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Comparative analysis of solvent-specific extraction efficiencies and bioactivities of *Pleurotus pulmonarius* (Fr.) Quél. from Ranni Forest Division of Kerala, India

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ABSTRACT

Pleurotus pulmonarius mushrooms, collected from the Ranni Forest Division in Kerala, India, were evaluated for their bioactive constituents and antioxidant, anti-inflammatory, and antidiabetic activities using five solvent extracts: methanol, ethyl acetate, acetone, chloroform, and hexane. Total phenolic, flavonoid, and alkaloid contents were quantified, and antioxidant potential was assessed through phosphomolybdenum, FRAP, DPPH, and ABTS assays. Methanol extracts demonstrated the highest total phenolic (0.8369 μg/ml) and flavonoid (0.8314 μg/ml) concentrations, while ethyl acetate was notably rich in alkaloids (0.5143 μg/ml). Methanol exhibited superior antioxidant activity in DPPH, FRAP, and phosphomolybdenum assays, whereas acetone was most effective in the ABTS assay. Methanol extracts also showed notable anti-inflammatory (IC50 = 3.35 μg/ml) and antidiabetic (IC50 = 1.39 μg/ml) activities. The study highlights *P. pulmonarius* as a promising natural source of bioactive compounds suitable for medicinal and nutritional applications.

Introduction

Edible fungi are excellent suppliers of valuable biologically active substances with an elaborate range of pharmacological uses, including antioxidant properties [1]. Nowadays, different types of mushrooms are studied to determine their antioxidant potency. Organic antioxidants are incredibly powerful in reducing damage-causing activities that are triggered by oxidative stress in our body [2], no matter whether they are found in chemical nature or simple extract forms. The human immune system has an innate antioxidative mechanism, which underpins many biological functions, including anti-mutagenic [3], anti-carcinogenic, and anti-ageing responses. Antioxidants regulate or eliminate unstable free radicals [4], generally before reaching cell targets. It has been proven that free radicals induce cellular destruction using the processes of covalent adhesion and peroxidation of lipids [5], which leads to tissue injury. Naturally occurring antioxidants are catching researchers' attention quickly due to their capability to scavenge free radicals. Thus, the use of naturally occurring antioxidants in nutrition, beauty products, and medicinal products has surged significantly [6], due to their enormous potential for rectifying the imbalance.

Studies on veggies, fruits, and mushrooms have revealed an abundance of phenolic compounds [7] such as phenols, flavonoids, alkaloids, and tannins; carotenoids, tocopherol, ascorbic acid, etc [8], each of which serves as an essential defensive substance [9], for the wellness of people. Consumption of natural antioxidants has been linked to a reduction in death from fatal diseases [10]. Hepatic infections continue to be a serious health problem today; mushrooms strengthen liver function in hepatitis B patients and enhance the immune system [11], as well as reducing ageing problems and encouraging a long lifespan.

In the search for natural bioactive substances, mushrooms have proven to be one of the most promising options [12]. The mushroom fruiting bodies require less time to produce [13]; this is due to the rapid colonization of mycelia, resulting in the production of a large amount of active substances [14]. Mushrooms are a great source of plant-based antibiotics; glucans on the outer layer of cells are well-known for immune-regulating functions [15]; several additional metabolites are transcribed and released from the mycelia, which suppress microbial infectious agents [16]. The mushrooms comprise a variety of

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Fig. 1. *Pleurotus pulmonarius* mushroom samples.

polyphenolic components that are known to be powerful antioxidant substances, which can scavenge harmful, unstable free radicals via single-electron exchange [17]. The majority of living organisms have special mechanisms for protecting them against free radical damage [18], including oxidant enzymes like SOD (superoxide dismutase) and CAT (catalase), as well as chemical substances like ascorbic acid, tocopherol, carotenoids, polyphenolic compounds, and glutathione [19]. Those mechanisms are not adequate to fully inhibit destruction [20]. However, antioxidant medications and food items containing antioxidants are essential elements of the human dietary regimen [21] for preventing or reducing the effects of oxidation [22].

The cultivation of *Pleurotus* mushrooms has increased dramatically over the past several years. It is considered to be the world's third-most commercially cultivated fungus. The crop's popularity has grown largely because of its simple method of production, high production potential, and nutritious and curative properties [23]. Antioxidants found in nature are gaining popularity as powerful antioxidants for eliminating the free radicals that are produced by constantly changing pollutants in the atmosphere and lifestyle habits [24], which interfere with the human body's defence systems. The human immune system has well-constructed antioxidant defence systems to safeguard against damage from oxidative stress [25], which encompass enzyme-mediated defences such as glutathione peroxidase, superoxide dismutase, and catalase, as well as non-enzymatic defences such as vitamins (A, C, E), carotenoids, organic phenols, flavonoids, alkaloids and other antioxidant substances [26].

According to Mustonen *et al.* [27], probable negative effects on mice's cardiac and skeletal muscles were noticed after feeding 9 g/kg of mushroom powder daily for five days. The researchers also discovered a drop in lymphocytes and white blood cell counts in the animals. Even though the majority of mushrooms' safety profile has not yet been fully assessed [28], it is widely accepted that medications produced from naturally occurring substances are safer than manufactured alternatives.

The research study investigated the phenolic constituents and antioxidant activity (DPPH scavenging activity, ABTS test, FRAP test, and phosphomolybdenum assay) of five distinct solvent extracts of *Pleurotus pulmonarius* mushroom. To determine the potential for human intake and health, their enzymatic and non-enzymatic antioxidant characteristics were investigated. While previous studies have examined the antioxidant properties of commercially cultivated *Pleurotus* species, there is limited information on wild *P. pulmonarius* from specific ecological niches in India. Our study investigates *P. pulmonarius* from the Ranni Forest Division of Kerala, which represents a unique ecological zone with distinct environmental conditions that may influence the phytochemical composition of mushrooms. Furthermore, we provide a comprehensive comparative analysis of extraction efficiency across five different solvents, which offers practical insights for optimizing the extraction of bioactive compounds from wild mushrooms.

