Assessment of phytochemicals of an indigenous white rot fungus isolated from AJK, Pakistan

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Abstract

Medicinal mushrooms offer a wide range of pharmacological properties as well as other significant health advantages owing to the presence of phytochemicals and other chemicals. This study was conducted for the chemical analysis of *Polyporus versicolor*, a white rot fungus and mushroom specie, naturally growing in the forests of AJK and Pakistan. For proximate analysis, *P. versicolor* extracts were prepared in different solvents (methanol, ethanol and aqueous) and utilized to assess the level of various primary parameters as well as phytochemicals like flavonoid, tannins and phenols. The extracts were further characterized with HPLC and FTIR analysis. The results demonstrate the higher moisture contents and dietary fibers in methanolic extract, higher crude lipids in ethanolic extract and higher carbohydrates & protein in aqueous extract. Significantly high amount of total phenols (7.26 mg/g), total flavonoids (14.53mg/g) and total tannins (4.36mg/g) observed in methanolic extract compared to other two extracts. The HPLC analysis revealed the presence of quercetin (a flavonoid) at a substantial level. FTIR analysis further confirms the representative functional groups of the flavonoids in the samples. These findings confirm the presence of medicinally important constituents in the *P.versicolor.* Further studies are suggested for further exploitation of its biological applications.

Key Words: White rot fungi, Phytochemicals, *P.versicolor,* flavonoid

Introduction

In the realm of natural products with therapeutic potential, medicinal mushrooms have emerged as a fascinating and promising area of research. With a rich history deeply rooted in traditional medicine across diverse cultures, these fungi have garnered attention for their potential bioactive compounds and their impact on human health. The exploration of medicinal mushrooms has become particularly pertinent in the context of advancing scientific methodologies, allowing for a comprehensive assessment of their diverse activities at both molecular and cellular levels. *Polyporus versicolor* also known as *Tramerer versicolor* and *Coriolus versicolor* found throughout the world. It commonly grow on the trunk of the trees and has various medicinal

uses. It is white rot fungi and belongs to the Basidiomycetes division of the fungi. *P. versicolor* contains various biologically active substances including amino acids, proteins and polysaccharopeptide (PSP) [1-3].

Mushrooms, mainly edible, are rich in protein contents with reported cytotoxic properties. There are many from these known for immune-modulatory effects due to fungal immunomodulatory proteins (FIPs). The mechanism of action of these proteins can be diverse but still not well understood. Some of the proteins like lectins, binds reversibly to the mono-saccharides and oligosaccharides with high affinity with ability to identify various proteoglycans and carbohydrates expresses at the surface of cells [4, 5].

Methodology

Collection and Preparation of samples

Polyporus versicolor, also known as *Trametes versicolor* and *Coriolus versicolor,* specimens were collected from the multiple sites in AJK, Pakistan. The specimens were photographed, carefully dried using fans, packed, and subsequently analyzed using a range of molecular techniques, from simple to advance. The desiccated samples were pulverized using an electric grinder, filtered through an 80-mesh screen, and stored in plastic bags at a reduced temperature for future uses.

Chemical analysis

The moisture contents of samples were measured by weighing them before and after overnight high-temperature heating. Crude lipid was measured using 5 grams of sample in 100 ml of ether through the soxhlet device and dietary fiber were measured by using method reported [6]. The mineral contents were analyzed by using an atomic absorption spectrophotometer (Hitachi Model, 170–10). The phenol concentration in mushroom samples was measured by using different solvents and previously established method [7]. After mixing 100 microliters of an extract with three milliliters of distilled water, 0.5 milliliters of Folin-Ciocalteu reagent was added. After 3 minutes, 2 milliliters of 20% sodium carbonate was added and stirred. The color was synthesized and measured at 650 nm using a Shimadzu UV- 1800 spectrophotometer. Gallic acid was utilized as the reference compound to create a standard curve with an R2 of 0.9926. The total phenolic content was measured in milligrams per 100 grams of dry matter using gallic acid equivalents (GAE) [8].

