

Evaluation of the Phytochemical and Antioxidant Properties of *Polyporus leptocephalus* (Jacq.) Fr

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ABSTRACT

Mushrooms have a long history of uses for their medicinal and nutritional properties. Recently, wild mushrooms acquired considerable attention since they provide beneficial effects to humans in terms of health promotion, longevity, and reduction of chronic diseases like cancer. The bioactive compounds isolated from wild mushroom *Polyporus leptocephalus* (Jacq.) Fr growing in the spawning lands of Thattekad forest, Kerala has been studied, together with their biological activities. Phytoconstituents with antioxidant activities have been highlighted. The secondary metabolites obtained were characterized using Soxhlet extractor. The phytochemical screening of the mushroom extract quantified a significant amount of alkaloids, tannins, flavonoids, and total phenols. The antioxidant activity of evaluated mushroom extracts gave positive results with free radical scavenging activity. Thus, the result obtained from this study has shown the wild mushroom *Polyporus leptocephalus* (Jacq.) as a potential reservoir of innovative therapeutic solutions and as a healthy food supplement rich in natural antioxidants for human health.

Keywords : Mushrooms, *Polyporus leptocephalus*, Phytochemical, Antioxidant.

INTRODUCTION

Since time immemorial, mushrooms have been part of human culture. It is believed that mushrooms provided strength for warriors in battle, and the Romans perceived them as the "Food of the Gods".¹ It is not just because of its unique organoleptic characters, but also due to the rich content of substances like amino acids, fatty acids, vitamins, sterols, and essential minerals, etc.² The nutritional value of mushrooms can be compared to those of eggs, milk, and meat.³ The total energetic value of mushroom caps is between 250 and 350 cal/kg of fresh mushrooms.⁴ The inclusion of whole mushrooms in the diet may have efficacy as superior dietary supplements attributed to magnificent medicinal values. Therefore, undoubtedly mushrooms are a valuable asset for the welfare of humans.

Apart from the exceptional nutritional value, mushrooms are rich in health-promoting phytochemicals which is currently getting much attention due to their biological effects, which include antioxidant, antibacterial, antifungal, antiviral, antiparasitic, anti-inflammatory, antiproliferative, anticancer, antitumor, cytotoxic, DNA damaging, anti-HIV, hypocholesterolaemia, antidiabetic, anticoagulant, hepatoprotective, etc.⁵ Scientists have underpinned the argument that oxidative damage caused by unstable molecules known as free radical is a significant causative factor in the development of human chronic diseases and that antioxidants are capable of preventing or ameliorating these disease processes.⁶ Numerous synthetic antioxidants can effectively improve defense mechanisms, but because of their adverse toxic

effects under certain conditions, preference is given to natural compounds. Consequently, the requirements for natural or alternative sources of antioxidant-rich biochemicals are identified in mushrooms. Interestingly, mushrooms got higher antioxidant properties than in most vegetables and fruits that could be attributed to their bioactive compounds, such as phenolics, polysaccharides, tocopherols, flavonoids, carotenoids, glycosides, ergothioneine, and ascorbic acid. A significant advantage in antioxidant compounds extraction from mushrooms is that fruit bodies or mycelium can be manipulated using biotechnology to produce active compounds in a relatively short period.⁷

Wild mushrooms have manifold impacts on biology, ecology, and economy.⁵ They are superior to other food items with respect to vitamins, minerals, and other nutraceuticals. They are naturally gluten-free and make a delicious and nutritious addition to a gluten-free diet.⁸ The Indian sub-continent is blessed with diverse agroclimatic zones that harbor a treasure trove of fungal diversity. Approximately 70,000 species of fungi out of ~1.5 million fungi in existence are identified of which one third is said to be found in India.⁹ It comprises a vast and yet largely untapped source of powerful new pharmaceutical products.¹⁰ In particular, and most importantly for modern medicine, they represent an unlimited source of antioxidant-rich foods and sources of novel molecules. Thus, the objectives of our study were designed to quantify the phytochemical constituents of the few collected wild mushrooms followed by the estimation of the antioxidant potentiality of the same.

