Research Paper

Comparative Phytochemical Profiling and Characterization of *Pleurotus ostreatus*, *Pleurotus florida* & *Agaricus bisporus* Edible Mushrooms of Chhattisgarh, India

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Abstract:

Edible mushrooms are of great value in the global food market. They are known to have amazing nutraceutical importance and possess very high antioxidant, antidiabetic, and antimicrobial properties. Some regions of Chhattisgarh, India, are quite known to be rich in diverse mushroom species consumed and marketed by locals. Understanding their phytochemical diversity is essential for validating traditional claims and exploring potential medicines or nutraceuticals. A Few edible mushrooms, such as *Pleurotus ostreatus*, *Pleurotus florida*, and *Agaricus bisporus*, are considered in this study. To date, there are very few scientific comparative studies of the phytochemical composition of *Pleurotus ostreatus*, *Pleurotus florida*, and *Agaricus bisporus* species. Therefore, the present study seeks to comparatively profile the phytochemical composition of these mushroom species, with a special focus on flavonoid abundance, and to characterize their bioactive constituents. Initially, *Pleurotus ostreatus*, *Pleurotus florida*, and *Agaricus bisporus* were cultivated under sustainable conditions in the Bishrampur region of Surajpur District, Chhattisgarh, India. The harvested fruiting bodies were subsequently subjected to solvent extraction using established extraction protocols reported in the literature. To comparatively evaluate the phytochemical composition of these mushroom species, both qualitative and quantitative phytochemical analyses were performed following standard methodologies. Total flavonoid content was quantified using UV–Visible spectrophotometric analysis, while Thin Layer Chromatography (TLC) was employed to verify the presence of flavonoids and other major phytochemical constituents. The results demonstrated that *Pleurotus ostreatus* contained significantly higher concentrations of flavonoids, phenolics, tannins, alkaloids, and other secondary metabolites than *Pleurotus florida* and *Agaricus bisporus.* Among the different solvent extracts evaluated, the methanolic extract of *P. ostreatus* exhibited the highest total flavonoid content and overall phytochemical abundance. Based on these findings, *P. ostreatus* was selected for further investigation, and flavonoids were isolated from its methanolic extract using a standard flavonoid isolation protocol. The isolated flavonoid-rich fraction was subsequently characterized using liquid chromatography–mass spectrometry (LC–MS), enabling the identification and profiling of several potential bioactive metabolites. This comprehensive characterization provided deeper insights into the flavonoid composition and metabolite profile of *P. ostreatus*, highlighting its potential as a valuable source of bioactive phytochemicals. This study demonstrates that *Pleurotus ostreatus* possesses a substantially richer phytochemical profile than the other mushroom species investigated, with its methanolic extract exhibiting the highest flavonoid content and overall phytochemical abundance. These findings highlight *P. ostreatus* as a promising source of bioactive compounds with significant potential for the development of pharmaceutical, nutraceutical, and functional food formulations. Furthermore, the comprehensive phytochemical profiling and metabolite characterization presented in this study provide a valuable baseline for future bioactivity-guided investigations, quality standardization, and the identification of novel mushroom-derived therapeutic compounds. The study also underscores the importance of adopting sustainable cultivation practices and conserving the rich mushroom biodiversity of Chhattisgarh, India, thereby promoting the sustainable utilization of indigenous fungal resources while supporting regional bioeconomy and natural product-based drug discovery.

Keywords: Chhattisgarh, Medicinal, Nutraceutical, *Pleurotus ostreatus*, Phytochemical, Sustainable

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1. Introduction

1.1 Edible Mushrooms and Their Nutritive Values & Economic Importance

Edible mushrooms, the first ever documented fleshy fungi, exist in both tropical and moderate regions and differ extensively in shape, structure, and color. They constitute saprophytic and symbiotic species of significant environmental and nutritive importance related primarily to the *Ascomycotina* and *Basidiomycotina* genera (Rizzo, 2021). Renowned for their therapeutic and environmental roles, mushrooms have a broad spectrum of biological activities, including antimicrobial, antioxidant, anti-inflammatory, hepatoprotective, anticancer effects, etc., which have led to growing scientific and pharmaceutical interest owing to their diverse bioactive compounds (Katoch, 2023). While many edible species grow naturally, some edible mushrooms in Chhattisgarh are grown on a commercial scale, such as *Pleurotus ostreatus*, *Pleurotus florida,* and *Agaricus bisporus*. A few are also known to have potential benefits such as antioxidant, anti-inflammatory, and antidiabetic properties, etc., and can be considered for consumption due to their health benefits. Common mushroom cultivation includes selection of suitable species, preparation and sterilization of substrates, spawning, incubation, and harvesting under controlled environments, while post-harvesting processing reduces spoilage and improves marketability (Anusiya et al., 2021).

Mushrooms are globally recognized for their distinctive properties and wide range of potential uses in meals, nutritional value, and therapeutics. In developing countries that prefer cereal-based diets, mushrooms serve as an important additional food source and dietary supplement (Kaliyaperumal, 2019). Mushrooms are traditionally known for promoting health and longevity and are now receiving growing scientific attention as functional foods. Edible mushrooms contain several important bioactive compounds, such as terpenes, polysaccharides, glycoproteins, phenolic compounds, and peptides, which make them valuable for both nutrition and medical research (Losoya-Sifuentes, 2025).

Mushrooms have a major economic role in promoting agricultural sustainability and protecting the environment, in addition to their immediate use in numerous sectors. The cultivation of mushrooms can provide the development of employment opportunities for tribal people, and small-scale farmers and entrepreneurs could promote rural development and food security. The mushroom's value in the global economy is expected to increase as research into its nutritional, therapeutic, and ecological benefits continues. The mushroom sub-sector has challenges such as a lack of knowledge and abilities among mushroom farmers, as well as a lack of facilities. Implementing environmentally friendly production methods improves market connections and adds value throughout the production (Jain, 2024).

1.2 Chhattisgarh: A Growing Market for Culturing Edible Mushrooms

Chhattisgarh is one among those states with a high mushroom biodiversity; studies from the Bastar Plateau, northern hill zones, and rural areas have discovered several species, including edible, medicinal, poisonous, and mycorrhizal varieties (Kumar, 2022). The study found around 83 different mushroom species growing in forests of Chhattisgarh. Based on their utility, around 35-40% of the identified species were edible, 10-15% medicinal, and the other 45-55% were non-edible species. Common edible mushrooms discovered in the region were *Termitomyces spp.*, *Rhizopogon spp.*, *Calocybe indica*, *Pleurotus spp.*, *Volvariella volvacea*, and *Hypsizygus ulmarius*. Ecologically, the mushrooms primarily functioned as saprophytic decomposers, mycorrhizal symbionts, and nutrient recyclers, providing important contributions to forest ecosystem stability (Singh et al., 2020).

Chhattisgarh's agricultural environment supports a diverse mushroom ecosystem, which includes both wild edible and cultivated species. Tribal people in Chhattisgarh, including the Surguja, Raigarh, and Bastar districts, are dependent on wild mushrooms seasonally during monsoon for consumption and income generation. In this region, tribal people collect various kinds of wild mushrooms from forest regions for both their own consumption and sale, including *Termitomyces heimii*, *Astraeus hygrometricus*, *Russula sp.,* etc. Despite the state's rich natural resources, commercial agriculture remains limited, highlighting significant potential for boosting cultivation technology and market connections to improve both food security and rural livelihoods in Chhattisgarh (Thakur & Singh, 2021).