Despite numerous studies on cultivated *Pleurotus* species, wild strains remain underexplored, particularly in biodiverse regions like the Western Ghats. Previous research has focused largely on commercial varieties, with limited data on the phytochemical richness and therapeutic potential of wild *P. pulmonarius* strains. Studies have shown that regional and environmental factors can significantly influence metabolite profiles in wild mushrooms, yet similar insights into Indian forest-origin strains are lacking. This gap is especially pronounced for mushrooms sourced from the Ranni forest division, a region known for its unique ecological conditions.

Given this context, we hypothesize that *P. pulmonarius* collected from the Ranni forest may possess distinct phytochemical properties and enhanced bioactivities compared to cultivated strains. Therefore, the present study aims to (i) quantify the major bioactive compounds in extracts of *P. pulmonarius* using different solvents, (ii) evaluate their antioxidant, anti-inflammatory, and antidiabetic activities in vitro, and (iii) identify the most effective extract for potential nutraceutical development.

Materials and methods

Sample collection and culture

The mushrooms were collected in June 2022 from the Ranni Forest Division in Pathanamthitta District, Kerala (9° 22′ N, 76° 46′ E). Dr. Kumar from the Department of Botany at Madras Christian College in Chennai taxonomically identified the mushroom (Fig. 1). A genomic strain has been submitted to GenBank under accession number QQ727110. The collected *Pleurotus pulmonarius* mushroom was then cultivated on paddy straw to enhance the quantity. This involved preparing the spawn, substrate, and mushroom beds. The first flush of mushrooms was harvested on the 21st day, followed by two subsequent harvests. A total of approximately 25 kg of mushrooms were gathered from these three consecutive harvests, utilizing 20 prepared beds. After harvesting, the mushrooms were cleaned and air-dried in the shade. They were then crushed into a fine powder using a blender and stored in an airtight container.

Extraction of active compounds

The powdered sample was placed into a Soxhlet apparatus extraction thimble. Five solvents, hexane, ethyl acetate, methanol, chloroform, and

acetone were used for the extraction. The resulting residues were in a condensed form. For dilution, the herbal extracts were mixed with appropriate solvents to achieve a concentration of 1 mg/ml. Their antioxidant effectiveness was then assessed using a variety of procedures.

Antioxidant activity

Mushrooms possess antioxidant properties that inhibit oxidation through various mechanisms. Generally, mushrooms contain two types of antioxidants: primary (free radical scavengers) and secondary (defensive) [29,30]. Secondary antioxidants are formed by deactivating metals and blocking lipid hydroperoxides, while primary antioxidants are regenerated through various processes. Mushroom compounds exhibit antioxidant capabilities by acting as cell signals, thereby altering gene expression and activating enzymes to eliminate reactive oxygen species [31]. Edible mushrooms, particularly *P. pulmonarius*, have been linked to a hypocholesterolemic response [32] and the inhibition of lipid oxidation, a process closely associated with cellular oxidative stress. While oxidation is crucial for organisms to generate energy for bodily functions, a common method to assess antioxidant capacity is by scavenging free radicals [33], which in turn inhibits lipid oxidation. Free radicals, particularly those derived from oxygen, are detrimental to human health due to their unpaired electrons and high reactivity [34]. During oxidative stress, these free radicals damage cells, tissues, organs, proteins, RNA, and DNA, leading to cellular destruction and apoptosis. This cellular damage can contribute to various health issues, including cardiovascular disorders, diabetes, premature aging, and various types of cancer [35].

Estimation of total phenol

The spectrophotometric technique was employed to quantify the total phenolic content [36]. First, 1 mL of the sample (1 mg/mL) was mixed with 1 mL of Folin-Ciocalteu's phenol reagent. After five minutes, 13 mL of deionized water and 10 mL of 7% sodium carbonate were added and stirred. The resulting solution was then incubated in a dark room at 23°C for approximately 90 minutes. Finally, the absorbance of the mixture was measured at 750 nm. A calibration curve was generated using an alcohol solution as the standard, and the total phenol content was expressed in mg catechol/g of dry weight. All determinations were performed in triplicate and are presented as mean ± SD.

Estimation of total flavonoid

The total flavonoid content was determined spectrophotometrically with slight modifications [37]. One gram of powdered sample was weighed, and 200 mL of 80% aqueous methanol was added. The mixture was ground using a mortar and pestle, and the resulting solution was filtered to obtain a clear filtrate. In a test tube, 0.5 mL of the extract was mixed with 0.3 mL of 5% sodium nitrite and 3 mL of distilled water. The mixture was allowed to stand at room temperature for 5 minutes, after which 0.6 mL of 10% aluminum chloride solution was added. Six minutes later, 1 mL of 1 M NaOH was introduced, and the final volume was adjusted to 10 mL with distilled water. After 15 minutes of incubation, the absorbance was measured at 510 nm. Quercetin was used as the standard.

Estimation of alkaloids

The extracts were evaporated to dryness, dissolved in 2 N HCl, and filtered. A 1 mL aliquot of the filtrate was transferred into a separating funnel and washed three times with 10 mL chloroform. Subsequently, 0.1 N sodium hydroxide, 5 mL bromocresol green, and 5 mL phosphate buffer were added, and the mixture was thoroughly shaken. Extraction was then carried out sequentially with 1, 2, 3, and 4 mL portions of chloroform, each with vigorous shaking. The combined chloroform extracts were collected in a 10 mL volumetric flask and diluted to volume with chloroform. An atropine standard (1 mg dissolved in 1 mL) was used to construct the calibration curve, and absorbance was measured at 470 nm.

