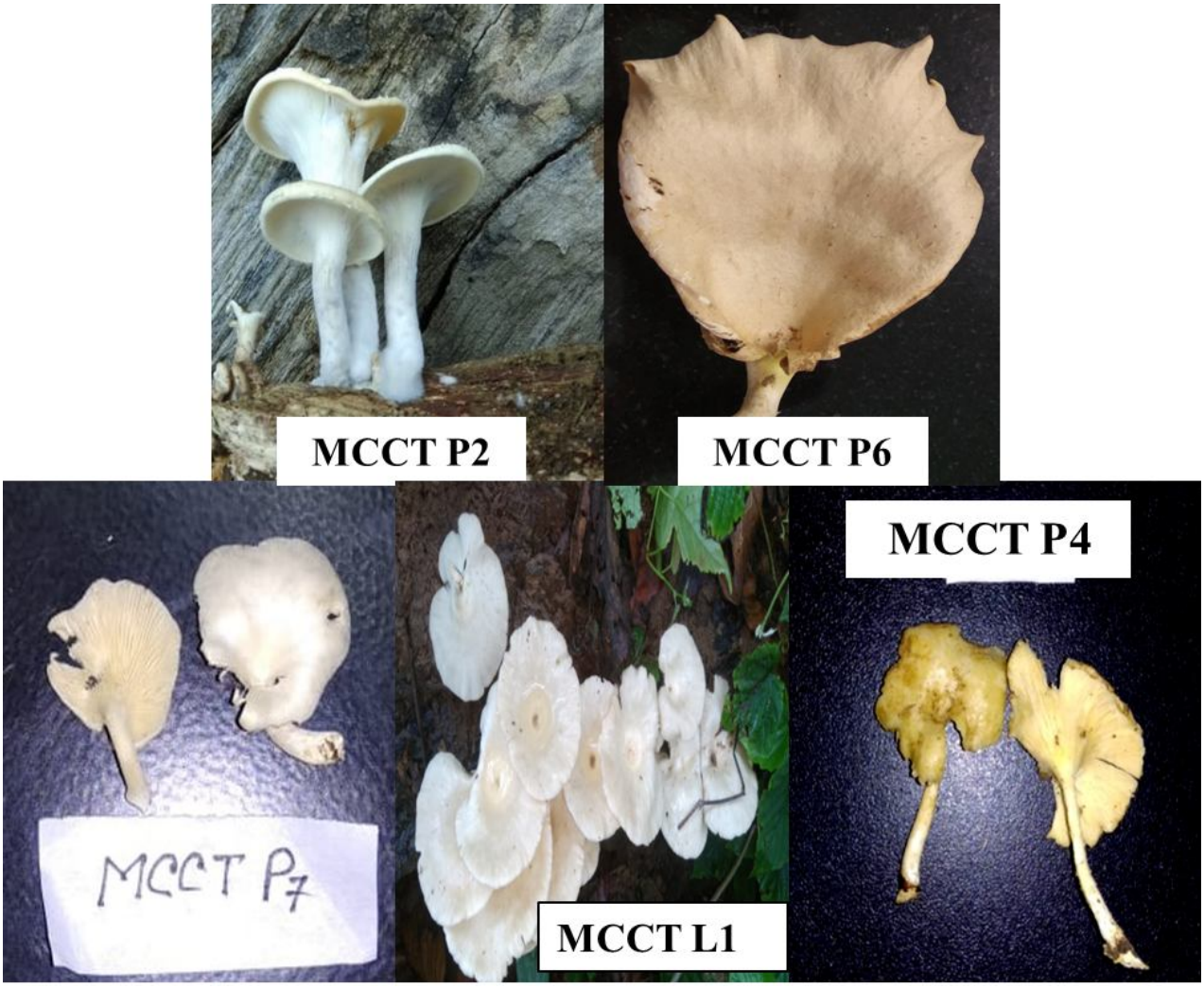

Fig. 1: Collected mushroom samples.
MCCT P2: *Lentinus tigrinus*; MCCT P6: *Pleurotus giganteus*; MCCT P7: *Lentinus tuber-regium*; MCCT L1: *Lentinus sajor-caju*; MCCT P4; *Pleurotus ostreatrus*

