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Evaluating Bioactive Polysaccharides in Marketed Mushrooms: Towards Natural Alternatives to Synthetic Drug

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ABSTRACT

Bioactive compounds in mushrooms are recognized for their therapeutic potential and are seen as promising alternatives to synthetic medications, which these synthetic medications may lead to adverse health effects. Commercially available mushrooms are particularly noteworthy for their potential to produce extracellular and intracellular polysaccharides. This research investigates the bioactive compounds in various commercial mushrooms to assess their potential as safer alternatives to synthetic drugs. The study aims to extract and analyze bioactive compounds from different commercial mushrooms, addressing the need for safer medicinal alternatives. Five mushroom types—Monkey mushroom, Abalone mushroom, Yellow oyster mushroom, Enoki mushroom and Shiitake mushroom, were selected for analysis. Tests were conducted to assess carbohydrate content, antioxidant capacity, and the presence of polysaccharides and phenolics using the Phenol Sulphuric Acid Assay. Phytochemical screenings were also performed to determine the overall phytoconstituents. The Monkey mushroom showed the highest intracellular polysaccharide content, while the Yellow oyster mushroom had the lowest. Additionally, the Monkey mushroom exhibited the highest phenolic content, whereas the Enoki mushroom had the lowest. These results suggest that commercial mushrooms, especially the Monkey mushroom, contain significant levels of bioactive compounds, supporting their potential use as safer alternatives to synthetic medications. Future research should explore the mechanisms underlying the bioactive properties of these compounds and their clinical applications, while expanding the scope to include a broader range of mushroom species and extraction methods.

1. Introduction

Mushrooms have long been recognized for their medicinal properties, with numerous studies demonstrating their potential to serve as a natural source of bioactive compounds [1]. These compounds, including polysaccharides and phenolic substances, have been shown to possess anti-

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inflammatory, antimicrobial and antioxidant activities [2]. In the search for alternative therapies to synthetic medications, the evaluation of bioactive compounds in commercially available mushroom species, such as monkey mushroom, abalone mushroom, yellow oyster mushroom, enoki and shiitake, is of great interest [1–3].

Mushrooms have been part of human diets since ancient times; the ancient Greeks thought they bestowed strength upon warriors, while the Romans referred to them as the "Food of the Gods." Throughout the centuries, Chinese culture has valued mushrooms as a health food, considering them an "elixir of life." They have played a significant role in human history and have been appreciated by many of the world's major civilizations [4].

Given their long-standing use in traditional medicine across various cultures, mushrooms are now increasingly being recognized for their potential as modern therapeutic agents, particularly as alternatives to synthetic medications. Given the adverse effects associated with synthetic medications, such as liver toxicity and drug resistance, there is a growing need to explore safer, natural alternatives, with mushrooms emerging as a promising candidate. Furthermore, Radrigán *et al.,* [5] highlighted the potential of natural compounds derived from plants and fungi as effective and safer alternatives to synthetic medications.

Bioactive polysaccharides from fungi, specifically mushrooms, have gained attention because of their diverse therapeutic properties. These compounds exhibit various biological activities, including antitumor, antioxidant, anticoagulant, antidiabetic, and immunomodulatory effects [6]. Recent research has highlighted their neuroprotective potential against oxidative stress, apoptosis, and neuroinflammation, suggesting their application in treating neurodegenerative diseases [7].

Phenolic compounds on the other hand, are widely distributed in plants and possess significant benefits. Phenolic compounds are associated with various health benefits, including improved insulin sensitivity and enhanced glucose uptake, as indicated by studies on their interactions with other bioactive molecules like leucine and resveratrol. Phenolic compounds are known for their antioxidant properties that can help reduce oxidative stress and inflammation in the body [8].

In this study, we aimed to evaluate the bioactive polysaccharide and phenolic contents, as well as the antioxidant activity, of aqueous extracts obtained from five commercially available mushroom species: abalone mushroom, enoki mushroom, monkey mushroom, shiitake mushroom, and yellow oyster mushroom. Utilizing standardized extraction techniques and analytical assays, this study provides valuable findings that could contribute to the search for natural alternatives to synthetic medications.

Despite the known health benefits of mushrooms, comparative analyses of bioactive polysaccharide and phenolic contents across different commercially available mushroom species, particularly using aqueous extracts, remain scarce. Prior studies have predominantly focused on the bioactive compounds of mushrooms using solvent-based extraction methods. This study contributes new insights by evaluating the antioxidant activity of aqueous extracts from five commercially available mushroom species, an area less explored in current literature.

Current treatments for neurodegenerative diseases often rely on synthetic drugs, which are associated with adverse side effects and drug resistance. Natural compounds, such as polysaccharides and phenolics, offer a safer alternative but remain underutilized due to insufficient comparative analyses. This study fills this gap by examining the antioxidant and neuroprotective properties of commercially available mushrooms, providing a foundation for their potential therapeutic applications.