Estimation of antioxidant activity

Radicle scavenging activity (DPPH assay)

The antioxidant activity of the mushroom extracts was evaluated using the DPPH radical scavenging assay [38]. Sample solutions were prepared by diluting the extracts with methanol to a final volume of 100 µL, using different concentrations for each extract. Ascorbic acid served as the standard reference. To each sample, 5 mL of 0.1 mM DPPH solution in methanol was added and thoroughly mixed. The reaction mixtures were incubated at 27°C for 20 minutes in the dark. Absorbance was then measured at 517 nm to determine the free radical scavenging activity. All measurements were performed in triplicate, and the results were expressed as mean ± SD. The percentage of DPPH radical scavenging was calculated using the following formula:

$$\textbf{(\%)} = \textbf{[(Control – Sample) / Control]} \times \textbf{100}$$

ABTS scavenging activity

The ABTS radical scavenging activity was evaluated following the standard protocol with slight modifications [39]. The ABTS working solution was prepared by mixing 2.46 mL potassium persulfate with 1 mL ABTS solution and incubating the mixture in clear glass vials at room temperature in the dark for approximately 16 hours. The absorbance of the resulting ABTS- $^{+}$ solution was adjusted using a spectrophotometer prior to sample analysis. For the assay, 1 mL of mushroom fruiting body extract was added to the ABTS working solution and incubated at room temperature in the dark for 2 hours. The reduction in absorbance was measured at 734 nm, and antioxidant activity was expressed as Trolox equivalent antioxidant capacity (TEAC). All experiments were performed in triplicate, and data are presented as mean ± SD.

Phosphomolybdenum assay

The amount of green phosphomolybdenum complexes produced by the samples was used to measure their activity [40]. Mix 1 ml of reagent solution (28 ml Na_3PO_4 (sodium phosphate), 0.6 ml H_2SO_4, and 4 mL $(NH_4)_6Mo_7O_{24}$ (ammonium molybdate)) with 100 µl of fruiting body extract in 1 mM dimethyl sulfoxide. Following their sealing, the vials were incubated for ninety minutes at 95°C in a water bath. When the mixture attained the appropriate temperature, a blank sample was added to calculate the absorption at 695 nm. Assays were run in triplicate, and data expressed as mean ± SD.

Ferric reducing antioxidant power (FRAP)

The method is based on the reduction of Fe^{3+} TPTZ complex (colorless complex) to Fe^{2+} tripyridyltriazine (blue colored complex) formed by the action of electron-donating antioxidants at low pH. This reaction is monitored by measuring the change in absorbance at 593 nm. The Ferric reducing antioxidant power (FRAP) reagent was prepared by mixing 300 mM acetate buffer, 10 ml TPTZ in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ in the proportion of 10:1:1 at 37°. Freshly prepared working FRAP reagent was pipetted using a 1-5 ml variable micropipette (3.995 ml) and mixed with 5 µl of the appropriately diluted samples and mixed thoroughly. An intense blue color complex was formed when ferric tripyridyl triazine (Fe^{3+} TPTZ) complex was reduced to ferrous (Fe^{2+}) form, and the absorbance at 593 nm was recorded against a reagent blank (3.995 ml FRAP reagent+5 µl distilled water) after 30 min

incubation at 37°. All the determinations were performed in triplicate. The calibration curve was prepared by plotting the absorbance at 593 nm versus different concentrations of $FeSO_4$. The concentrations of $FeSO_4$ were, in turn, plotted against the concentration of standard antioxidant trolox. The FRAP values were obtained by comparing the absorbance change in the test mixture with those obtained from increasing concentrations of Fe^{3+} and expressed as mg of Trolox equivalent per gram of sample.

In vitro anti-inflammatory activity of Pleurotus pulmonarius crude extracts

Protein denaturation method [41]

The anti-inflammatory protein denaturation method is based on the principle that anti-inflammatory agents can inhibit the denaturation of proteins, a process commonly associated with inflammation. Protein denaturation occurs when external stressors, such as heat, pH changes, or chemicals, disrupt the secondary and tertiary structures of proteins, leading to a loss of their biological functions.

In this method, a protein such as bovine serum albumin is used as a model to study the effects of test compounds, including exposure to heat or altered pH. Anti-inflammatory compounds work by stabilizing protein structures or counteracting the factors causing denaturation, thereby reducing the denaturation process. The degree of inhibition is evaluated by measuring changes in turbidity or spectrophotometric absorbance, with results expressed as the percentage inhibition of protein denaturation. This straightforward and reproducible technique is commonly used to screen plant extracts, pharmaceuticals, and natural products for their potential anti-inflammatory activity, serving as an initial step before more complex biological evaluations.

A volume of 2 ml of each sample extract and standard (Diclofenac sodium) at varying concentrations was measured. This was then combined with 2.8 ml of phosphate-buffered saline (pH 7.4) to create a reaction mixture with a total volume of 5 ml. For the control, 2 ml of triple-distilled water, 0.2 ml of a 1–2% BSA solution, and 2.8 ml of phosphate-buffered saline were mixed to achieve a total volume of 5 ml. The reaction mixtures were incubated at 37 ± 2°C for 30 minutes, followed by heating in a water bath at 70 ± 2°C for 15 minutes. After cooling, the absorbance was measured at 280 nm using a suitable UV/Vis spectrophotometer, with triple-distilled water as the blank. Each concentration was tested in triplicate; percentage inhibition values are mean ± SD

The following equation was used to determine the % inhibition of protein denaturation.

$$\text{Percentage inhibition} = [A_B - A_S] \times 100 / A_C$$

Were,

A_C - Absorbance of control
A_S - Absorbance of the test sample
A_B - Absorbance

Then plant extract concentration for 50% inhibition (IC_{50}) was determined by plotting the percentage inhibition concerning control against the concentration

In vitro antidiabetic activity of Pleurotus pulmonarius crude extracts

Alpha amylase inhibition assay [42]

Maltose is a reducing disaccharide. It reduces pale yellow-colored alkaline 3,5-dinitrosalicylic acid to orange-colored 3-amino, 5-nitrosalicylic acid. The intensity of the color is proportional to the concentration of maltose, and its absorbance is measured colorimetrically at 540 nm.

A 20 mM phosphate buffer (pH 6.9) was used to dissolve 1 ml of porcine pancreatic α-amylase (PPA; A05329G191). A standard concentration of 1 μg/ml was used to prepare the stock solution of the compounds, from which various concentrations were made. To obtain a clear solution, potato starch (0.5% w/v) was dissolved in 20 mM phosphate-buffered saline (pH 6.9) and then placed in a boiling water bath.