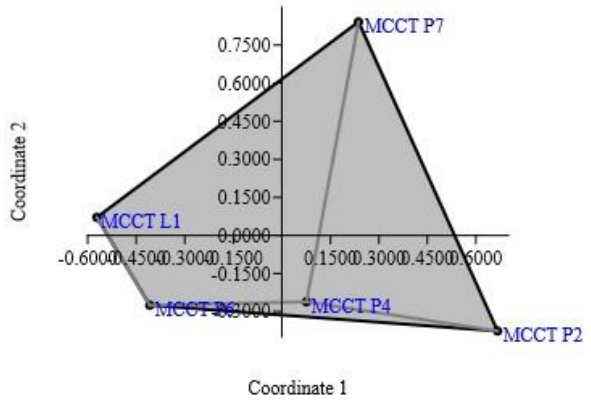

Fig. 2: Principal coordinate analysis (PCoA) of different phytochemical properties presents in the collected mushroom samples *Lentinus tigrinus* (MCCT P2), *Pleurotus ostreatus* (MCCT P4), *Pleurotus giganteus* (MCCT P6), *Lentinus tuber-regium* (MCCT P7), and *Lentinus sajor-caju* (MCCT L1).

of 1.40E-06. Levene's test indicated homogeneity in variance (0.00312), signifying relatively consistent variance in β-carotene content across the samples.

In the study, the ascorbic acid content was found to vary among the mushroom samples, ranging from 0.33 mg/g (*Lentinus tigrinus*, MCCT P2) to 2.33 mg/g (*Lentinus sajor-caju*, MCCT L1). Significant differences were observed (*p*-value: 0.0038), with some variance noted (0.0918) across the samples. The present findings confirm that there was variation in ascorbic acid content among the mushroom samples.

Principal Coordinates Analysis (PCoA) was performed on the phytochemical data of five mushroom samples. The PCoA plot displayed

(Fig. 2) a clear separation of samples along the first two principal coordinates. Notably, *Pleurotus giganteus* (MCCT P6) and *Lentinus sajor-caju* (MCCT L1) formed distinct clusters, indicating their unique phytochemical profiles. Conversely, *Pleurotus ostreatus* (MCCT P4), *Lentinus tigrinus* (MCCT P2) and *Lentinus tuber-regium* (MCCT P7) shared a common cluster, suggesting similarity in their phytochemical composition. These results underscore the significant variations in phytochemical constituents among the studied mushroom species, potentially reflecting differences in nutritional and medicinal properties.

Mushrooms, as important macro-fungi, serve multiple purposes, including food, medicine, bio-control agents, and as a source of bioactive compounds used in the pharmaceutical industry, primarily due to their therapeutic benefits (Okwu, 2001; Lee and Hong, 2011). They possess a wide array of secondary metabolites, including phenols, flavonoids, saponins, alkaloids, and tannins, with variations in their quantities. The presence of these secondary metabolites strongly indicates the medicinal importance of mushrooms. In the current study, the phytochemical constituents of various species of *Pleurotus* and *Lentinus* mushrooms were evaluated. Qualitative phytochemical screening of five wild edible mushrooms revealed the presence of flavonoids, saponins, phenol, tannins, terpenoids, alkaloids, ascorbic acid, lycopene and β-carotene, as detailed in Table 1. These findings align with previous reports in the literature (Odangowei *et al*. 2019; Egwim *et al*. 2011).

In the quantitative analysis of phytochemical constituents, it was found that the phenol content was the highest (ranging from 3.33 to 6.8 mg GAE/g) in the examined mushroom samples. This observation is corroborated by various researchers studying different *Pleurotus* and *Lentinus* species, including Yim *et al*. (2010), Egwim *et al*. (2011), Wandati *et al*. (2013), Unekwu *et al.* (2014), and Yildiz *et al*. (2017). Previous studies have demonstrated that phenol has the potential to prevent damage to vital organs such as the liver, lungs, and kidneys. The phenolic component is the primary contributor to its antioxidant activity, as noted by Jayakumar *et al*. (2009), Elsayed *et al*. (2014), and Singh *et al*. (2015). Furthermore, phenol can serve as a reducing agent for hydrogen donors and as a quencher for singlet oxygen, in addition to its potential metal-chelating properties, as highlighted by Rahimah *et al*. (2019) and Boonsong *et al*. (2016).

Flavonoids represent one of the most diverse groups of natural compounds known for their wide range of chemical and biological activities, including scavenging free radicals, antiallergenic effects, antiviral properties, anti-inflammatory actions, and vasodilation (Pereira *et al*., 2009; Parajuli *et al*., 2012; Madhavi *et al*., 2014). Consequently, the extracts obtained from the mushrooms under investigation hold promise as potential alternatives for the treatment of diseases linked to excessive free radical generation and associated damage.

The current study revealed that *Lentinus tuber-regium* and *Pleurotus ostreatus* contained notably high levels of flavonoids. These results are consistent with the findings of Ogidi *et al.* (2021), where a similar flavonoid content of 0.05± 0.01% was reported, although the flavonoid content obtained in this research was considerably lower compared to other related studies conducted by Abugri and McElhenney (2013) and Unekwu *et al*. (2014).