2. Literature Review

2.1 Historical and Cultural Significance of Mushrooms

Mushrooms have served as a source of medicine for our ancestors for thousands of years. The Greek physician Hippocrates, around 450 BCE, recognized the amadou mushroom (Fomes fomentarius) for its potent anti-inflammatory properties and its use in cauterizing wounds. In the 5th century, the alchemist Tao Hongjing documented several medicinal mushrooms, including ling zhi (*Ganoderma lucidum*) and zhu ling (*Dendropolyporus umbellatus*), which were reportedly utilized by Shennong many centuries earlier. Additionally, Ötzi, the Ice Man, who lived approximately 5,300 years ago, carried amadou and a birch polypore in a pouch, which he likely used for survival in the Alps of northern Italy. Indigenous peoples of North America also employed puffball mushrooms (from the *Calvatia* genus) as natural wound healers. Despite the long-standing use of mushrooms across various cultures, it has only recently that modern science has begun to rediscover the profound medicinal potential that ancient civilizations recognized long ago [9].

One such mushroom that exemplifies this rediscovery is *Hericium erinaceus*, commonly known as lion's mane. This edible and medicinal mushroom has a long history in traditional Chinese medicine, where it has been utilized for its therapeutic properties, particularly for gastrointestinal disorders, such as gastritis and inflammatory bowel diseases. Numerous studies have highlighted its potential for healing, with some components exhibiting strong antineoplastic capabilities against gastric and colorectal cancer. The historical use of *H. erinaceus* is well documented; for example, it has been traditionally prescribed for conditions such as chronic gastritis and gastric ulcers. Its medicinal reputation has persisted, leading to modern scientific investigations that validate the ancient knowledge of its healing properties. Despite the strong need for clinical studies, many preclinical experiments have demonstrated the beneficial effects of *H. erinaceus* extracts on gastrointestinal diseases, emphasizing its anti-inflammatory and gastroprotective qualities [10].

Moreover, the historical significance of mushrooms extends beyond their medicinal applications. Psychoactive fungi and plants have long been integrated into the ritual life, medicine, and leisure of various human populations globally. In ancient Costa Rica, the consumption of psychoactive substances, including mushrooms, was linked to shamanistic healing practices and social-ceremonial events, emphasizing their importance in the cultural and spiritual fabric of the time. Although there is limited documentary evidence of such practices, archaeological findings suggest that these substances played a crucial role in the lives of indigenous societies [11].

Approximately 14,000 species of mushrooms have been discovered so far, of which around 2,200 species are identified as edible mushrooms. Among these, about 650 species have been widely studied, cultivated, and consumed for health and medical applications [12].

Abalone mushroom, enoki mushroom, monkey mushroom, shiitake mushroom, and yellow oyster mushroom are among the commercially available mushroom varieties that are of interest in this research. Abalone mushroom is a type of oyster mushroom known for its culinary and potential medicinal properties. Enoki mushroom is a popular edible mushroom with a long history of use in traditional medicine. Monkey mushroom, also known as bearded tooth mushroom, has been used in traditional Chinese medicine for its purported health benefits. Shiitake mushroom is a widely cultivated species prized for its culinary and medicinal uses, while yellow oyster mushroom is a variety of oyster mushroom valued for its distinctive color and flavor [2,13].

As we explore the multifaceted historical and cultural significance of mushrooms, it becomes clear that they have been deeply embedded in the medicinal, social, and spiritual aspects of human civilization. This ongoing investigation into their therapeutic potential further underscores the relevance of mushrooms as natural alternatives to synthetic medications. This historical use of

mushrooms as medicine by our ancestors also lays the foundation for the current scientific investigation into their bioactive compounds.

2.2 Bioactive Compounds in Mushrooms

A bioactive compound is a substance having biological activity affecting directly a living organism [14]. Bioactive compounds are recognized for their beneficial effects on human health. Unlike macro- and micronutrients—such as carbohydrates, proteins, fats, minerals, and vitamins—that are essential for life and bodily functions, phytochemicals are not strictly necessary for survival [15]. Bioactive compounds can be categorized into three primary groups: (i) terpenes and terpenoids, which comprise approximately 25,000 compounds; (ii) phenolic compounds, with around 8,000 varieties; and (iii) alkaloids, totaling about 12,000 compounds.

The synthesis of these bioactive compounds occurs through four metabolic pathways in plant secondary metabolism: (i) the malonic acid pathway; (ii) the mevalonic acid pathway; (iii) the shikimic acid pathway; and (iv) the non-mevalonate pathway. Common groups of bioactive compounds found in foods include phytosterols (e.g., stigmasterol in soybeans), terpenoids (e.g., limonene in citrus fruits), polyphenols (e.g., chlorogenic acids in raspberries), glucosinolates (e.g., sulforaphane in broccoli), alkaloids (e.g., caffeine in coffee beans), capsaicinoids (e.g., capsaicin in peppers) and carotenoids (e.g., β-carotene in carrots) [16,17].

Other than the three primary groups (terpenes and terpenoids, phenolic compounds and alkaloids), there is also separate category of bioactive compounds known as polysaccharides.

Polysaccharides derived from edible mushrooms are complex molecules with variations in their monosaccharide composition, content, and the conformation of their main chains, differing among various strains, mycelium, or fermentation broths of the same strain. For instance, *Ganoderma* is a notable edible mushroom recognized for its high medicinal value, with scientific studies focusing on its polysaccharide structures. The most common monosaccharides found in the polysaccharides of *Ganoderma* include glucose, mannose, galactose, and fucose, along with other monosaccharides like xylose, arabinose, rhamnose and ribose. These monosaccharides are linked together through different glycosidic bonds to form complex chemical structures [18,19].