1.3 Potential Uses of Edible Mushroom Varieties of Chhattisgarh

Pleurotus ostreatus, *Pleurotus florida*, and *Agaricus bisporus* are among the most widely cultivated edible mushrooms and are recognized not only for their nutritional value but also for their diverse health-promoting properties. The present study investigates the qualitative and quantitative phytochemical composition of these traditionally cultivated mushroom species to establish a scientific

basis for their potential therapeutic applications. Extracts prepared using different solvents were evaluated to compare their phytochemical profiles, with particular emphasis on bioactive constituents such as flavonoids and other secondary metabolites known to contribute to pharmacological activities (Debnath et al., 2019). These phytochemicals have been associated with a wide range of biological effects, including antioxidant, antibacterial, antidiabetic, anti-inflammatory, and immunomodulatory properties, thereby highlighting their potential for pharmaceutical and nutraceutical development. Moreover, the comprehensive phytochemical profiling generated in this study provides a valuable resource for future bioactivity-guided investigations, metabolite standardization, and the development of mushroom-derived functional ingredients. As environmentally sustainable functional foods requiring comparatively low agricultural inputs, edible mushrooms also represent an eco-friendly approach to improving nutritional security while promoting the conservation and sustainable utilization of indigenous mushroom biodiversity in Chhattisgarh, India.

Pleurotus and *Agaricus* species play a significant role in enhancing food and nutritional security, improving public health, and supporting sustainable economic development in India. Their remarkable ability to bio converts agricultural and agro-industrial residues into value-added products, including nutrient-rich foods, bioactive compounds, bioenergy, and medicinal metabolites, underscores their ecological and economic importance within a circular bioeconomy. Owing to their rich repertoire of bioactive phytochemicals, these mushrooms have emerged as promising candidates for the development of functional foods, nutraceuticals, and pharmaceutical products. Nevertheless, further investigations are required to elucidate the molecular mechanisms underlying their pharmacological activities, assess their long-term safety and efficacy, and establish standardized protocols for their therapeutic applications. Such studies will not only advance the fields of biotechnology, microbiology, pharmacology, and natural product research but also facilitate the sustainable exploitation and global commercialization of mushroom-derived bioactive compounds, thereby reinforcing the importance of these edible fungi as valuable resources for future healthcare and industrial innovations (Ustun et al., 2018).

1.4 Mushrooms: A Choice as Traditional Herbal Medicines

Mushroom benefits have been used as traditional herbal medicines across the world since early times, including in India, China, and other Asian countries. Mushrooms have a wide range of therapeutic properties. Some of them are *Pleurotus* and *Agaricus* mushroom species, which are widely known for their high medicinal potency. Also have a variety of health-promoting benefits and are currently referred to as functional foods due to their positive impact on human health (Golak-Siwulska et al., 2018). They are widely accepted as therapeutic herbs and have great potential in the herbal and drug development fields. They have several phytochemical substances, including alkaloids, flavonoids, glycosides, and triterpenes, etc., and in this study, we observed a richer potency of flavonoids in *Pleurotus* species than in *Agaricus* species. These biomolecules support the development of functional foods, nutraceuticals, and medicinal products. The rapidly growing global market for novel mushroom-based products highlights its medicinal and economic value. However, further study is needed to improve medicinal benefit and discover new species with therapeutic promise. Integrating traditional herbal knowledge with current drug discovery will assist in developing safe, effective, and long-lasting mushroom-derived treatments (Torres-Martinez et al., 2021).

To assess the potential of selected mushroom species as natural sources of flavonoids in nutraceutical and therapeutic applications. The current study aims to cultivate selected mushroom species. To evaluate and compare the flavonoid content and phytochemical profile of selected flavonoid-rich cultivated mushroom species by using different solvent extractions by UV-visible spectrophotometry and thin-layer chromatography (TLC), using quercetin as the standard. The study shows the bioactive compound profile of *Pleurotus ostreatus*, *Pleurotus florida*, and *Agaricus bisporus*, commonly consumed edible mushroom species. Mushroom species have been effectively cultivated in a controlled environment, and extracts were made using different solvents such as methanol, ethanol, and distilled water to evaluate the efficacy through the maceration process of extraction. The yield % of each extract was recorded to evaluate solvent efficacy, followed by qualitative and quantitative phytochemical screening to detect the presence or absence of essential bioactive compounds such as

alkaloids, flavonoids, phenols, terpenoids, tannins, etc. Further, the Total Flavonoid Content (TFC) was calculated in order to measure therapeutic capability and establish a correlation between phytochemical richness and potential therapeutic efficacy. The comparison study found significant differences between each of the species in terms of phytochemical composition and extract yield, indicating nutritional and therapeutic importance (Chaudhary et al., 2023).

Based on these findings, the mushroom species with greater quantities of bioactive compounds and higher extract production can be further investigated for therapeutic studies, such as antibacterial and antioxidant evaluations, to validate its medicinal potential. This systematic comparison technique provides an effective basis to identify the most potent species for nutraceutical or pharmaceutical development (Sharma et al., 2021). Additionally, the study highlights the socioeconomic importance of mushroom cultivation, identifying oyster and button mushrooms as cost-effective, protein-rich food sources having considerable promise for rural income generation, value-added product development, and sustainable agriculture. The outcomes of this study are expected to promote the use of edible mushrooms as natural sources of bioactive compounds, thus promoting future developments in functional food formulation, natural drug discovery, and community-based mushroom cultivation initiatives aimed at improving health and livelihood sustainability (Chowdhury & Paul, 2020).

2. Material & Methods:

2.1 Mushroom sample cultivation, collection, and preparation

The fruiting bodies of *Pleurotus ostreatus*, *Pleurotus florida*, and *Agaricus bisporus* used in the present study were cultivated under controlled environmental conditions in a home-based indoor cultivation unit located at Jainagar, Bishrampur, Surajpur District, Chhattisgarh, India (23.17° N, 82.99° E). Following harvest, the mushroom specimens were morphologically identified and taxonomically authenticated by Dr. Prashant Kumar Sharma, Scientist (Biotechnology), Biotech Lab Training & Demonstration Centre, Collectorate Campus, Ambikapur, Surguja, Chhattisgarh, India. The authenticated fruiting bodies were subsequently processed for phytochemical extraction and comparative biochemical characterization, ensuring the authenticity and reproducibility of the experimental findings.

2.2 Sample preparation of edible mushrooms

Freshly harvested mushroom fruiting bodies were thoroughly washed with distilled water to remove adhering soil particles, debris, and other surface contaminants. The cleaned samples were subsequently shade-dried at ambient temperature (25–35°C) until a constant weight was achieved to preserve their bioactive constituents. The dried fruiting bodies were then pulverized into a coarse powder using a laboratory grinder, and the resulting powder was stored in sterile, airtight containers under dry conditions to prevent moisture absorption and degradation of phytochemicals. The powdered mushroom samples were subsequently subjected to solvent extraction using three different solvents, following established extraction protocols, to obtain crude extracts for comparative phytochemical and biochemical analyses (Rubina & Aboltins, 2021).

2.3 Extraction of Crude Phytochemicals from Edible Mushrooms

For crude phytochemical extraction, 20 g of powdered fruiting bodies from each mushroom species (*Pleurotus ostreatus*, *Pleurotus florida*, and *Agaricus bisporus*) were extracted separately using ethanol, methanol, and distilled water as extraction solvents. The extraction was performed using the maceration technique, in which the powdered samples were immersed in the respective solvent and intermittently stirred while being maintained at room temperature for an appropriate extraction period to facilitate the efficient release of bioactive phytochemicals. Following extraction, the mixtures were filtered through Whatman No. 1 filter paper to remove insoluble residues. The filtrates were subsequently concentrated and dried in a vacuum oven maintained at 60–65°C until a constant weight was obtained.