A reaction mixture was prepared by combining 400 μl of starch solution, 160 μl of distilled water, and 40 μl of extracts. To initiate the reaction, 200 μl of the enzyme solution was added, and the tubes were incubated at room temperature (25°C) for three minutes. The addition of the enzyme solution occurred on a one-minute cycle following the start of the reaction. After incubation, 200 μl of the mixture was transferred into a separate test tube containing 100 μl of DNS color reagent (prepared by dissolving 50.68 g sodium potassium tartrate in 70 ml of 2 M NaOH with 0.026 mM of 3,5-dinitrosalicylic acid). The mixture was then kept in a water bath at 85–90°C for 15 minutes. After cooling, the solution in each tube was diluted with 900 μl of distilled water, and the absorbance was measured at 540 nm. For each sample concentration, a blank was prepared by replacing the enzyme solution with 200 μl of distilled water. Acarbose, a well-known α-amylase inhibitor, was used as the standard drug. All reactions were performed in triplicate, and IC_{50} values were calculated from mean ± SD data.

$$\text{The α-amylase inhibitory activity} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

The concentration of acarbose and mushroom extracts required to inhibit 50% of α-amylase activity under the conditions was defined as the IC_{50} value. The α-amylase inhibitory activities of plant extract and acarbose were calculated, and their IC_{50} values were determined. Absorbance of control = 0.998nm

Statistical analysis

All experiments were conducted in triplicate, and the results are expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to determine the significance of differences among the various extract treatments. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

Table 1
The total phenol, flavonoid, and alkaloid content in the fruiting body of five solvent extracts of *Pleurotus pulmonarius*.

Sl/No.	*P. pulmonarius* extracts	TP concentration in the extract (μg/ml)	TF concentration in the extract (μg/ml)	TA concentration in the extract (μg/ml)
1	Acetone	0.4521 ± 0.0127	0.5043 ± 0.0032	0.3523 ± 0.0041
2	Chloroform	0.3508 ± 0.0016	0.2287 ± 0.004	0.483 ± 0.0022
3	Ethyl acetate	0.5376 ± 0.0072	0.5299 ± 0.015	0.5262 ± 0.0166
4	Hexane	0.2448 ± 0.0044	-	-
5	Methanol	0.8582 ± 0.0292	0.8434 ± 0.0198	0.2785 ± 0.0039

TP;Total Phenol, TF;Total Flavanoid, TA;Total Alkaloid

Results

Total phenolic compounds

The whole phenolic components of the mushroom extracts made from *Pleurotus pulmonarius* fruiting bodies are displayed in Table 1. The methanol extract exhibited significantly higher total phenolic content (0.8369 ± 0.02 μg/ml) and flavonoid content(0.8314 ± 0.02 μg/ml) compared to other solvents ($p < 0.05$), suggesting its enhanced extraction efficiency for polyphenols. While alkaloids are mostly present in ethyl acetate and chloroform extracts, followed by acetone, methanol, and hexane. Fig. 2 shows the graphical data used to compute the

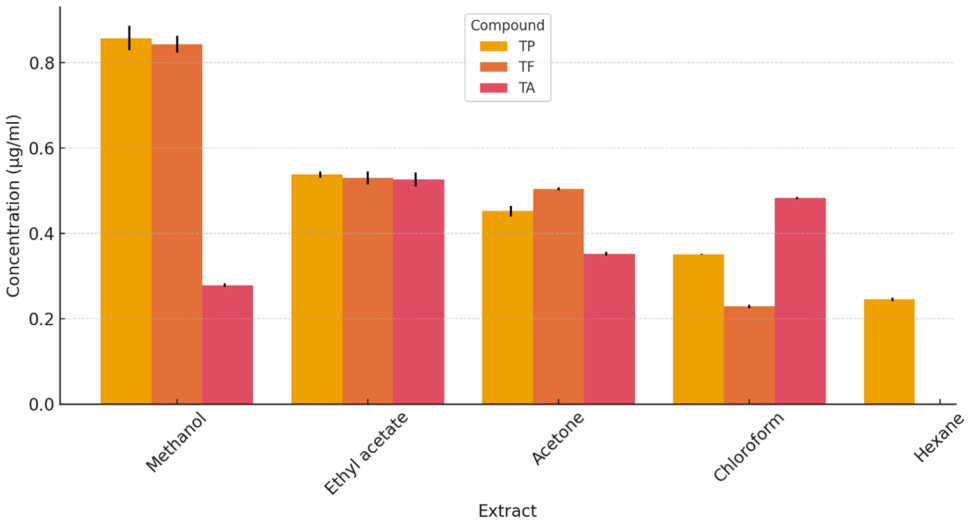

Fig. 2. Estimation of phytoconstituents. The X axis illustrates the solvent extracts used, and the Y axis shows the activity of the phytoconstituents in the five extracts of *P. pulmonarius*.

Table 2
The DPPH activity in the fruiting body of five different solvent extracts of P. pulmonarius.

Sl/ No.	Conc. (µg/ml)	Absorbance of sample(nm)					
		Ascorbic acid	Methanol extract	Ethyl Acetate extract	Chloforom extract	Acetone extract	Hexane extract
1	0.2	0.058	0.0844 ± 0.0017	0.0978 ± 0.0038	0.1245 ± 0.0064	0.186 ± 0.0052	0.2949 ± 0.0049
2	0.4	0.041	0.0793 ± 0.0043	0.0825 ± 0.0042	0.0966 ± 0.0046	0.138 ± 0.0088	0.2747 ± 0.0112
3	0.6	0.030	0.0664 ± 0.0014	0.0784 ± 0.0022	0.0825 ± 0.004	0.1071 ± 0.0036	0.2166 ± 0.0088
4	0.8	0.019	0.048 ± 0.0021	0.0693 ± 0.0029	0.0764 ± 0.001	0.0925 ± 0.0024	0.1888 ± 0.0045
5	1.0	0.010	0.0383 ± 0.0034	0.0803 ± 0.0021	0.0668 ± 0.0021	0.0863 ± 0.0018	0.1321 ± 0.0068
	IC50 value	0.5122 µg/ml	0.6356 µg/ml	0.7113 µg/ml	0.9333 µg/ml	0.9685 µg/ml	1.1655 µg/ml