MATERIALS AND METHODS

Sample material

Polyporus leptocephalus (Jacq.) Fr was harvested from the lowland areas of Thattekad forest (10°08'N 76°41'E and altitude of 35 m to 528 m above sea level) located near Kothamangalam, Ernakulam district in the sprawling lands of Kerala. The identity of the collected mushroom was authenticated by Dr. Madhusudhan, Department of Botany, St. Albert's College, Ernakulam. Mushrooms as fruiting bodies (pileus and stipe) were cleaned under running tap water to remove the adhered dust particles and air-dried under room temperature. The dried material was ground using the electronic grinder, sieved through a 0.5-mm mesh, and stored in air-tight plastic bags in a desiccator at room temperature until further analysis.

Extract preparation

Fine dried mushroom powder sample (10 g) was extracted with 80% ethanol (250 ml) for 5 hours using a Soxhlet extractor. The extracts were filtered using Whatman No.1 filter paper. The ethanol from the filtrate was removed using a rotary evaporator to get the dry extract. The extracts were stored in plastic bottles and stored at 4 °C to prevent oxidative damage until further analysis.

Determination of total phenolic content

Total phenolic compounds in the sample extracts were estimated by using the Folin-Ciocalteu assay.¹¹ 0.1 ml of mushroom extracts was added to the test tubes containing 0.1 ml of gallic acid, which is used as the reference standard compound and made up to the volume of 3.5 ml using distilled water. Then, 0.125 ml of Folin's reagent was added and incubated at room temperature for 6 minutes. 1.25 ml of 7% sodium carbonate was added sequentially in each tube and again incubated at room temperature for 90 minutes. The absorbance was recorded at 725 nm against the reagent blank.

Determination of total flavonoid content

The total flavonoid content of sample extracts was determined using a slightly modified method described by Zhishen *et al.*¹² A 0.5 ml extract was mixed with 2 ml of distilled water and subsequently with 75 µl of 5% sodium nitrate solution and incubated at room temperature for five minutes. 150 µl of a 10% aluminum chloride solution was added and allowed to stand for 6 minutes. The pink-colored solution formed after adding 0.5 ml of 1 M NaOH was spectrophotometrically measured at 415 nm. Catechol was used as a standard compound for the quantification of total flavonoid content.

Determination of total tannin content

To estimate total tannin content, the modified Prussian blue method was used.¹³ 0.1 ml of sample extract was mixed with 3 ml of distilled (deionized) water. Then, to the sample, 1 ml potassium ferricyanide was added, followed by 1ml ferric chloride and immediately vortexed. 15 minutes after adding the reagents to the sample, 5 ml of stabilizer (30 ml distilled water, 10 ml 85% H₃PO₄, 10 ml 1% gum arabic) was added and again vortexed, such that the colors were stable, so the

timing was not critical. The absorbance was measured spectrophotometrically at 700 nm.

Determination of total alkaloid content

The total alkaloid contents in the samples were measured using a 1,10-phenanthroline method described by Singh *et al.*¹⁴ with slight modifications. The extract was obtained by dissolving 100 mg extract in 10 ml of 80% ethanol and filtered through a muslin cloth. It is then centrifuged at 5000 rpm for 10 minutes. The supernatant so obtained was used for the further estimation of total alkaloids. To 1 ml plant extract, 1 ml of 0.025 M ferric chloride in 0.5 M HCl and 1 ml of 0.05 M of 1,10-phenanthroline in ethanol was added. The mixture was incubated for 30 minutes in a hot water bath with a maintained temperature of 70 ± 2 °C. The absorbance of the red-coloured complex was measured at 510 nm against the reagent blank.