The dried crude extracts were weighed to determine the extraction yield and to compare the extraction efficiency of different solvents for *P. ostreatus*, *P. florida*, and *A. bisporus*, as presented in Table 1 (Sharma & Cannoo, 2017; Morosanova et al., 2020). The dried extracts were stored in airtight containers under appropriate storage conditions until further qualitative and quantitative phytochemical analyses. The percentage extraction yield was calculated using the following equation (Masjedi et al., 2022):

$$\text{Extraction Yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of dry mushroom powder}} \times 100$$

2.4 Comparative Qualitative Phytochemical Analysis of Edible Mushrooms

The crude extracts of *Pleurotus ostreatus*, *Pleurotus florida*, and *Agaricus bisporus*, prepared using different extraction solvents, were subjected to comparative qualitative phytochemical analysis following standard protocols. Preliminary phytochemical screening was performed to detect the presence of major classes of bioactive constituents, including flavonoids, phenolic compounds, tannins, alkaloids, saponins, terpenoids, glycosides, steroids, and other secondary metabolites, as summarized in Table 2. The analysis involved a series of established chemical assays, in which the presence of individual phytochemical groups was confirmed based on characteristic colour changes, precipitate formation, or other specific reaction patterns (Pipriya & Tiwari, 2019). Comparative evaluation of the solvent extracts enabled the identification of variations in phytochemical composition among the three mushroom species, providing valuable insights into their relative phytochemical richness and their potential as sources of biologically active compounds. These findings also served as the basis for selecting the most suitable mushroom species and extraction solvent for subsequent quantitative analysis and advanced metabolite characterization.

2.5 Quantitative determination of total flavonoid content (TFC) of edible mushrooms

The total flavonoid content (TFC) of *P. ostreatus*, *P. florida*, and *A. bisporus* mushroom extracts was determined by following the aluminum chloride colorimetric method and using quercetin equivalents (QE) as a standard in different types of solvent extracts. It was observed that there was a significant difference in the amount of flavonoids between the mushroom species and solvents (Table 3). Master stock solutions of the extracts were formulated in methanol, ethanol, and distilled water, while a fresh 2% $AlCl_3$ reagent was prepared and kept in an amber bottle. To create working standards of 2-10 µg/mL, a working standard stock solution of quercetin was diluted and mixed with 2.0 mL of 2% $AlCl_3$ reagent. The reaction mixtures were incubated in the dark at room temperature for 15 min, after which the absorbance was recorded at 430 nm using a UV–Visible spectrophotometer. A quercetin calibration curve was constructed by plotting absorbance against concentration, and the corresponding regression equation ($y = mx + c$) and coefficient of determination (R^2) were used to quantify the flavonoid content of the samples. For analysis, 1.0 mL of each mushroom extract was mixed with 2.0 mL of 2% aluminium chloride ($AlCl_3$) reagent and incubated under identical experimental conditions. The total flavonoid content (TFC) was calculated using the following equation:

$$\text{TFC (mg QE/g)} = \frac{C \times V}{m}$$

where C represents the flavonoid concentration obtained from the quercetin standard calibration curve (mg/mL), V is the volume of the extract (mL), and m is the mass of the dried extract (g). The flavonoid content of each sample was expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g) to facilitate comparative evaluation of the different mushroom extracts (Khan et al., 2018). The quantitative estimation enabled the identification of the extract exhibiting the highest flavonoid concentration, thereby providing a basis for its selection for subsequent isolation and advanced metabolite characterization.

2.6 UV–Visible Spectrophotometric Analysis of Edible Mushroom Extracts

The UV–Visible spectrophotometric analysis of the mushroom extracts was performed to evaluate their absorption characteristics and to identify the presence of flavonoid-associated chromophores. Methanolic, ethanolic, and aqueous extracts of *Pleurotus ostreatus*, *Pleurotus florida*, and *Agaricus bisporus* were scanned over a wavelength range of 200–800 nm using a UV–Visible spectrophotometer, with the respective extraction solvents serving as blanks. The absorbance spectra were recorded, and the characteristic maximum absorption wavelength (λ max) of each extract was determined. Particular attention was given to the absorption region corresponding to flavonoid compounds. The formation of flavonoid–aluminium chloride ($AlCl_3$) complexes was confirmed by the appearance of distinct absorption peaks within the 415–430 nm region, indicating the presence of flavonoid constituents in the mushroom extracts (Li, Li, et al., 2020). Comparative analysis of the absorption spectra enabled the assessment of variations in flavonoid abundance among the three mushroom species and across different extraction solvents, thereby supporting the quantitative flavonoid estimation and the selection of the most promising extract for subsequent phytochemical isolation and LC–MS characterization.

2.7 Thin-Layer Chromatographic (TLC) Analysis of Edible Mushroom Extracts

Thin-layer chromatography (TLC) was employed as a rapid qualitative screening technique to confirm the presence of flavonoids in the mushroom extracts. Pre-coated silica gel plates served as the stationary

phase, while a solvent system comprising chloroform: methanol: formic acid (8.2:1.5:1, v/v/v) was used as the mobile phase (Kagawad, Gharge, et al., 2021). Quercetin was selected as the reference standard owing to its well-established role as a representative flavonoid marker for the identification of polyphenolic compounds.

Aliquots of the mushroom extracts and the quercetin standard were carefully applied onto the TLC plates using fine capillary tubes. The plates were developed in a saturated chromatographic chamber until the solvent front reached an appropriate distance, after which they were removed, air-dried, and examined under ultraviolet (UV) light at 254 nm and 366 nm. The separated constituents were identified by comparing their retardation factor (Rf) values, fluorescence characteristics, and colour patterns with those of the quercetin standard (Puri, 2019). Comparative TLC profiling enabled rapid assessment of flavonoid distribution among the different mushroom species and extraction solvents, thereby providing preliminary evidence for selecting the extract exhibiting the highest flavonoid richness for subsequent detailed chemical characterization.

2.8 Selection of Mushroom Species and Isolation of the Flavonoid-Enriched Fraction

The selection of the most suitable mushroom species for advanced phytochemical characterization was based on a comprehensive comparative evaluation of extraction yield, qualitative phytochemical composition, total flavonoid content (TFC), UV–Visible spectrophotometric analysis, and TLC fingerprinting. Among the investigated species, *Pleurotus ostreatus* consistently exhibited the highest extraction efficiency, superior phytochemical richness, and greater flavonoid content than *Pleurotus florida* and *Agaricus bisporus*. Consequently, *P. ostreatus* was selected for the isolation of a flavonoid-enriched fraction and subsequent LC–MS characterization.

The dried powdered fruiting bodies of *P. ostreatus* were extracted with methanol using the maceration technique. The resulting methanolic extract was subjected to flavonoid fractionation following a modified flavone/flavanol isolation procedure adapted from the method described by Agrawal and Paridhavi (2007). Briefly, the extract was gently heated and cooled before treatment with lead acetate to precipitate tannins and other interfering phenolic constituents. The precipitated impurities were removed by filtration, and the filtrate was subsequently acidified with hydrochloric acid (HCl) to facilitate the liberation of flavonoid aglycones. The acidified solution was extracted with alcohol, and the alcoholic phase containing flavonoid constituents was separated. Further purification was achieved through fractional crystallization to obtain a flavonoid-enriched fraction suitable for downstream analyses.