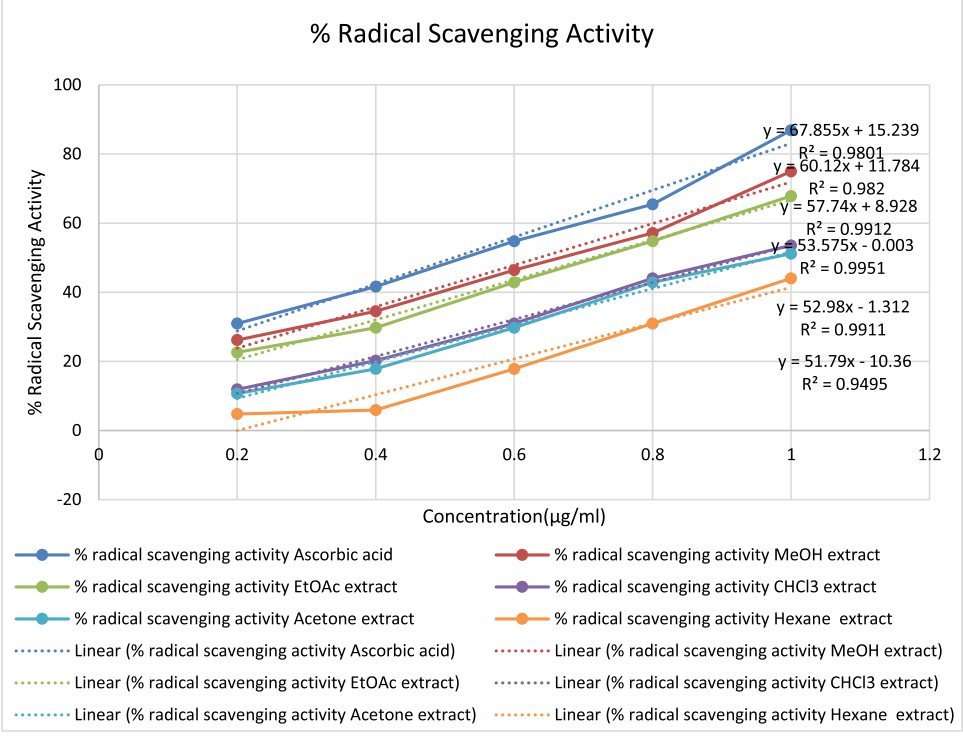

Fig. 3. Demonstrates the DPPH radicle activity and IC50 value of 5 solvent extracts of *P. pulmonarius. The* X axis represents the extract concentration in µg/ml and the Y axis represents the % of absorption in OD value.

Table 3
The ABTS radicle activity in the fruiting body of different solvent extracts of P. pulmonarius.

Conc. (µg/ml)	Ab std (nm)	% RSA	Ab Hexane	% RSA	Ab $CHCl_3$	% RSA	Ab Ethyl Acetate	% RSA	Ab Methanol	% RSA	Ab Acetone	% RSA
0.2	0.062	37.37	0.0925 ± 0.0015	8.08	0.0773 ± 0.0022	20.2	0.0735 ± 0.0038	27.27	0.0649 ± 0.0025	34.34	0.067 ± 0.0013	30.3
0.4	0.059	40.4	0.0855 ± 0.0035	15.15	0.0739 ± 0.0047	24.24	0.0673 ± 0.0032	30.3	0.0599 ± 0.003	38.38	0.0681 ± 0.0037	32.32
0.6	0.056	43.43	0.0835 ± 0.0034	18.18	0.0691 ± 0.0023	28.28	0.0617 ± 0.003	34.34	0.0586 ± 0.0016	40.4	0.0654 ± 0.0014	35.35
0.8	0.053	46.46	0.0755 ± 0.0018	23.23	0.0672 ± 0.0018	30.3	0.0642 ± 0.0008	36.36	0.0591 ± 0.0025	41.41	0.0623 ± 0.0027	38.38
1	0.05	49.49	0.0679 ± 0.0035	27.27	0.0644 ± 0.0013	34.34	0.0591 ± 0.0018	38.38	0.0549 ± 0.0014	45.45	0.0579 ± 0.0051	40.4
IC50 value		5.168 µg/ml		9.805 µg/ml		9.56 µg/ml		8.895 µg/ml		6.962 µg/m		8.578 µg/m

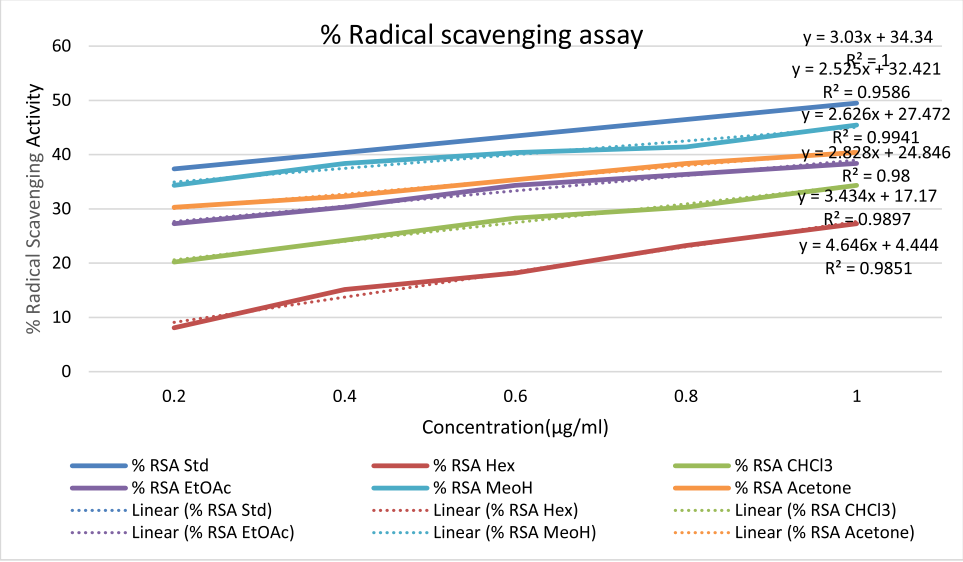

Fig. 4. Demonstration of ABTS radicle activity and IC50 value of 5 solvent extracts of *P. pulmonarius. The* X axis represents the extract concentration in µg/ml, and the Y axis represents the % of absorption in OD value.