Mushroom polysaccharides significantly stimulate the activation and differentiation of immune cells, influencing the levels of various immune factors and cytokines. The high content of β-glucans in these polysaccharides is associated with substantial physiological activity, capable of promoting the secretion of pro-inflammatory and anti-inflammatory cytokines. This regulatory function plays a crucial role in the immune system's ability to respond to pathogens and inflammation [20].

Polysaccharides derived from edible mushrooms have demonstrated efficacy in suppressing inflammatory conditions, including inflammatory bowel disease (IBD). For instance*, Helvella leucopus* polysaccharides have been shown to downregulate pro-inflammatory cytokines while promoting beneficial bacterial populations in the gut [21,22].

Polysaccharides from mushrooms have garnered attention for their anti-tumor and immunomodulatory properties. They have been investigated as potential adjuvant therapies for various cancers, enhancing the sensitivity of cancer cells to treatments and improving overall therapeutic outcomes [23,24].

Mushroom polysaccharides possess significant antioxidant properties, helping to mitigate oxidative damage caused by reactive oxygen species (ROS). Various studies have identified specific polysaccharides with strong radical scavenging activities, suggesting their role in supporting the body's antioxidant defense systems [25,26].

Research indicates that polysaccharides from edible mushrooms can exhibit hypolipidemic effects, potentially reducing the risk of cardiovascular diseases. These polysaccharides contribute to regulating lipid levels and improving insulin resistance, making them valuable for managing metabolic health [27,28].

In summary, the bioactive compounds present in mushrooms, particularly polysaccharides, offer substantial health benefits and potential applications in medicine and nutrition. Their complex interactions with the immune system and gut microbiota underscore their importance as natural bioactive agents.

2.3 Comparative Analysis with Synthetic Medications

When evaluating the comparative benefits of bioactive polysaccharides from mushrooms against synthetic medications, it becomes evident that natural compounds offer a broader therapeutic potential with fewer adverse effects.

Synthetic medications such as anthracyclines (e.g., doxorubicin) and taxanes (e.g., paclitaxel) are widely used in chemotherapy but are associated with a range of severe side effects, including bone marrow suppression, neurotoxicity, and gastrointestinal damage, as well as hair loss [29–31].

These effects often limit the long-term use of synthetic drugs, despite their potent anticancer properties. On the other hand, polysaccharides derived from mushrooms, including beta-glucans and other bioactive compounds, have shown substantial efficacy in modulating immune responses, activating macrophages, T cells, and natural killer cells to enhance the body's ability to target and destroy tumor cells [32]. This immunomodulatory action is particularly significant because it offers a multi-targeted approach, unlike synthetic drugs that usually focus on a single biochemical pathway [33].

Moreover, synthetic medications often contribute to multi-drug resistance (MDR) in cancer, particularly through mechanisms such as drug efflux, where cancer cells expel the chemotherapeutic agents, rendering treatment ineffective [34]. In contrast, natural polysaccharides may help mitigate these issues by working synergistically with synthetic drugs to enhance their efficacy. For instance, polysaccharides have been found to sensitize cancer cells to chemotherapy by promoting apoptosis and reducing the activity of pro-survival pathways, such as NF-κB and STAT-3, that are commonly activated in drug-resistant cancers [35]. This dual functionality—targeting both the cancer cells and the mechanisms leading to resistance—suggests that bioactive polysaccharides could serve as valuable adjuncts in chemotherapy or as standalone treatments.

A key advantage of mushroom-derived compounds lies in their low toxicity profile compared to synthetic drugs. Doxorubicin, for example, while effective in reducing tumor size, can cause irreversible cardiotoxicity with prolonged use, a limitation that is less pronounced with natural compounds. Polysaccharides, by contrast, not only exhibit lower toxicity but are also more biocompatible, which enhances their suitability for long-term administration in chronic diseases like cancer [35]. Additionally, these natural compounds are often better tolerated by patients, which could potentially lead to better compliance and overall treatment outcomes.

In summary, while synthetic medications are powerful and targeted, their limitations in terms of toxicity and drug resistance highlight the growing interest in bioactive polysaccharides as a viable alternative. These natural compounds offer a more holistic approach by interacting with multiple cellular pathways, reducing toxicity and potentially overcoming resistance mechanisms. The evidence suggests that polysaccharides could complement or even replace some synthetic drugs in certain therapeutic contexts, particularly for patients who suffer from the side effects of conventional treatments.

2.4 Hot Water Extraction

There are many methods used nowadays to extract compounds from mushrooms. Among the methods used is Hot Water Extraction (HWE). This method used heat as a vital component for the extraction process.

HWE has emerged as a safe, cost-effective and environmentally friendly method for extracting bioactive compounds from plants, including mushrooms [36]. This technique utilizes water as the solvent, taking advantage of its ability to solubilize a wide range of polar compounds, including polysaccharides, proteins, and some phenolic compounds, which are often found in mushrooms and recognized for their medicinal properties [37].