The purified flavonoid fraction was subsequently characterized using liquid chromatography–mass spectrometry (LC–MS) to generate a comprehensive metabolite profile and identify potential bioactive flavonoid derivatives. This targeted isolation strategy minimized interference from non-flavonoid constituents and enhanced the reliability of metabolite identification, thereby providing a robust chemical basis for future bioactivity-guided studies and the development of mushroom-derived nutraceutical and pharmaceutical formulations (Agrawal & Paridhavi, 2007).

2.9 LC–MS Analysis and Metabolite Identification of *Pleurotus ostreatus*

The flavonoid-enriched methanolic fraction of *Pleurotus ostreatus* was analyzed using liquid chromatography–mass spectrometry (LC–MS) to identify bioactive flavonoids and other secondary metabolites. Chromatographic separation was performed on a Waters Alliance 2695 HPLC system equipped with an AccuCore C18 column (150 × 4.6 mm, 2.6 µm) maintained at 40°C. A 10 µL sample was injected and separated using a binary mobile phase of acetonitrile and water containing acetic acid over a 40 min run (Santosa et al., 2021).

The eluted compounds were detected using a Waters 2998 Photodiode Array (PDA) detector coupled with an electrospray ionization mass spectrometer (ESI–MS) operating in both positive and negative ionization modes over an m/z range of 100–750. Raw data were processed using ProteoWizard and MS-DIAL for peak detection, alignment, and noise reduction. Metabolite identification was performed by comparing the processed spectra with the MassBank of North America (MoNA) LC–MS spectral database, and only high-confidence matches were considered for compound annotation (Santosa et al., 2021).

The LC–MS analysis generated a metabolite fingerprint of the flavonoid-enriched fraction, enabling the identification of several bioactive flavonoids and related metabolites. These findings provide a valuable chemical basis for future bioactivity-guided studies and the development of mushroom-derived nutraceutical and pharmaceutical products.

3. Results:

This study comparatively evaluated the extraction yield, phytochemical composition, total flavonoid content (TFC), UV–Visible spectrophotometric characteristics, and thin-layer chromatography (TLC) profiles of *Pleurotus ostreatus*, *Pleurotus florida*, and *Agaricus bisporus*. Comparative analysis revealed that *Pleurotus ostreatus* exhibited superior extraction efficiency, greater phytochemical richness, and the highest flavonoid content among the investigated species. Based on these findings, *P. ostreatus* was selected for advanced chemical characterization. Subsequently, liquid chromatography–mass spectrometry (LC–MS) analysis was performed to profile and identify its bioactive metabolites, providing a comprehensive understanding of its phytochemical composition. The detailed results of the comparative analyses and LC–MS characterization are presented in the following sections.

3.1 Comparative extraction yields of edible mushrooms

The comparative extraction yields of *Pleurotus ostreatus*, *Pleurotus florida*, and *Agaricus bisporus* obtained using different solvents such as methanol, ethanol, and distilled water were shown in Table 1. Using 20 g of dried mushroom powder (W_1) and 200 ml of each respective solvent, the extraction efficiency was evaluated using the percentage yield obtained from a standard solid to solvent ratio of 1:10 (w/v). The final weight of dried extract (W_2) was obtained after maceration, filtration, and vacuum oven drying. The percentage yield is now calculated using the formula [Yield = Weight of dried extract W_2 / Weight of mushroom powder W_1 × 100]. The results are shown below.

Table 1: Comparative Extraction Yields of edible mushrooms

S.No.	Mushroom Species	Solvent (200 ml)	(W_1)	(W_2)	Yield (%)
1.	***Pleurotus ostreatus***	**Methanol**	20 g	**3.6 g**	**18.0 %**
2.		Ethanol		3.2 g	16.0%
3.		Distilled Water		3.5 g	17.5%
4.	*Pleurotus florida*	Methanol		3.3g	16.5%
5.		Ethanol		2.9 g	14.5%
6.		Distilled Water		3.5 g	17.5%
7.	*Agaricus bisporus*	Methanol		3.2 g	16.0%
8.		Ethanol		3.0 g	15.0%
9.		Distilled Water		3.3 g	16.5%

Key to the table: W_1 = Weight of mushroom powder, W_2 = Weight of dried extract

Yield (%) = Weight of dried extract W_2/ Weight of mushroom powder W_1 × 100

According to the above results the extraction efficiency varied based on solvent polarity and among the species *P. ostreatus* showed highest yield at 18.0 % using methanol, while *P. florida* and *A. bisporus* reached their maximum yields of 17.5 % in distilled water and 16.5 % in methanol respectively. Overall, methanol come out as the most efficient solvent system for maximum crude extract recovery across all the solvents.

3.2 Qualitative phytochemical screening of edible mushrooms

The qualitative phytochemical screening of *Pleurotus ostreatus, Pleurotus florida,* and *Agaricus bisporus* in different solvent systems was carried out by standard biochemical assays for each phytoconstituent. The presence and absence of alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, glycosides, carbohydrates, proteins, steroids, reducing sugars, etc. were obtained for each extract. The comparative phytochemical profiles are presented in Table 2, showing variations in phytochemical compositions among the three mushroom species and extraction solvents.

Table 2: Phytochemicals screened from different species of edible mushrooms

S.No.	Phyto-constituents	Biochemical Tests	*Pleurotus ostreatus*			*Pleurotus florida*			*Agaricus bisporus*		
			ME	E	DW	ME	E	DW	ME	E	DW
1.	Alkaloids	Dragendorff's reagent	+	+	+	+	+	+	-	-	-
2.	Carbohydrates	Molisch	+	+	+	+	+	+	+	+	+
3.	Flavanoids	Alkaline Reagent	+	+	+	+	+	+	-	-	-
4.	Glycosides	Keller- Killani	+	+	+	+	+	+	+	+	+
5.	Phenols	Ferric Chloride Test	+	+	+	+	+	+	+	+	+
6.	Quinone	Conc. Sulphuric Acid	+	+	+	-	+	+	+	+	+
7.	Saponins	Frothing	-	+	+	+	+	+	+	-	-
8.	Steroids	Salkowski	+	+	+	+	+	+	+	+	+
9.	Tannins	Ferric Chloride	+	+	+	+	+	+	+	+	+
10.	Terpenoids	Salkowski	+	+	+	+	+	+	+	+	+

Key to the table: (+) Positive, (-) Negative, ME-Methanol Extract, E-Ethanol Extract, DW-Aqueous Extract

Among the different mushroom species, *Pleurotus ostreatus* and *Pleurotus florida* showed a comparatively richer phytochemical profile than *Agaricus bisporus,* as shown in the above results. Carbohydrates, glycosides, phenols, steroids, tannins, and terpenoids were identified in all methanolic, ethanolic, and aqueous extracts of all three species. Alkaloids and flavonoids were present in all extracts of *Pleurotus* species but were absent in *Agaricus bisporus*. Quinones were detected in all extracts of *P. ostreatus* and *A. bisporus,* whereas the methanolic extract of *P. florida* tested negative. Saponins showed variation in species and solvents, being identified in the ethanolic and aqueous extracts of *P. ostreatus*, all extracts of *P. florida*, and only the methanolic extract of *A. bisporus.* Overall, the results showed that *Pleurotus ostreatus* and *Pleurotus florida* have a richer qualitative phytochemical compared with *A. bisporus,* suggesting that these mushrooms are rich sources of several bioactive compounds.