concentrations of phenol, flavonoid, and alkaloids in the extract. Statistical analysis confirmed that the differences in activity among the

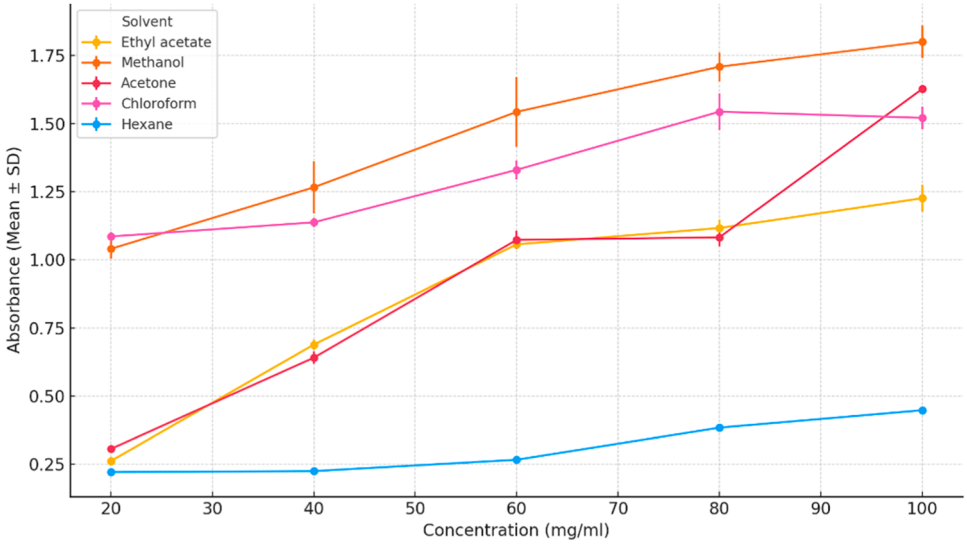

Fig. 5. Phosphomolybdenum assay of different concentrations in 5 solvent extracts of *P. pulmonarius.* The X axis represents the extract's concentration in mg/ml, and the Y axis represents the % of absorption in nm.

Table 4
The phosphomolybdenum assay activity in the fruiting body of five different solvent extracts of P. pulmonarius.

Sl/ No.	Conc. (mg/ ml)	Ethyl acetate	Methanol	Acetone	Chloroform	Hexane
1	20	0.2622 ± ± 0.0161	1.0403 ± 0.0359	0.3065 ± ± 0.0043	1.0856 ± 0.0154	0.2214 ± ± 0.0078
2	40	0.6888 ± ± 0.0189	1.2659 ± 0.0954	0.6408 ± ± 0.0232	1.1374 ± 0.0176	0.225 ± 0.003
3	60	1.0566 ± ± 0.0056	1.543 ± 0.1281	1.0731 ± ± 0.0342	1.3301 ± 0.0342	0.2667 ± ± 0.0136
4	80	1.1165 ± ± 0.0307	1.7087 ± 0.0531	1.0823 ± 0.032	1.5437 ± 0.0672	0.3842 ± ± 0.0047
5	100	1.2259 ± 0.05	1.8007 ± 0.0597	1.6271 ± ± 0.0159	1.5211 ± 0.0418	0.448 ± 0.0108

extracts were significant ($p < 0.05$), with methanol extract consistently showing the most potent bioactivity across all assays, as determined by ANOVA and Tukey's test.

DPPH assay

In the DPPH assay, the methanol extract demonstrated the strongest free radical scavenging activity (IC_{50} = 0.6356 μg/ml), significantly outperforming acetone (IC_{50} = 1.2451 μg/ml) and hexane (IC_{50} = 2.0968 μg/ml). These results correlate with its high phenolic content, indicating a strong antioxidant mechanism, as shown in Table 2. Hexane extract demonstrates the least effective scavenging function. These data suggest that methanol and ethyl acetate extracts perform better in terms of scavenging reactive radicals. Fig. 3 shows the graphical representation of the DPPH scavenging activity of five *P. pulmonarius* solvent extracts. Statistical analysis confirmed that the differences in activity among the extracts were significant ($p < 0.05$), with methanol extract consistently showing the most potent bioactivity across all assays, as determined by ANOVA and Tukey's test.

ABTS assay

When potassium persulfate and ABTS react, a blue or green ABTS chromophore is formed in ABTS radical scavenging test. Here, acetone and methanol successfully scavenged the ABTS radicals. Chloroform was the extract with the least activity among the five solvents, which represented in Table 3. Fig. 4 shows the graphical illustration of the ABTS experiment. Statistical analysis, using ANOVA and Tukey's test, confirmed these differences were significant ($p<0.05$).

Phosphomolybdenum assay

The current study used the phosphomolybdenum test to assess the overall antioxidant capacity of different mushroom solvent extracts. This process reduces Mo (IV) to Mo (V), producing Mo (V) green phosphate compounds with a maximum absorption of 695 nm. At different concentrations, the most effective extracts of *P. pulmonarius* were methanol and chloroform, followed by acetone and ethyl acetate. Across all concentrations, the hexane extract is the least active. Fig. 5 displays the graphical data of the 5 extracts. The methanol extract consistently showed the most potent bioactivity across all assays. This was statistically significant ($p<0.05$), as confirmed by ANOVA and Tukey's test, which revealed significant differences in activity among the extracts (Table 4).

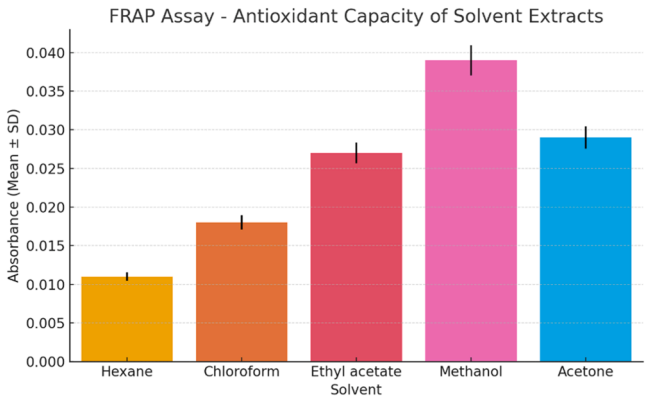

Fig. 6. FRAP activity and absorbance of 5 solvent extracts of *P. pulmonarius*. *The* X axis represents the extract concentration in μg/ml, and the Y axis represents the absorbance value.