HWE is not as effective at extracting non-polar compounds and prolonged exposure to high temperatures can degrade some heat-sensitive compounds, researchers have been exploring ways to optimize extraction parameters, such as temperature, time, and solvent-to-solid ratio, to maximize yield and minimize degradation [38]. This technique has been successfully employed to extract bioactive polysaccharides, such as beta-glucans, from various mushroom species, highlighting its potential in harnessing the therapeutic properties of these fungi [36].

An excerpt from Chen *et al.,* [39] highlights the benefits of hot water extraction for polysaccharide extraction: "Among these methods, hot water extraction technology has been widely chosen for the extraction of polysaccharides owing to its simple operation, low price, and easy mass production."

2.5 Antioxidant Activity

Cell reinforcements are substances that can avoid or moderate the harming impacts of free extremists and oxidative pressure on typical cells. Additionally, a few investigations have shown that the cancer prevention agent properties of mushroom separates are essentially identified with their polyphenol content [40].

To assess the antioxidant activity of the mushroom extracts, researchers often employ assays such as the Phenol-Sulphuric Acid Assay and the Folin-Ciocalteu Reaction Total Phenolic Assay [41,42].

3. Materials and Methods

3.1 Materials

Fresh samples of the commercialized mushroom species were obtained from local markets which is *Hericium erinacues* (monkey head mushroom), *Pleorotus ostreatus* (abalone mushroom), *Pleorotus citrinopileatus* (yellow oyster mushroom), *Flammulina velutipes* (Enoki mushroom) *and Letinula edodes* (shiitake mushroom).

The experiments used five replicates per mushroom species, with each assay conducted in triplicate. Controls included distilled water as a negative control and gallic acid as a positive control for phenolic assays. Mushroom extracts were prepared by boiling 1 g of dried sample in 20 mL of distilled water at 100°C for 3 hours, followed by filtration using Whatman No. 1 paper. Absorbance values were measured at 492 nm for polysaccharides and 620 nm for phenolic content.

Essential supplies included a grinder, water bath, Whatman No. 1 filter paper, Falcon tubes, distilled water, conical flasks, Petri dishes, pipettes, cotton wool, aluminium foil, muscline cloth, test tubes and disposable pipettes.

Various chemicals were utilized in the experiments, including phenol reagent, sulphuric acid, Wagner's reagent, Benedict's reagent, and 2% copper (II) sulfate. Additionally, a 95 % ethanol solution, sodium hydroxide, neutral 5 % ferric chloride, glucose solution and Folin-Ciocalteu reagent were employed. Other chemicals included 2 % sodium carbonate solution, gallic acid, chloroform, biuret reagent and Sudan (III) reagent.

The study also utilized a laminar flow chamber, incubator, micropipette, oven, cork borer, forceps, electronic balance, hot plate, spatula, UV-spectrophotometer and centrifuge.

3.2 Sample Preparation and Extraction

The fruiting bodies of these mushrooms was diced into small pieces and was dried in an oven at 60°C for about 3 days (Figure 1). Once dried, the mushroom samples were grinded into a fine powder using a grinder.

Fig. 1. Dried mushroom sample

The powdered mushroom samples, comprising both cap and stipe portions, were weighed to approximately 17.5 grams. These powdered samples were then subjected to HWE, with each gram of the sample being boiled in 20 mL of sterile distilled water for a duration of 3 hours using a water bath at a temperature of 100 °C (Figure 2).

After the extraction process, the mixtures were cooled down to the room temperature and then filtered using Whatman No. 1 filter paper. It was used for the polysaccharide and phenolic content analyses as well as antioxidant activity assays.

Fig. 2. Hot water extraction using double boiling method

3.3 Determination of Polysaccharide Content (Phenol Sulphuric Acid Assay)

The polysaccharide content of the mushroom extracts was determined using the phenol-sulphuric acid assay. This colorimetric method involves the reaction of polysaccharides with phenol and sulphuric acid, which results in the formation of a yellow-orange complex.

The polysaccharide content of the mushroom extracts was determined using a modified version of the phenol-sulphuric acid colorimetric assay, as described by Fournier. Glucose solutions at various known concentrations were prepared in sterile distilled water and used as standards for the analysis. For the assay, approximately 1 mL of the test mushroom extracts were gradually combined with 2.5 mL of concentrated sodium hydroxide and 0.5 mL of a 4 % phenol reagent. These mixtures were then left to stand at room temperature overnight before the absorbance readings were taken at 492 nm. The absorbance measurements were performed using a 96-well microplate reader, with 100 µL of each test solution loaded into the wells.

All tests were carried out in triplicate. The absorbance values of the test solutions were then measured against a blank sample and plotted against the known glucose concentrations to determine the polysaccharide content of the mushroom extracts.

3.4 Determination of Phytochemical Composition

The freshly prepared, water-based extracts of the mushroom samples were subjected to comprehensive phytochemical screening to identify the presence and diversity of active chemical constituents using well-established analytical methods. These qualitative tests were designed to detect and characterize the occurrence of a wide range of important phytochemical classes, including phenols, flavonoids, tannins, alkaloids, steroids, saponins, glycosides and carbohydrates. This preliminary phytochemical profiling provided valuable insights into the potential bioactive compounds present in the mushroom extracts, which could inform further quantitative analysis and evaluation of their therapeutic properties.