3.3 Quantitative determination of total flavonoid content (TFC) of edible mushrooms

The total flavonoid content of the mushroom extracts was determined using the $AlCl_3$ colorimetric method with quercetin as the standard reference compound. The quercetin calibration curve (Figure 1) illustrates the excellent linearity, indicated by a high coefficient of determination (R^2), confirming the reliability of the method for flavonoid quantification. The quantified TFC values varied among *Pleurotus ostreatus*, *Pleurotus florida,* and *Agaricus bisporus* mushroom species. Indicating differences in flavonoid content across species and solvents. As shown below in Table 3.

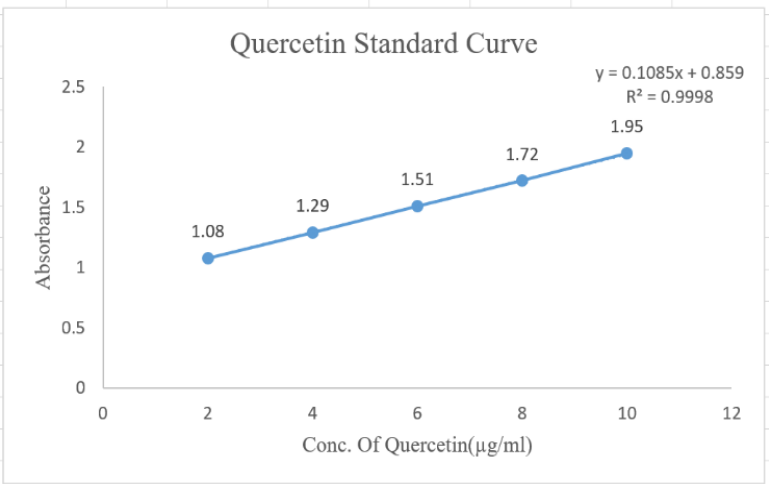

Figure 1: Quercetin Standard Curve Resolution

Table 3: Shows flavonoid content of edible mushrooms in different solvents

Key to the table: ME-Methanol, E-Ethanol, DW-Distilled Water,

Mushroom Sample Extract	Solvent Used	Absorbance (430 nm)			Mean ± SD	Conc. (µg/mL)	Flavanoid (mg QE/g)
Pleurotus ostreatus	**ME**	**2.42**	**2.51**	**2.53**	**2.49±0.06**	**15.03**	**22.54**
	E	1.66	1.69	1.69	1.68±0.02	7.57	11.36
	DW	0.29	0.24	0.24	0.26±0.03	-5.52	-8.28
Pleurotus florida	ME	2.22	2.16	2.31	2.23±0.08	12.64	18.96
	E	1.37	1.24	1.20	1.27±0.09	3.79	5.68
	DW	0.13	0.13	0.12	0.13±0.01	-6.72	-10.08
Agaricus bisporus	ME	0.82	1.0	1.1	0.97±0.14	1.02	1.53
	E	1.17	1.26	1.13	1.19±0.07	3.05	4.58
	DW	0.25	0.22	0.22	0.23±0.02	-5.80	-8.70

1) (-) Negligible Results: Below detection limit,

2) TFC = (C × V) / m, Where, C= Conc. of flavonoid in the Sample, V= Volume of the extract used, M= Mass of the dried extract.

The total flavonoid content varied among the studied mushroom species and extraction solvents. As shown above in Table 3, the methanolic extract of *Pleurotus ostreatus* showed the highest flavonoid content (22.54 mg QE/g), followed by the methanolic extract of *Pleurotus florida* (18.96 mg QE/g). Ethanolic extracts showed moderate flavonoid levels, whereas the aqueous extracts of all three mushroom species showed negligible results. Overall, methanol was the most efficient solvent system for flavonoid extraction in this order: *Pleurotus ostreatus*, *Pleurotus florida*, and *Agaricus bisporus*.

3.4 UV–visible absorption of edible mushrooms

To evaluate the presence of bioactive compounds, UV-visible spectroscopic analysis was performed to identify the absorption peaks of *Pleurotus ostreatus*, *Pleurotus florida,* and *Agaricus bisporus* mushroom extracts using three distinct solvent systems such as methanol, ethanol, and distilled water, as shown in Table 4. The prepared extracts were then subjected to spectrophotometric scanning to detect the distinct absorption bands observed between 260 nm and 280 nm, indicating the presence of phenolic and flavonoid compounds across the sample. Variation in both peak intensities and absorption maxima among the mushroom extracts indicates differences in the phytochemical composition and solvent efficiency. The UV-visible spectrum profiles align with the qualitative phytochemical screening, confirming the presence of phenolic and flavonoid constituents.

Table 4: Shows UV-visible spectroscopy results of edible mushrooms in different solvents.

Mushroom Sample Extract	Solvent Used	Primary λ max (nm)	Absorbance (Abs.)	Secondary Peak (nm)	Absorbance (Abs.)
Pleurotus ostreatus	ME	235	1.25	275	0.35
	E	232	1.91	275	1.00
	DW	238	3.75	278	1.95
Pleurotus florida	ME	235	1.76	275	0.65
	E	N/A	N/A	N/A	N/A
	DW	238	3.52	278	1.45
Agaricus bisporus	ME	235	2.44	275	1.10
	E	232	1.55	275	0.60

	DW	238	3.61	278	1.65

Key to the table: ME-Methanol, E-Ethanol, DW-Distilled Water, λ max (nm): The wavelength of maximum absorption, N/A: Not applicable round off range

The above result in Table 4 shows two distinct absorption zones for the mushroom extracts was observed. The primary bands at 232-238 nm and secondary peaks at 275-278 nm confirm the presence of phenolic and flavonoid constituents, validating the qualitative phytochemical screening. Among the extraction solvents, distilled water showed the highest efficiency, yielding maximum absorbance across all the species, and the aqueous extract of *Pleurotus ostreatus* showed the highest primary (3.75) and secondary (1.95) absorbance. The ethanolic extract of *Pleurotus florida* showed no detectable peaks, indicating its bioactive fractions. These variations in peak intensities and λ max demonstrate that solvent polarity can influence the recovery of any specific phytochemicals from these edible mushrooms. Further, while methanolic extracts showed better results in preliminary analysis, the maximum UV-visible absorbance observed in aqueous extracts suggests that the specific UV absorbing chromophores and highly polar phenolic glycosides in these mushrooms dissolve well in distilled water due to its higher dielectric constant and polarity.

3.5 TLC: Thin layer chromatography of edible mushrooms

The TLC analysis was performed to evaluate and compare the phytochemical profiles of *Pleurotus ostreatus*, *Pleurotus florida,* and *Agaricus bisporus* mushroom extracts. Using quercetin as a standard reference for the identification of flavonoid-like compounds. The mushroom extracts and the standard were spotted onto the silica gel TLC plates and developed using a mobile phase consisting of chloroform: methanol: formic acid (8.2:1.5:1), v/v/v). This solvent system was adapted as a standardized method for the separation of flavonoids in natural extracts that was reported by Kagawad, Gharge, et al. (2021). The total distance travelled by the solvent front was recorded at 5.6 cm. By measuring the distance travelled by each spot, the respective retardation factor Rf values were calculated to determine the presence and diversity of the phytochemical constituents within each mushroom species.