Table 5
The antioxidant activity of the FRAP assay in the fruiting body of five different solvent extracts of P. pulmonarius.

Extract	Absorbance value	FRAP value (mg Trolox equivalent)
Hexane	0.0111 ± 0.0006	16.16
Chloroform	0.0172 ± 0.0012	23.08
Ethyl acetate	0.0276 ± 0.0014	31.98
Methanol	0.0384 ± 0.0028	43.84
Acetone	0.0292 ± 0.001	33.95

Table 6
IC50 value of in vitro anti-inflammatory activity of extracts.

Extract	IC_{50} Value (μg/ml)
Diclofenac sodium	2.529± 0.011
Methanol extract	3.347±0.012
Ethyl acetate extract	4.296±0.022
Chloroform extract	5.259±0.013
Acetone extract	4.752±0.011
Hexane extract	4.752± 0.021

FRAP assay

The total antioxidant potential of the sample was determined using the ferric reducing ability of plasma FRAP assay by Benzie and Strain as a measure of antioxidant power. The assay was based on the reducing power of a compound (antioxidant). A potential antioxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}). Methanol shows the highest reducing power, followed by acetone and ethyl acetate. Fig. 6 shows the graphical representation of the FRAP assay of 5 solvent extracts (Table 5).

In vitro anti-inflammatory activity of Pleurotus pulmonarius extracts

The crude extracts of *Pleurotus pulmonarius* exhibited concentration-dependent anti-inflammatory activity. The standard compound showed maximum inhibition (78.79%) at 1 μg/ml. Among tested extracts, the methanol extract was most potent, showing 68.74% inhibition at 1 μg/ml (IC_{50} = 3.35 μg/ml). Ethyl acetate extract had moderate activity (58.29% inhibition, IC_{50} = 4.30 μg/ml). Acetone and hexane extracts displayed similar effectiveness (58.29% and 54.07% inhibition, respectively; IC_{50} = 4.75 μg/ml). The chloroform extract had the lowest activity, with 48.64% inhibition (IC_{50} = 5.26 μg/ml). (Table 6) (Fig. 7)

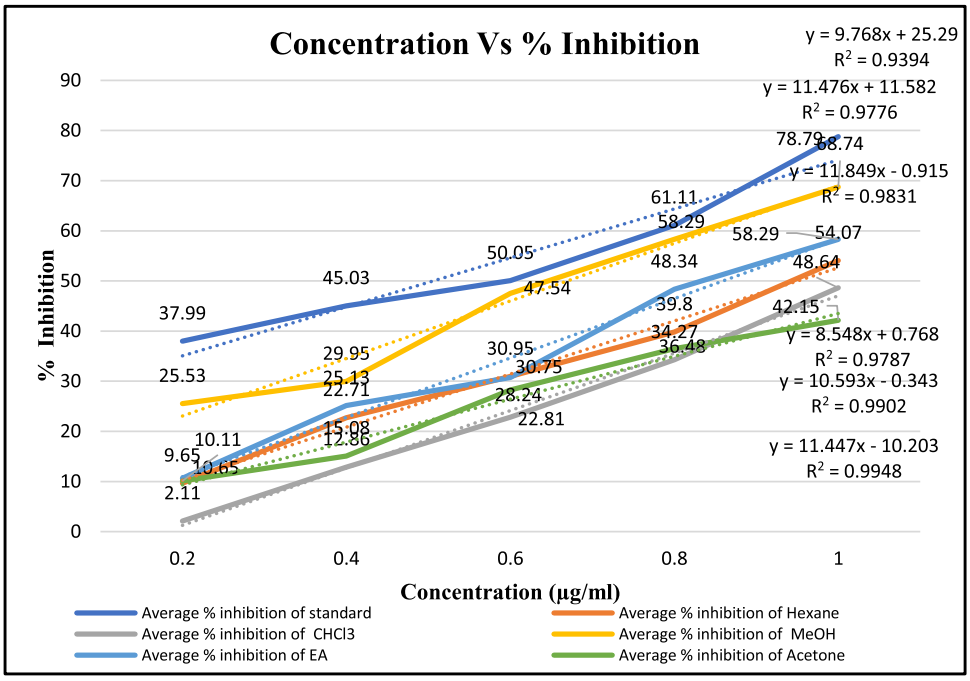

Fig. 7. Anti-inflammatory activity of five extracts of *Pleurotus pulmonarius*using different concentrations of sample.

Table 7
IC_{50} value of *in vitro*anti-diabetic activity of extracts.

Extract	IC_{50} Value (µg/ml)
Acarbose	0.619±0.014
Methanol extract	1.39±0.021
Ethyl acetate extract	2.91±0.021
Chloroform extract	3.31±0.024
Acetone extract	2.63±0.021
Hexane extract	17.19±0.041

In vitro anti-diabetic activity of Pleurotus pulmonarius extracts

Pleurotus pulmonarius is recognized for its nutritional and medicinal value, notably its antidiabetic potential. This study evaluated the anti-diabetic effects of extracts through α-amylase inhibitory activity assays in vitro, using acarbose as the standard. Extracts at concentrations from 0.2 to 1 µg/ml were tested to determine IC_{50} values. Methanol extract exhibited the highest antidiabetic potency (IC_{50} = 1.39±0.021 µg/ml), followed by acetone (IC_{50} = 2.63±0.021 µg/ml) and ethyl acetate (IC_{50} = 2.91±0.021 µg/ml). Hexane extract showed the lowest activity, with an