3.4.1 Alkaline reagent test

This test was conducted to evaluate the presence of flavonoids in the mushroom extracts. A few drops of sodium hydroxide were added to the extract, resulting in an intense yellow colour that turned colourless upon adding a diluted acid, indicating the presence of flavonoids.

3.4.2 Ferric chloride test

The purpose of this test was to assess the presence of phenols and tannins. It involved mixing 1 mL of the extract with approximately 2 mL of distilled water, 3 drops of 10% Ferric Chloride solution and 3 drops of Potassium Ferrocyanide. The development of a blue-green colour suggested the presence of polyphenolic compounds.

3.4.3 Froth test

This test was performed to screen for the presence of saponins. Approximately 1 mL of the extract was diluted with 5 mL of distilled water and shaken vigorously for 30 seconds. After allowing the mixture to rest for 20 minutes, a stable foam layer on the surface indicated the presence of saponins.

3.4.4 Terpenoid test

This test was utilized to confirm the presence of terpenoids in the extracts. A 1 mL portion of the extract was dissolved in 2 mL of chloroform and concentrated with sulphuric acid to form a layer. The appearance of a reddish-brown colour at the interface confirmed the presence of terpenoids.

3.4.5 Salkowski test

This test aimed to evaluate the presence of steroids in the extracts. A few drops of sulphuric acid were added to 2 mL of the extract and shaken vigorously. After leaving the tubes upright for a few minutes, the solution turned bluish-red, slowly changing to violet-red, with the sulphuric acid layer exhibiting a green fluorescence.

3.4.6 Wagner's test

This test was conducted to assess the presence of alkaloids. A few drops of Wagner's solution were added to 5 mL of the extract. The formation of a reddish-brown precipitate confirmed the presence of alkaloids.

3.4.7 Biuret test

This test was used to detect the presence of peptides in the extracts. Approximately 2 mL of extract was heated with 1 drop of 2 % $CuSO_4$ (copper (II) sulfate solution), followed by 1 mL of 95 % ethanol and excess KOH. The formation of violet-coloured coordination complexes in an alkaline solution confirmed the presence of peptides.

3.4.8 Sudan test

This test aimed to screen for the presence of lipids in the extracts. About 2 mL of extract was mixed with 5 drops of Sudan reagent. After a brief incubation, red-stained oil floating in the solution indicated the presence of lipids.

3.4.9 Benedict test

This test was conducted to evaluate the presence of reducing sugars. Approximately 2 mL of extract was added to 1 mL of Benedict's solution and boiled at 100°C for 2 minutes. The appearance of a brick-red color indicated the presence of reducing sugars.

3.5 Antioxidant Activity Analysis: Total Phenolic Content (Folin-Ciocalteu Assay)

The total phenolic content of the mushroom extracts was determined using a modified version of the Folin-Ciocalteu assay as described by Singleton and Rossi in 1965. Approximately 9 mL of each mushroom sample was placed into a labelled Falcon tube. Subsequently, 7 mL of 7% sodium carbonate was added to the sample, followed by the addition of 3 mL of Folin-Ciocalteu reagent. The mixture was then incubated for 90 minutes to allow for proper color development (Figure 3).

After the incubation period, the samples were transferred to a 96-well microplate, and the absorbance was measured at 620 nm using a microplate reader. A blank sample was analyzed prior to the mushroom samples to calibrate the instrument. Each sample was tested in triplicate, and the average absorbance value was recorded. The phenolic content was quantified using a standard curve generated with gallic acid, allowing for comparison of the phenolic content across different mushroom species.

Fig. 3. Extracts tested with sodium carbonate and Folin-Ciocalteu reagent

4. Results and Discussion

4.1 Biomass of Dried Weight of Marketed Mushrooms

All the mushrooms were locally sourced. Despite having the same wet biomass, the dry biomass varied among the mushroom types due to differences in size and water absorption characteristics (Table 1). Notably, the Monkey mushroom exhibited the highest dry biomass, indicating it absorbs less water and has a denser flesh compared to the other varieties.

Table 1
Total dried fruiting body of mushrooms in 500 grams of wet weight

Types of Mushrooms	Wet Biomass (g)	Dry Biomass (g)
Abalone Mushroom	500	57.86
Enoki Mushroom	500	21.50
Monkey Mushroom	500	84.79
Shiitake Mushroom	500	45.35
Yellow Oyster Mushroom	500	28.91

4.2 Phytochemical Test

The results indicate that all mushroom extracts exhibited similar characteristics across the phytochemical tests. Negative results for the alkaline, Salkowski, and Wagner tests imply the absence of cholesterol, alkaloids, and alkaline properties in the extracts (Table 2). However, saponins, reducing sugars, lipids, terpenoids, and polyphenols were present in all extracts.