Table 5: Thin layer chromatography (TLC) analysis—Rf values of edible mushrooms

Key to the table: Rf: Retardation factor, Trace: Faint spot observed, ND= Not determined

Mushroom Sample Extract	Component	Distance Travelled by Component (cm)	Distance Travelled by Solvent (cm)	Rf Value
Quercetin (Standard)	Single Spot	5.0	5.6	0.89
P. ostreatus	Component 1 (Lower)	2.2	5.6	0.39
	Component 2 (Upper)	3.5	5.6	0.63
P. florida	Component 1 (Lower)	2.0	5.6	0.36
	Component 2 (Upper)	4.0	5.6	0.71
A. bisporus	Component 1 (Lower)	0.2	5.6	0.04
	Component 2 (Upper)	Trace	5.6	Trace (ND)

The above results show distinct chromatographic profiles for the selected edible mushroom extracts. The quercetin standard reference showed a single spot with a Rf value of 0.89, confirming its purity and suitability for the TLC system. *Pleurotus ostreatus*, *Pleurotus florida*, and *Agaricus bisporus* mushroom species showed two well-distinct spots (Rf = 0.36-0.71), indicating the presence of multiple phytochemical compounds. Whereas *Agaricus bisporus* showed only a faint trace spot, suggesting a comparatively lower abundance of detectable compounds. Although the Rf values of the mushroom extracts did not exactly align with the quercetin standard, the TLC results suggest the presence of flavonoid-like compounds and differences in the phytochemical composition of the

mushroom extracts and show greater phytochemical diversity in the *Pleurotus* species.

3.6 LC–MS analysis of edible mushrooms

The flavonoid-rich methanolic extract of the *Pleurotus ostreatus* mushroom was subjected to LC-MS analysis to identify the metabolites present in the extract. Chromatographic separation was carried out using a reverse-phase C18 column, followed by mass spectrometric detection. The acquired mass spectral data were processed for peak detection, alignment, data refinement, and metabolite identification using spectral database matching based on accurate mass and retention time. The identified metabolites detected in the methanolic extract of *P. ostreatus* are presented below in Table 6.

Table 6: Tentatively identified metabolites in flavonoid-rich *Pleurotus ostreatus* mushroom extract by LC-MS analysis

S.No.	RT (Min)	Phytochemicals	M/F	Chemical Class	m/z
	0.37	Thein-2-yl acetate	C6H6O2S	Sulfur-containing aromatic ester	142.99
	1.39	2,4-Dichoro-cis, cis -muconate	C6H4Cl2O4	Organic acid derivative	210.99
	1.39	Spermidine	C7H19N3	Polyamine	146.15
	1.40	3-Oxododecanoic acid	C12H22O3	Fatty acid derivative	215.19
	1.41	L-Tryptophan amide	C11H14N2O	Amino acid derivative	204.13
	1.44	4-Trimethyl ammoniobutanoate	C7H16NO2	Quaternary ammonium compound	147.15
	1.44	Ochrofluorine A	C29H29N	Alkaloid derivative	439.30
	1.47	Jasmonic acid	C11H16O	Organic acid derivative	165.16
	1.59	L-Ornithine	C5H12N2O2	Amino acid derivative	133.11
	1.59	N-(Cyclohexylmethyl) -N-methylbenzenamine	C14H21N	Aromatic amine	204.17
	1.73	Methyl 2-diazoacetamidobenzoate	C9H6N3O3	Aromatic ester derivative	215.09
	2.11	Catechol	C6H6O2	Phenolic compound	111.07
	2.54	Imepitoin	C9H15N3	Nitrogen-containing heterocycle	166.17
	20.10	Limonene	C10H16	Amino acid derivative	137.14
	27.53	Cyclohexanol	C6H12O	Quinone derivative	101.13
	29.77	o-Anisidine	C7H9NO	Amino acid derivative	124.11
	31.10	5-Valerolactone	C5H8O2	Organic acid derivative	101.03
	32.09	p-Benzoquinone	C6H4O2	Alcohol derivative	109.03
	35.66	Taurine	C2H7NO3S	Monoterpene derivative	126.06

Key to the Table: RT: Retention time, M/F: Molecular formula, m/z: Mass to charge ratio

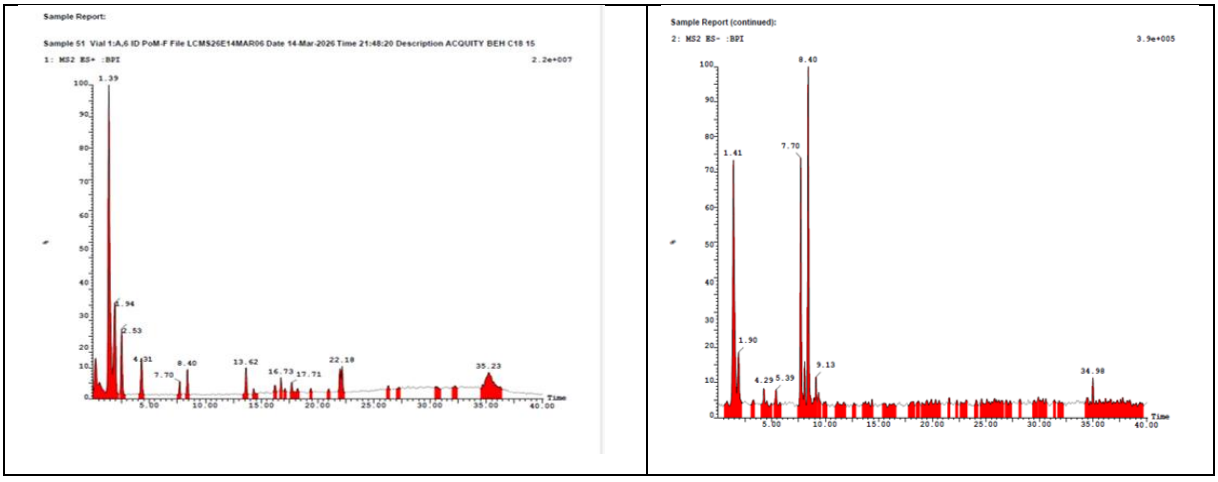

Figure 2: LC–MS chromatograms of flavonoid-rich *Pleurotus ostreatus* mushroom extracts in both a) MS chromatogram recorded in positive electrospray ionization model (MS2 ES+) and b) MS chromatogram recorded in negative electrospray ionization model (MS2 ES$^-$)

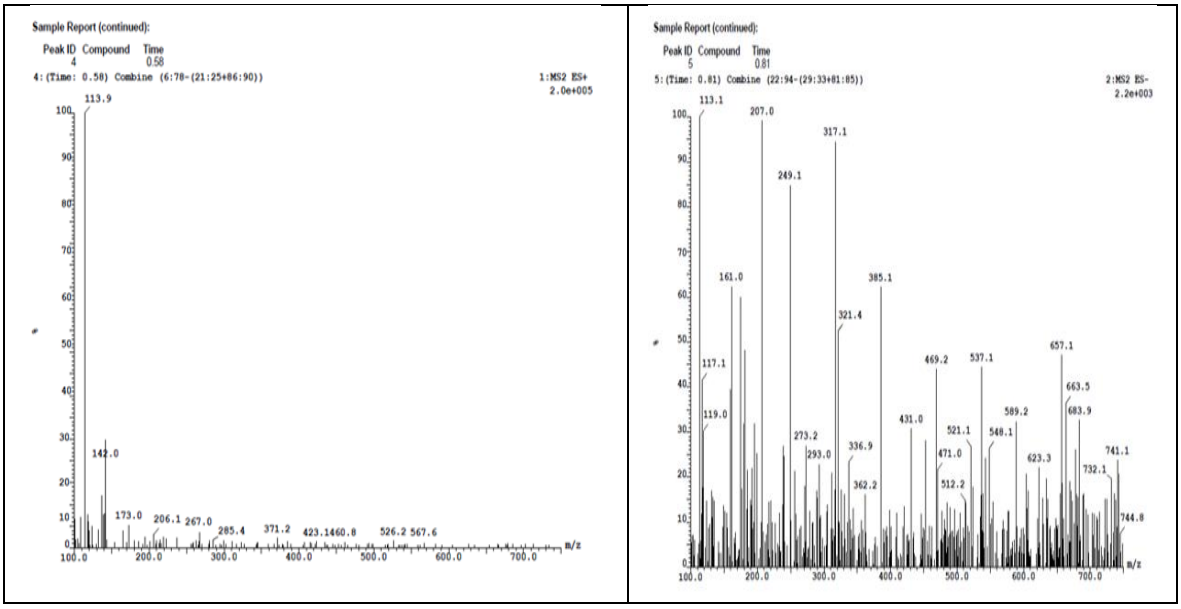

Figure 3: Representative m/z spectra of *Pleurotus ostreatus* phytochemical fractions a) Positive mode (MS2 ES$^+$) at 0.58 min and b) Negative mode (MS2 ES$^-$) at 0.81 min