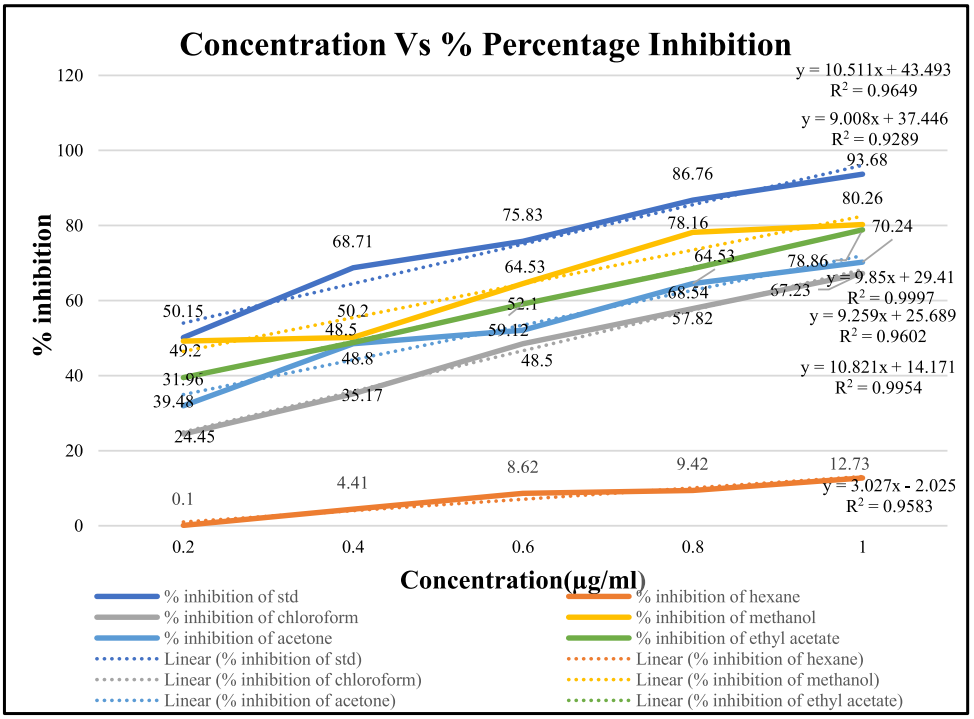

Fig. 8. Anti-diabetic activity of the extracts of *Pleurotus pulmonarius*using different concentrations of sample.

IC_{50} of 17.19±0.041 μg/ml, indicating minimal antidiabetic potential relative to other extracts. (Table 7) (Fig. 8).

Discussion

In recent years, mushroom consumption has increased significantly due to their nutritional and pharmacological benefits. Mushrooms are rich in phenolic compounds, such as flavonoids and alkaloids, known for potent antioxidant, anti-inflammatory, and antidiabetic properties [1, 7–9]. Our study revealed that methanol extracts had the highest total phenolic content, aligning with previous findings by other researchers demonstrating methanol's effectiveness in extracting polyphenolic antioxidants [45].

The strong correlation between phenolic content and antioxidant capability suggests flavonoids and phenolic acids are significant contributors to observed antioxidant activities [43,44]. In our experiments, methanol, ethyl acetate, and acetone extracts exhibited robust scavenging activity, especially methanol, which demonstrated substantial efficacy in neutralizing free radicals through DPPH assays (IC50 = 0.6356 μg/ml), consistent with similar studies [46].

ABTS assay results showed that acetone and methanol extracts effectively scavenged radicals, indicating potential use in managing oxidative stress-related conditions [47]. Our phosphomolybdenum assay identified methanol and chloroform extracts as possessing the highest antioxidant capabilities, further supporting previous research highlighting methanol extracts' potent antioxidative effects [48].

In evaluating the anti-inflammatory activity, the methanol extract showed notable potency (IC50 = 3.35 μg/ml), supporting its potential therapeutic use in inflammatory conditions. Similarly, the antidiabetic assessment demonstrated methanol extract's significant α-amylase inhibitory activity (IC50 = 1.39 μg/ml), comparable to standard antidiabetic drugs like acarbose.

These findings underscore *P. pulmonarius* extracts' potential as natural therapeutic agents in dietary supplements and pharmaceutical formulations, particularly emphasizing methanol extracts due to their superior antioxidant, anti-inflammatory, and antidiabetic activities [49, 50].

Conclusion

This study comprehensively highlights the pharmacological potential of *Pleurotus pulmonarius*, particularly in its antioxidant, anti-inflammatory, and antidiabetic properties. The methanol extract consistently demonstrated the highest efficacy across multiple assays, revealing a rich profile of phenolic and flavonoid compounds responsible for these bioactivities. The observed IC50 values in DPPH, ABTS, anti-inflammatory, and α-amylase inhibition assays establish its comparative potency and reinforce its promise as a multi-functional therapeutic agent.

The novelty of this study lies in its investigation of a wild strain of *P. pulmonarius* collected from the Ranni forest, an ecologically unique and underexplored region of the Western Ghats. Such wild-sourced mushrooms are known to possess distinct phytochemical characteristics due to environmental influences, offering bioactivity profiles that may differ significantly from those of cultivated strains. By providing a detailed phytochemical and bioactivity profile of this strain, our research contributes new knowledge to the field of mushroom biotechnology and natural product pharmacology.

Given the growing demand for natural health-promoting products, this study not only supports the ethnopharmacological relevance of wild mushrooms but also underscores their commercial viability as functional foods and nutraceuticals. Future directions should involve bioassay-guided fractionation and structural elucidation of the active compounds, validation through in vivo models, and standardization for potential clinical use. Furthermore, sustainable harvesting and cultivation techniques must be developed to protect biodiversity while enabling broader application of this natural resource.

Overall, the present work sets a strong foundation for integrating forest-derived edible fungi like *P. pulmonarius* into modern health systems, paving the way for eco-conscious drug discovery and the development of dietary interventions aimed at mitigating oxidative stress, inflammation, and metabolic disorders.

Data availability statement

The data will be made available on request.

Funding statement

No funding was received for this research.

CRediT authorship contribution statement

S Aswathy: Investigation, Data curation. **J Manjunathan:** Supervision, Conceptualization. **Madhu Kanta:** Resources. **G Vijetha:** Methodology, Formal analysis. **K Anu:** Writing – original draft, Methodology. **Yuvaraj Dinakarkumar:** Writing – review & editing, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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