Table 2
Total dried fruiting body of mushrooms in 500 grams of wet weight

Test	Abalone	Enoki	Monkey	Shiitake	Yellow
Saponins	(+)	(+)	(+)	(+)	(+)
Alkaline	(-)	(-)	(-)	(-)	(-)
Salkowski	(-)	(-)	(-)	(-)	(-)
Ferric Chloride	(+)	(+)	(+)	(+)	(+)
Wagner	(-)	(-)	(-)	(-)	(-)
Biuret	(+)	(+)	(+)	(+)	(+)
Sudan	(+)	(+)	(+)	(+)	(+)
Benedicts	(+)	(+)	(+)	(+)	(+)
Terpenoids	(+)	(+)	(+)	(+)	(+)

4.3 Phenol Sulphuric Acid Assay

The glucose standard curve generated from the data in Table 3 and the corresponding graph demonstrates a clear linear correlation between glucose concentration and absorbance at 492 nm in the phenol-sulphuric acid assay. The absorbance increased consistently with rising glucose levels, from 0.663 at 1 mg/mL to 3.933 at 9 mg/mL, reinforcing the linear trend in the graph (Figure 4). This standard curve serves as a crucial tool to quantify the carbohydrate content in the mushroom samples, as the equation of the line and R^2 value can be used to accurately determine the carbohydrate levels.

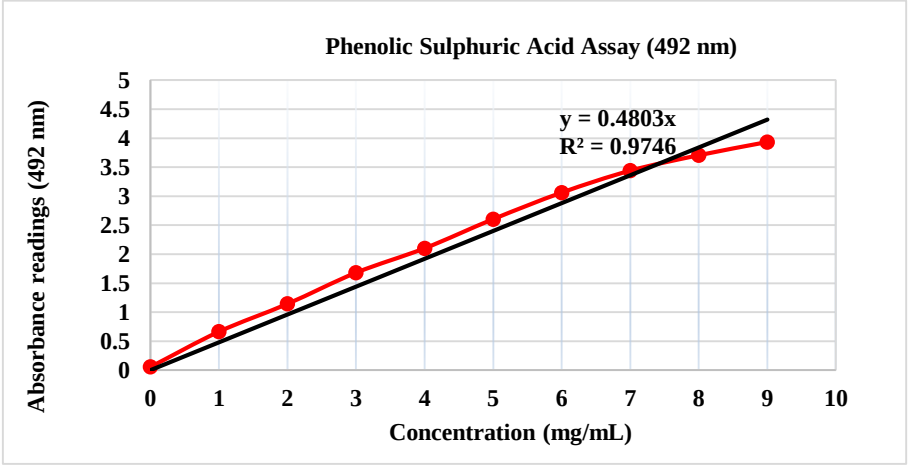

Fig. 4. Glucose standard curve of phenol sulphuric acid assay

Table 3
The absorbance of different concentration of glucose solution at 492 nm in phenol sulphuric acid assay

Concentration (mg/mL)	Absorbance at 620 nm
Blank	0.055
1	0.663
2	1.143
3	1.170
4	2.101
5	2.603
6	3.061
7	3.439
8	3.705
9	3.933

The results in Table 4 summarize the total carbohydrate content across five mushroom species, measured using the phenol-sulphuric acid assay. The data reveal significant variation, with the Monkey Head Mushroom exhibiting the highest carbohydrate concentration at 5.868 ± 0.175 mg/mL, and the Yellow Oyster Mushroom having the lowest at 0.809 ± 0.379 mg/mL.

The carbohydrate content in Abalone Mushroom is relatively high at 3.300 ± 0.125 mg/mL, while Enoki and Shiitake Mushrooms demonstrate moderate values of 2.240 ± 0.020 mg/mL and 1.539 ± 0.095 mg/mL, respectively. These differences may be attributed to structural and biochemical variations between the mushrooms, as well as their polysaccharide storage capacity.

Table 4
The absorbance reading and the total carbohydrate content (mg/ml) of different types of mushrooms at 492 nm in phenol sulphuric acid assay

Types of samples	Carbohydrate (mg/mL)	Average ± standard deviation
Abalone Mushroom	3.304	3.300 ± 0.125
	3.423	
	3.173	
Enoki Mushroom	2.262	2.240 ± 0.020
	2.222	
	2.235	
Monkey Head Mushroom	5.918	5.868 ± 0.175
	6.013	
	5.674	
Shiitake Mushroom	1.565	1.539 ± 0.095
	1.619	
	1.434	
Yellow Oyster Mushroom	0.705	0.809 ± 0.379
	0.493	
	1.230	

The bar chart visually represents the carbohydrate content (Figure 5), clearly highlighting the Monkey Head Mushroom as the highest and the Yellow Oyster Mushroom as the lowest. The larger standard deviation in Yellow Oyster Mushroom indicates greater variability in carbohydrate content compared to the more consistent readings in Abalone and Monkey Head Mushrooms.