Among them catechol, jasmonic acid, spermidine, taurine, and L-ornithine were particularly noteworthy. Reported to have potential antioxidant, antimicrobial, anti-inflammatory, and antidiabetic properties. Their identification was based on retention time (RT), molecular formula (M/F), chemical classes, and mass to charge ratio (m/z). The occurrence of these metabolites supports the results obtained from phytochemical screening, TFC, and TLC analysis, confirming the richness of bioactive constituents in the extract.

Overall, among the *Pleurotus ostreatus*, *Pleurotus florida*, and *Agaricus bisporus* mushroom species, *Pleurotus ostreatus* showed higher extraction yield, stronger phytochemical responses, greater total flavonoid content, and higher UV-visible absorbance than the other mushroom species. Methanol was found to be the most effective solvent for extracting flavonoid-rich compounds. TLC analysis further supported these findings, as the methanolic extract of *Pleurotus ostreatus* exhibited Rf values comparable to the quercetin standard, indicating flavonoid-like compounds and other bioactive phenolic constituents.

4. Discussion

Edible mushrooms are globally known from generations not only as nutritional food sources but also as a rich source of medicinal compounds considered functional foods and high-end novel nutraceuticals as reported by Kumar, Sharma, et al. (2021). Three typical mushroom species, such as *Pleurotus ostreatus*, *Pleurotus florida*, and *Agaricus bisporus*, were studied for a comparative analysis of their phytochemical profiles. Although these species of macrofungi are from different taxonomic groups and ecological habitats, they all have an ability to produce beneficial secondary metabolites. This study illustrates that different extraction solvent system polarities affect the isolation of health-promoting compounds through a complete evaluation that includes crude extraction, qualitative screening, total flavonoid content (TFC), UV-visible spectrophotometric profiling, thin-layer chromatography (TLC), and liquid chromatography mass spectrometry (LC-MS).

4.1 Comparative extraction yields of edible mushrooms

The extraction efficiency of bioactive compounds depends on the nature and polarity of the solvent system used, as it determines how well different phytochemical fractions dissolve. In this study, methanol showed the most efficient solvent system across all the three mushrooms. The highest yield percentage was obtained in *Pleurotus ostreatus* (18.0%), then followed by *Pleurotus florida* (16.5%) and *Agaricus bisporus* (16.0%). After methanol, distilled water extracts also showed strong yields (17.5%) for both the *Pleurotus* species, while ethanol resulted in the lowest recovery percentages. This pattern is close to the findings of Raman et al. (2020), their study reported that highly polar organic solvents like methanol disrupt the fungal cell wall

effectively, which allows intracellular compounds to release easily. Further, Vamanu (2019) showed in his studies that hot water extraction helps isolate large structural polysaccharides from organic systems like methanol, which is better for extracting low molecular weight phenolics and free lipophilic metabolites. As per the studies of Kalac (2013), it was reported that ethanol showed a lower extraction yield. This can be because of its lower dielectric constant compared to methanol and water because it limits its ability to dissolve highly polar glycoconjugates.

4.2 Qualitative phytochemical screening of edible mushrooms

The qualitative phytochemical screening study showed distinct chemical variations among *Pleurotus ostreatus*, *Pleurotus florida*, and *Agaricus bisporus* mushroom species. The key constituents like carbohydrates, glycosides, phenols, steroids, tannins, and terpenoids were found in all extracts, alkaloids and flavonoids were found in *Pleurotus* species such as *Pleurotus ostreatus* and *Pleurotus florida* and were absent in *Agaricus bisporus.* This variation aligns with the study of Arbaayah and Umi (2013) in which they proved that the genus *Pleurotus* is biologically and genetically prone to produce better nitrogenous and polymeric secondary metabolites than *Agaricus* species. As per the study of Suseem et al. (2018), the solvent-dependent analysis of saponins was present in ethanolic and aqueous extracts of *P. ostreatus* but absent in methanolic extracts, showing solvent polarity effects on the solubility of cell membrane components. Additionally, in the study of Chenegre (2021), quinones present in *P. ostreatus* and *A. bisporus* but not in the methanolic extract of *P. florida* highlight interspecies variations in active components.

4.3 Quantitative determination of total flavonoid content (TFC) of edible mushrooms

The quantitative analysis of TFC using the standard $AlCl_3$ colorimetric method confirmed the qualitative screening results. The methanolic extract of *Pleurotus ostreatus* showed the highest flavonoid content (22.54 mg QE/g), followed by the methanolic extract of *Pleurotus florida* (18.96 mg QE/g). In comparison to the *Pleurotus* species, *Agaricus bisporus* showed minimal results. The aqueous extracts of all the three species showed negligible results below the detection level. The results of my study are supported by Gil-Ramírez et al. (2016), which states that standard methanol is optimized for extracting mushroom flavonoids due to its hydrophobic-hydrophilic nature, which supports the high concentration of flavonoids in methanolic extract *P. ostreatus.* As confirmed by Effiong et al. (2024), the aqueous extract showed lower flavonoid recovery than organic extracts, indicating that solvent polarity influences flavonoid extraction and the organic media can provide greater efficiency for flavonoid recovery. Further, in the study of Kozarski et al. (2015), moderate flavonoid levels obtained in an ethanolic solvent system, as shown in the results (11.36 mg QE/g for *P. ostreatus*), suggest that ethanol can serve as a safe and next-best extraction solvent even if it shows slightly lower efficiency compared to methanol.

4.4 UV-visible absorption of edible mushrooms

The UV-visible absorption analysis is known to be a reliable tool to identify the light-absorbing structures in the extracts. The primary absorption bands between 232 and 238 nm and secondary distinct peaks observed at 275-278 nm provide clear spectroscopic proof of aromatic phenolic rings and flavonoid structures. *P.ostreatus* generated the highest absolute absorbance values (primary: 3.75, secondary: 1.95), particularly in distilled water extracts, while organic fractions are dominated by total yield and TFC metrics. A physical chemistry concept that was supported by Gan et al. (2013) explained that this result is obtained by the high dielectric constant of water, which easily dissolves highly polar phenolic acids and water-soluble glycosylated compounds that have intense UV absorption. Aligning with the solvent limits discussed by Prasad et al. (2015), the lack of detectable peaks in the ethanolic extract of *Pleurotus florida* illustrates either a very low concentration of free aromatic rings or a basic extraction failure that is solvent-specific. According to the study by Mabry et al. (2012), the consistent presence of the 275-278 nm absorption bands across the other samples successfully validates the qualitative screening matching the standard flavonoid identification profiles.