Evaluation of antioxidant activity *in vitro*

The antioxidant activity of the mushroom extract against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined by UV spectrophotometry at 518 nm. The activity was measured according to the method previously described by Pal *et al.*¹⁵ Different concentrations of the mushroom extract were prepared using analytical methanol and vitamin C was used as an antioxidant standard. 1 ml from each extract and 3 ml of methanol were mixed with 0.5 ml of 1 mM DPPH in methanol and allowed to react at room temperature for 30 minutes. The same amount of methanol and DPPH were mixed to prepare the blank solution. The radical scavenging activity was calculated using the following formula:

$$\text{Radical Scavenging activity (\%)} = \frac{Ab - Aa}{Ab} \times 100$$

Where *Ab* is the absorption of the blank and *Aa* is the absorption of the sample. Extract concentration providing 50% inhibition (IC₅₀) was calculated from the plot of inhibition percentage against extract concentration.

Statistical analysis

All experiments were done in triplicate and the data were expressed as the mean ± standard error.

Result and Discussion

Forest fungi represent a socially as well as economically important forest non-timber resource that provides diverse bioactive substances and services, that includes food, medicine, and recreation worldwide. Nutritionally, they are an important source of proteins, vitamins, fats, carbohydrates, amino acids, and minerals i.e., a worthy alternative or substitute for meat and fish. The phytochemical studies in wild mushrooms have attracted the attention of modern scientists as a key to, better valorization of services provided by them.

The family Polyporaceae of the fungi kingdom is enriched by biotechnologically useful organisms, due to the presence of hyphae with well-developed glucan layer.¹⁶ *Polyporus leptocephalus*, commonly known as Blackfoot polypore is a non-edible species of mushroom in the genus *Polyporus*. Although there have been records of its distribution and morphological description, no detailed studies have been reported. Therefore, in the current work, it was decided to scientifically highlight the

phytochemistry and antioxidant potentiality of the wild mushroom *Polyporus leptocephalus* (Figure 1) collected

from the low-lying lands of Thattekad forest near to Kothamangalam in Ernakulam district, Kerala.



Figure 1: *Polyporus leptocephalus* in its natural habitat

Alkaloids are large, nitrogen-containing cyclic compounds and of all secondary compounds, historically and contemporaneously, alkaloids are molecules with highly important pharmacological benefits including antimalarial (e.g., quinine), anticancer (e.g., homoharringtonine),¹⁷ antibacterial (e.g., chelerythrine),¹⁸ and antihyperglycemic activities (e.g., piperine).¹⁹ In the current research work, wild mushroom *Polyporus leptocephalus* contained significant alkaloid content with a value of 222 $\mu\text{g}/\text{mg}$ dry weight. Alkaloids induce immunogenic cell death, regulate Na^+ ions and channels, and microbial activity. Alkaloids are also known to have inhibitory effects on angiogenesis and therefore *Polyporus leptocephalus* could be useful in inhibiting the growth of cancerous cells.

Increasingly, flavonoids are becoming the subject of medical research. They have been reported to possess many useful properties, including anti-inflammatory, estrogenic, enzyme inhibition, antimicrobial, antiallergic, vascular, and cytotoxic antitumor activity,²⁰ but the antioxidant activity is, without a doubt, the most studied one attributed to flavonoids. In the present investigation, an appreciable quantity of flavonoids with a value of 145 $\mu\text{g}/\text{mg}$ dry weight was obtained. These results are contrary to those reported in previous literature²¹ that regarded mushrooms as non-sources of flavonoids. They can inhibit enzymes such as prostaglandin synthase, lipoxygenase, and cyclooxygenase, closely related to tumorigenesis,²² and induce detoxifying enzyme systems such as glutathione S-transferase.²³

Tannins are water-soluble high molecular weight phenolic compounds found in many mushrooms that are

important in herbal medicine due to their wound healing properties.²⁴ They have been reported to selectively inhibit HIV replication.²⁵ Furthermore, there are studies on the effects of hydrolyzable tannins on the lipid bilayer membrane, causing dose-dependent damage that can be the reason for its antibacterial action.²⁶ The tannin composition of the sample extract in the present study was reported to be 22.03 $\mu\text{g}/\text{mg}$ which is significantly lower than the tolerable limit as set by WHO (2003), indicating that *Polyporus leptocephalus* to be safe for consumption and support it to be a potential pharmaceutical drug source.