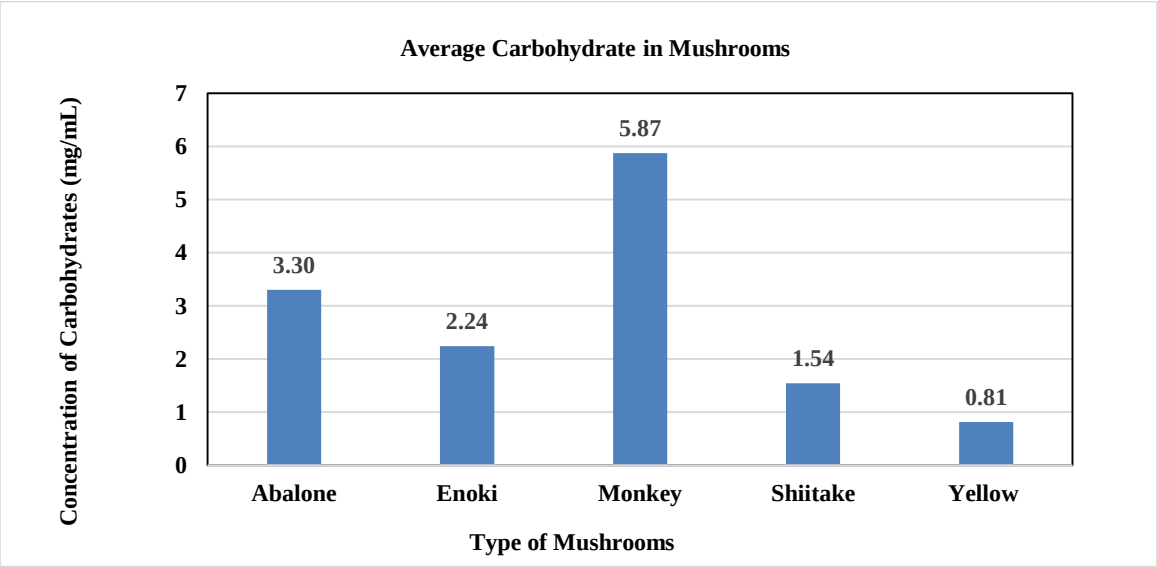

Fig. 5. The average reading of total carbohydrate content (mg/mL) of different types of mushrooms at 492 nm in phenol sulphuric acid assay

4.4 Total Phenolic Content Assay

The data in Table 5 highlight the absorbance values obtained from varying concentrations of Gallic Acid using the Total Phenolic Content Assay at 620 nm (Figure 6). This assay measures the total phenolic compounds in the samples, which are known to possess antioxidant properties. The absorbance values reflect the direct relationship between the concentration of gallic acid and the phenolic content.

Table 5
The absorbance of different concentration of gallic acid solution at 620 nm in total phenolic content assay

Concentration (mg/mL)	Absorbance at 620 nm
Blank	0.055
1	0.172
2	0.233
4	0.441
6	0.674
8	0.895
10	1.240

As the concentration of Gallic acid increases, there is a corresponding increase in absorbance values. Starting from a blank reading of 0.055, the absorbance progressively rises with each increase in concentration. The lowest concentration of 1 mg/mL shows an absorbance of 0.172, while the highest concentration of 10 mg/mL gives an absorbance of 1.240.

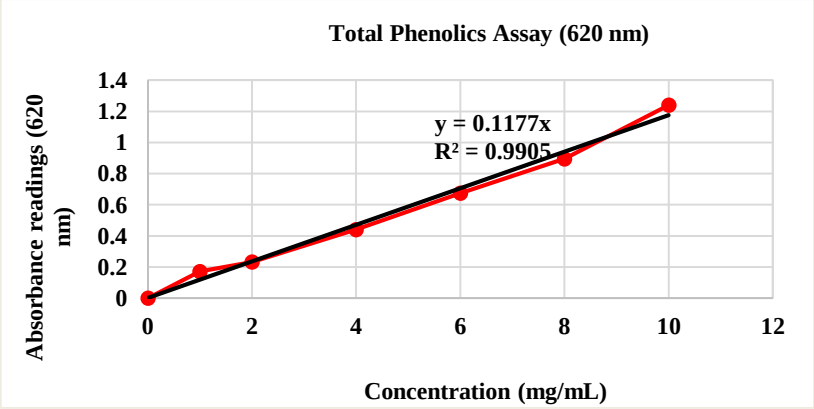

Fig. 6. Gallic acid standard curve of total phenolics content assay

The data in Table 6 show the total phenolic content of various mushrooms, obtained using the Total Phenolic Content Assay at 620 nm. Phenolic compounds are known for their antioxidant properties, and their concentration is an indicator of the mushrooms' potential health benefits.

The results reveal that Monkey Head Mushroom had the highest phenolic content, averaging 15.035 ± 0.448 mg/mL (Figure 7). This is significantly higher than the other mushrooms tested, suggesting that Monkey Head Mushroom may possess the strongest antioxidant properties. The phenolic content observed in Monkey Head mushrooms (15.035 ± 0.448 mg/mL) is comparable to antioxidant levels in curcumin extracts, known for their neuroprotective effects. Additionally, the antioxidant activity measured aligns with ascorbic acid-rich fruits, supporting the potential of mushrooms as a viable alternative to synthetic drugs [40,43].