4.5 Thin Layer Chromatography (TLC) of edible mushrooms

Thin-layer chromatographic separation is done for physical proof of the diversity of compounds present in the mushroom extracts. For the successful separation of complex crude extracts, the mobile phase system used in this study is chloroform: methanol: formic acid (8.2:1.5:1), v/v/v), which highlights the greater phytochemical profile of the *Pleurotus* samples through two distinct and clear spots (Rf = 0.36-0.71) compared to the faint trace spot observed for *Agaricus bisporus.* The separation

of bands at intermediate retardation factors (Rf = 0.39 and 0.63 for *P.ostreatus*) points directly to the presence of moderately polar flavonoid derivatives. These spots did not perfectly match the pure quercetin standard (Rf= 0.89), this chromatographic study align with the mushroom flavonoid separation studies published by Kagawad, Kadam, et al. (2021), where migration delay is very common in natural extracts where flavonoids exist as complex glycosides or organic acid conjugates which increases their polarity and lowers their Rf value. In the study of Marston (2011) and Sherma (2016), the interactions on the silica gel layer typically cause natural derivatives to move slower than pure standards.

4.6 LC-MS Analysis of edible mushrooms

Methanolic extract of *Pleurotus ostreatus* was chosen for metabolite profiling through high-resolution LC-MS analysis because of its superior results across the preliminary screening, spectrophotometric and chromatographic analyses. The mass spectral analysis successfully identified a diversity of biologically active compounds such as catechol, jasmonic acid, spermidine, taurine, and L-ornithine, which are particularly noteworthy. Similar classes of metabolites have been reported in edible mushrooms by Ren et al. (2023), who demonstrated that LC-MS is an effective approach for identifying bioactive compounds responsible for the therapeutic properties of mushrooms. The identification of catechol supports the high quantitative TFC and intense UV-visible phenolic absorption bands observed in our study; catechol is widely recognised as a highly efficient free-radical scavenger, a bioactivity highlighted by Robaszkiewicz et al. (2010). Additionally, catechol has been documented to possess antimicrobial properties through reactive oxygen species (ROS) generation, as well as potent antidiabetic efficacy through strong alpha-glucosidase and alpha-amylase inhibition documented by Li, Zhang, et al. (2021) and El-Sawi et al. (2023).

Spermidine and L-ornithine represent important polyamine and amino acid derivatives, respectively. Both of the compounds play an important role in cellular longevity pathways, neuroprotection, and immune regulation, as shown in the medicinal mushroom literature study by Kalac (2014). Notably, spermidine contributes to antidiabetic pathways by mitigating high glucose-induced oxidative damage and improving insulin signalling, alongside exhibiting intrinsic antibacterial properties against pathogenic bacteria, as shown in Kazakova et al. (2019) and Park et al. (2023). Further, taurine is a finding which gives strong therapeutic links to cardiovascular health and anti-inflammatory pathways Huxtable (2012). Taurine is widely used and known for its antidiabetic potential by modulating glycaemic control, enhancing insulin sensitivity and protecting pancreatic beta cells from oxidative stress, as documented by Ito et al. (2012). Further, an observation supported by Eng et al. (2021) that jasmonic acid is produced by fungi and associated with fungal physiological responses suggests the activation of complex environmental adaptation pathways within the mushroom mycelium. Heleno et al. (2021), reported that edible mushrooms are rich in phenolic compounds and other secondary metabolites with significant health promoting properties. These observations are consistent with metabolite profile obtained in the present study. Their detection supports the phytochemical analyses performed in the present study and indicates that the biological activity of *P.ostreatus* may result from the combined action of multiple metabolites. Overall, the clear validation of these diverse pharmacologically active compounds through LC-MS analysis perfectly aligns with our qualitative, spectrophotometric, and thin-layer chromatographic data, confirming *Pleurotus ostreatus* has potential for development into antimicrobial, antidiabetic and novel therapeutic or nutraceutical formulations.

The findings of the present study are consistent with previous reports by Rathore et al. (2017), and Bhagarathi et al. (2023), which highlighted the rich phytochemical composition and therapeutic potential of *Pleurotus ostreatus,* its methanolic extract possess a richer phytochemical profile compared with *Pleurotus florida*, and *Agaricus bisporus*. These findings provide a scientific basis for the future development of mushroom-based nutraceuticals and functional food products. However, additional *in vitro* and *in vivo* and pharmacological studies provide a scientific foundation for evaluating their potential use in the development of natural formulations for health promotion and disease management such as diabetes mellitus etc.

5. Conclusion

The diverse agro-climatic conditions of Chhattisgarh, India, provide an ideal environment for the cultivation of edible mushrooms that represent valuable natural resources with significant nutritional and therapeutic potential. Rich in high-quality proteins, vitamins, minerals, dietary fiber,

and a wide spectrum of bioactive phytochemicals, while being naturally low in fat, edible mushrooms have emerged as important functional foods with promising applications in the nutraceutical and pharmaceutical industries. In the present study, a comprehensive comparative phytochemical investigation of three commercially important edible mushroom species, *Pleurotus ostreatus*, *Pleurotus florida*, and *Agaricus bisporus*, was carried out using extraction yield analysis, qualitative phytochemical screening, total flavonoid content (TFC) estimation, UV–Visible spectrophotometry, thin-layer chromatography (TLC), and high-resolution liquid chromatography–mass spectrometry (LC–MS). This integrated analytical approach enabled systematic evaluation of their phytochemical diversity and metabolite composition, providing a robust scientific basis for assessing their therapeutic potential.

The results demonstrated that solvent polarity significantly influenced extraction efficiency, with methanol emerging as the most effective extraction solvent for all three mushroom species. The highest extraction yield was obtained from *Pleurotus ostreatus* (18.0%), followed by *Pleurotus florida* (16.5%) and *Agaricus bisporus* (16.0%). Comparative phytochemical screening further established that the *Pleurotus* species possessed a richer and more diverse phytochemical composition than *A. bisporus*. Among the investigated extracts, the methanolic extract of *P. ostreatus* exhibited the highest total flavonoid content (22.54 mg QE/g), corroborated by characteristic UV–Visible absorption bands (232–238 nm and 275–278 nm) and distinct TLC fingerprints (Rf = 0.36–0.71), confirming the abundance of flavonoids and other phenolic constituents.

Advanced LC–MS analysis of the flavonoid-enriched methanolic extract of *P. ostreatus* successfully identified several biologically relevant metabolites, including catechol, jasmonic acid, spermidine, taurine, and L-ornithine, providing comprehensive metabolite fingerprints of the selected species. The detection of these compounds offers a plausible chemical basis for the reported antioxidant, antimicrobial, antidiabetic, anti-inflammatory, neuroprotective, and other health-promoting properties of *P. ostreatus*. The integration of classical phytochemical screening with high-resolution metabolite profiling represents an important contribution toward the chemical characterization of edible mushrooms cultivated in Chhattisgarh and provides a valuable reference for future natural product research.

Overall, the present investigation establishes *Pleurotus ostreatus* as the most phytochemically rich and pharmacologically promising mushroom among the species evaluated. Its flavonoid-rich methanolic extract demonstrates considerable potential as a sustainable source of natural bioactive compounds for the development of functional foods, nutraceuticals, and pharmaceutical formulations. Furthermore, this study provides one of the first comprehensive comparative phytochemical and LC–MS-based metabolite profiles of these cultivated edible mushrooms from the Chhattisgarh region, thereby contributing to the scientific valorization of indigenous mushroom resources. Future investigations should focus on the isolation and structural elucidation of individual bioactive constituents, mechanistic studies, and comprehensive in vitro, in vivo, and clinical evaluations to validate their therapeutic efficacy, establish quality standards, and facilitate the development of commercially viable mushroom-derived health products.

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