When comparing our data with existing literature, several interesting insights emerge. Alkaloid content in mushrooms can vary widely, influenced by factors such as species, growth conditions, and geographical location. Our findings indicate that *Lentinus tuber-regium* (MCCT P7) exhibited a notably high alkaloid content at 1.83 mg/g, while *Pleurotus* giganteus (MCCT P6) displayed a lower content at 1.57 mg/g. In line with our results, previous research conducted by Ogbomida *et al*. (2018) reported similar trends, supporting the notion that *Lentinus tuber-regium* tends to have higher alkaloid levels. However, it's worth noting that our specific values differ from their findings, suggesting potential strain-specific or environmental variations. Conversely, our observation of *Pleurotus giganteus* having a relatively low alkaloid content aligns with earlier research conducted by Ogidi *et al*. (2021), which

adds reliability to our findings in this regard. These variations underscore the complexity of alkaloid profiles in mushrooms and emphasize the need for further investigation into the factors that influence alkaloid production.

Unekwu *et al*. (2014) demonstrated that two Nigerian species of *Pleurotus*, namely *Pleurotus ostreatus* and *Pleurotus pulmonarius*, exhibited a considerably higher tannin content ranging from 88.91 to 146.30 mg/g, which differed significantly from our present findings. Dandapat and Sinha (2015) revealed that *Pleurotus tuber-regium* contained 3.21± 0.30mg/100g of tannin content. However, our research findings showed a significantly higher amount of tannin content in two experimental samples of *Pleurotus*. Ogidi *et al*. (2021) reported that *Pleurotus ostreatus* contains 0.04% tannin and 0.06% terpenoid whereas our study observed that 0.19 % of tannin and 0.030 terpenoid content in *P. ostreatus*. There is no available data on the tannin and terpenoid content for *Lentinus tuber-regium* and *Pleurotus giganteus* in existing literature.

In our study, *Lentinus tuber-regium* (MCCT P7) exhibited the highest saponin content at 3.49 mg DE/g, while *Pleurotus giganteus* (MCCT P6) had the lowest content at 0.93 mg DE/g (Zhang *et al*. 2017). This substantial difference in saponin content is consistent with previous research findings. Studies by Zhang *et al*. (2017) reported varying saponin levels among different mushroom species, with values ranging from 0.8 mg DE/g to 4.5 mg DE/g. Similarly, Gupta *et al*. (2018) found diverse saponin content in edible mushrooms, with some species containing as little as 0.5 mg DE/g and others reaching up to 2.0 mg DE/g. These variations in saponin content highlight the species-specific nature of these bioactive compounds within mushrooms. Reis *et al*. (2011) reported that *Lentinus tigrinus* contained a significant amount of ascorbic acid, measuring 248.13 mg/100g, while β-carotene and lycopene levels were almost negligible, which contrasts with our current findings. Similarly, Hussein *et al*. (2015) found that *Lentinus sajor-caju* had 6.39 mg/100g of β-carotene and 3.10 mg/100g of lycopene, which slightly differs from our results. In contrast, Okunlola *et al*. (2021) discovered that *Pleurotus ostreatus* contained 0.37mg/100g of lycopene, 356.67mg/100g of β-Carotene, and 13.93mg/100g of ascorbic acid. Our findings, on the other hand, indicate higher levels of lycopene (0.024 mg/g), lower levels of β-Carotene (0.093 mg/g), and a substantially higher amount of ascorbic acid (1.33 mg/g) in *Pleurotus ostreatus* compared to their research.Based on the current findings, the mushrooms studied in this research serve as rich sources of bioactive compounds, which play a significant role in promoting health and well-being.

CONCLUSION

The analysis of various phytochemical constituents in five wild mushroom species from Tripura revealed significant variations in their chemical composition. These differences were evident in the phenol, flavonoid, alkaloid, tannin, terpenoid, and saponin content, as well as in the levels of ascorbic acid, β-carotene, and lycopene. The One Way ANOVA and Tukey test results demonstrated that these variations were statistically significant, indicating that the selected mushroom species have distinct phytochemical profiles.

Furthermore, Principal Coordinates Analysis (PCoA) highlighted the clear separation of these mushroom samples based on their phytochemical composition. This separation suggests that different mushroom species offer diverse nutritional and medicinal properties, making them valuable resources for various applications. Overall, *Lentinus tuber-regium* (MCCT P7) demonstrated higher values for several phytochemical constituents, indicating its potential as a rich source of these bioactive compounds.

It is worth highlighting that this study marks a significant milestone as the first report on the quantitative phytochemical estimation of *Pleurotus giganteus*. This discovery opens new horizons for understanding and harnessing the unique chemical compounds within this mushroom species.

Moreover, these findings emphasize the importance of exploring and understanding wild mushrooms' phytochemical diversity, as they may

contain promising bioactive compounds with potential health benefits and commercial value.

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DECLARATION

Conflict of Interest. The authors declare that there is no conflict of interest.

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