Table 6
The absorbance reading and the total carbohydrate content (mg/mL) of different types of mushrooms at 620 nm in total phenolic content assay

Types of samples	Phenolic compound (mg/ml)	Average ± standard deviation
Abalone Mushroom	8.929	9.586 ± 0.570
	9.932	
	9.898	
Enoki Mushroom	7.621	7.570 ± 0.088
	7.464	
	7.621	
Monkey Head Mushroom	14.503	15.035 ± 0.448
	15.140	
	15.463	
Shiitake Mushroom	13.560	13.850 ± 0.270
	14.095	
	13.891	
Yellow Oyster Mushroom	13.407	13.886 ± 0.443
	13.968	
	14.282	

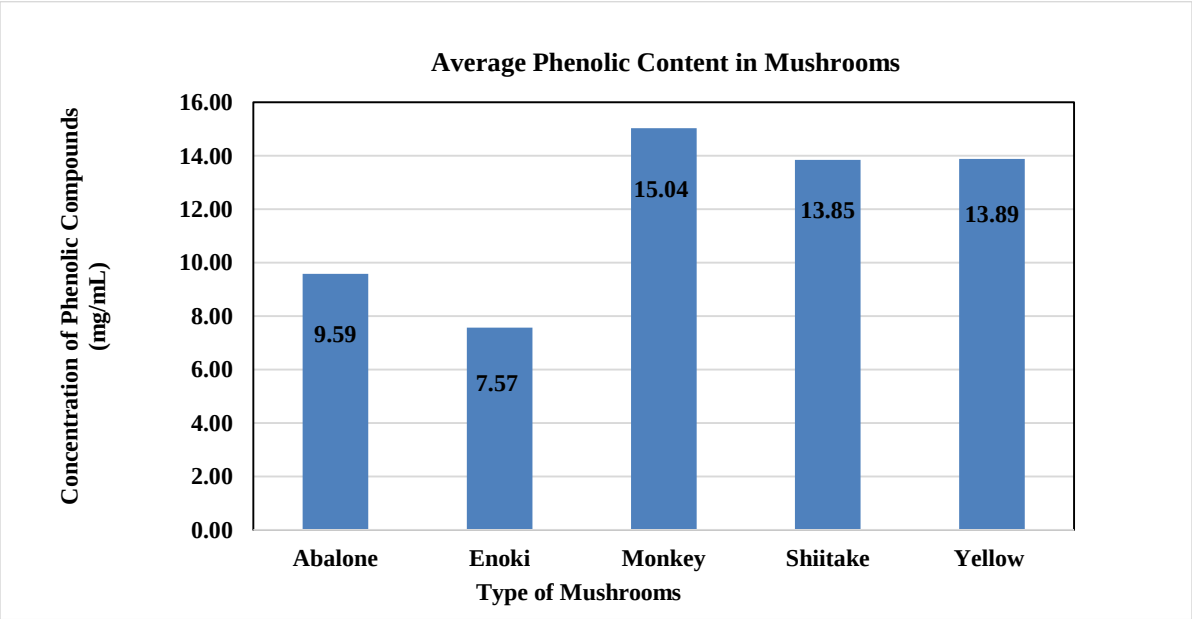

Fig. 7. The average reading of total phenolic content (mg/mL) of different types of mushrooms at 620 nm in Total Phenolic Content Assay

In contrast, Enoki Mushroom exhibited the lowest phenolic content, with an average of 7.570 ± 0.088 mg/mL. Although it has the least phenolic compounds among the samples, Enoki Mushroom still contains a significant amount, suggesting it also has antioxidant potential, though to a lesser extent compared to the other mushrooms.

The Abalone Mushroom, Shiitake Mushroom, and Yellow Oyster Mushroom fall between these two extremes. Abalone Mushroom had an average phenolic content of 9.586 ± 0.570 mg/mL, while Shiitake and Yellow Oyster Mushrooms recorded averages of 13.850 ± 0.270 mg/mL and 13.886 ± 0.443 mg/mL, respectively. These results highlight the diversity in phenolic content across different mushroom species, suggesting their bioactive properties and potential health benefits may vary.

5. Conclusion

In conclusion, the phenol-sulphuric acid assay revealed that Monkey Head mushrooms had the highest concentration of intracellular polysaccharides, while Yellow Oyster mushrooms showed the lowest levels. Additionally, the total phenolic content was found to be highest in Monkey Head mushrooms and lowest in Enoki mushrooms. These findings suggest that Monkey Head mushrooms are a particularly rich source of both polysaccharides and phenolic compounds.

The high polysaccharide content observed in Monkey Head mushrooms suggests significant clinical applications, particularly in immunomodulation and antioxidant therapies. Polysaccharides such as β-glucans activate immune cells, including macrophages and natural killer cells, via Dectin-1 and Toll-like receptor pathways, enhancing immune responses against cancer and chronic inflammation. Additionally, these compounds exhibit potential in regulating gut microbiota and reducing pro-inflammatory cytokines, offering new avenues for managing metabolic and inflammatory disorders.

Phenolic compounds, known for their antioxidant properties, could mitigate oxidative stress-related damage in neurodegenerative diseases such as Alzheimer's and Parkinson's. By scavenging reactive oxygen species and modulating pathways like NF-κB and Nrf2, these bioactive compounds may prevent neuronal apoptosis and promote neural repair. These mechanisms position mushroom-derived bioactives as promising candidates for integrative therapies in chronic and age-related diseases.

While this study provides valuable insights into the bioactive properties of five mushroom species, the limited scope restricts generalizability. Future studies should include underexplored mushroom species and samples from different ecological regions to provide a broader understanding of their therapeutic potential.

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