The phenolic compounds, especially phenolic acid is known to be a powerful chain-breaking antioxidant and they possess scavenging ability due to the presence of hydroxyl groups. The hydroxyl groups of phenolic compounds are good hydrogen donors that react with reactive oxygen and nitrogen species in a termination reaction, which breaks the cycle of a generation of new radicals. The antioxidant capacity of phenolic compounds is also attributed to their ability to chelate metal ions involved in the production of free radicals.²⁷ The hydrophobic benzenoid rings and hydrogen-bonding potential of the phenolic hydroxyl groups enhance the capacity to inhibit some enzymes involved in the radical generation, such as various cytochrome P450 isoforms, lipoxygenases, cyclooxygenase, and xanthine oxidase.²⁸ The current research has found evidence for total phenol content in *Polyporus leptocephalus* extract; however, in bare minimum amount with a value of 14.23 $\mu\text{g}/\text{mg}$ dry weight.

Table1: Quantification of phytochemicals in *Polyporus leptocephalus*

Sample	Phytochemical			
	Total Phenolics	Tannins	Alkaloids	Flavonoids
<i>P. leptocephalus</i>	----- $\mu\text{g}/\text{mg}$ -----			
	14.23 \pm 0.12	22.03 \pm 0.46	222 \pm 3.78	145 \pm 0.79

Each value is expressed as mean \pm standard error done in triplicates

DPPH free radical scavenging assay

High antioxidant activities in mushrooms can suppress active oxygen species, which are related to the aging process and increases the risk of chronic diseases.²⁹ The antioxidant activity of *Polyporus leptoccephalus* was measured by the ability to scavenge DPPH free radicals comparing with vitamin C. The scavenging effects of mushroom extracts and the standard on the DPPH radical were expressed as half-maximal inhibitory concentration (IC₅₀) values; the results are reported in table 2. According to the results obtained, the *Polyporus leptoccephalus* extract showed excellent radical scavenging activity of 73.16% with an IC₅₀ value of 128.23 μ g/ml almost similar to the standard compound ascorbic acid (120 μ g/ml).

Table 2: Antioxidant activity of *Polyporus leptoccephalus*

Concentration (μ g/ml)	Sample	
	<i>Polyporus leptoccephalus</i> Percentage inhibition	Ascorbic acid (standard)
100	47.07 \pm 0.79	46.54 \pm 0.11
200	58.23 \pm 0.007	65.94 \pm 0.25
300	73.16 \pm 1.12	81.64 \pm 1.01
IC ₅₀	128.23 μ g/ml	120 μ g/ml

Each value is expressed as mean \pm standard error done in triplicates.

Conclusion

The present work scientifically quantified the major bioactive phytochemicals in *Polyporus leptoccephalus*, as well as described the spectrum of various health benefits attributed to the quantified phytochemicals. The evidence from the current study intimates that the higher the bio-substances, the more significant the bio-therapeutic effects. The bioactive molecules like alkaloids, flavonoids, tannins, and phenols which were found in significant quantity displayed a critical role in enhancing the immune strength, lowering and preventing the risks of cancers, inhibiting the proliferation of tumour cell growth, protecting the cells from the damage of aging, etc. The study focused on the antioxidant assessment using DPPH assay that casts a new light on *Polyporus leptoccephalus* as an alternative source of food supplements or drugs in the future that would protect biomolecules against oxidation caused by free radicals. However, it deserves further investigations into the mechanisms of action of mushroom extracts, which could gain a better perception of the functional food and drug development process that is still needed in the future.

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