Determination of phytochemicals

The mushroom sample was extracted using three different solvents. The quantification of flavonoid contents in the extracts was performed using a previously published technique [9]. About 1 ml portion of the extract or a standard solution, quercetin (4mg/ml) was introduced into a 10 ml flask containing 4 ml of distilled water. Subsequently, 0.3 ml of a 5% $NaNO_2$ solution was added. 0.3 ml of a 10% solution of $AlCl_3$ was added after a duration of 5 minutes. Subsequently, 2 ml of 1M NaOH was added after 6 minutes, and the total volume was adjusted to 10 ml using distilled water. The spectrophotometer was used to measure the absorbance at a wavelength of 510 nm. The quantities of flavonoids in the samples were quantified as milligrams

of Quercetin equivalent per gram of sample [10]. The Folin Denis technique was used to measure the overall content of tannins in various mushroom extracts. The colorimetric determination of tannins was performed by measuring the blue color resulting from the reduction of phosphotungstomolybidic acid by tannins. A volume of 7.5 ml of distilled water was combined with 1.0 ml of both the extract and the standard solution of tannic acid. Subsequently, 1 milliliter of sodium carbonate was introduced, followed by the addition of 0.5 milliliters of Folin Denis reagent. The solution was diluted with distilled water to a final volume of 10 ml, and the absorbance was measured at a wavelength of 700 nm. The tannic acid concentration was expressed as milligrams of tannic acid equivalent per gram of extract [11].

Isolation of Phytochemicals

The 50 g of powdered mushroom's sample was dissolved in 500 mL ethanol (80%) and put on shaking incubator at 25 ^{0}C for 24 hrs. It was further centrifuged for 10 min at 10 °C with 10000 rpm speed. The mixture obtained was filtered with Whatman filter paper No.41. Filtrate was then kept at room temperature for the evaporation of solvent. The sample was stored at -20 °C before further use in column chromatography and HPLC analysis [12].

Liquid column chromatography (LCC) was performed with silica gel column having mesh size 70- 230 in 80 % methanol. The concentration was enhance by combining 6th and 7th elutions and run again through the silica column (mesh size 230-400) while the solvent system was kept same. The collected elutions were kept for the HPLC analysis following method reported by Fogarasi et al [13].

Analysis of flavonoids with High-Performance Liquid Chromatography

The HPLC analyses of the elutions were performed at system of Shimadzu (Tokyo, Japan) having C18 column (size 250 mm × 4.5 mm, 5 m) and UV/Visible detector. The mobile phase used for the elution of compounds consists of Acetonitrile and 0.1% phosphoric acid with ratio 36:64. The 20µl of each of the sample was injected at a flow rate of 1ml/min 20µl. The analyses of Flavonoids were performed at 280 nm & 285 nm while Quercetin was used as standard. All the analyses were performed in triplicates [14].

FT-IR analysis

The FTIR analysis was performed with FTIR instruments (Model 1:1 FS 25, Bruchure, Germany) to obtain its spectra. A small amount of sample was trampled into pellets with potassium bromide (KBr) and thin film was prepared for FT-IR analysis [15]. The IR transmittance data was collected within the wave number range of 4000 cm-1 to 500 cm-1. Each of the cases was tested in triplicates using KBr pellets, resulting in unambiguous observations. The spectra were compared to a reference that identifies the functional groups present in the samples.

Statistical analysis

Data obtained after the analysis of various organic compounds were further analyzed statistically by using one-way ANOVA and results were expressed in the form of mean, standard deviation, and percentage values by using the method reported earlier [16].

Results

For proximate analysis of *P. versicolor* different extracts were used to determine levels of moisture dietary fiber, ash, crudes, lipids, protein and carbohydrate contents (Table 1). The higher level of moisture content in *P. versicolor* was observed with methanol extraction, measuring 18.5±2.6. % This was followed by ethanol extraction, which yielded a moisture level of 14.6±1.5 % and aqueous extraction, which yielded a moisture content of 14.8±1.2 % Similarly, the extraction of dietary fiber was found to be higher with methanol, followed by ethanol with a value of 23.5±1.6 % and then aqueous extract with a value of 21.8±1.5 %. The ash level was highest in the aqueous extraction method, measuring 2.6±0.7%. It was slightly lower in the methanol extraction method, measuring 2.4±0.5 % and even lower in the ethanol extraction method, measuring 2.2±0.8 %. The higher proportion of Crude lipids was found in the methanol extraction (5.54±0.25 %) and ethanol extraction (5.72±0.12 %) of the examined mushroom, compared to the water extraction (3.83±0.15 %). The protein and carbohydrate content obtained from methanol extraction was somewhat lower compared to ethanol and aqueous extractions (Table 1). Previously, different mushroom species have been undergone for their proximate analysis. Mostly carbohydrate content was found maximum followed by protein content in wild edible mushrooms [17].

Table 1: Proximate analysis of *Polyporus versicolor* (%) for various parameters.

Sr. No.	Extracts	Moisture	Dietary Fiber	Ash	Crude Lipid	Protein	Carbohydrate
1	Methanol	18.5±2.6	24.6±2.8	2.4±0.5	5.54±0.25	30.43±1.2	48.5±2.5
2	Ethanol	14.6±1.5	23.5±1.6	2.2±08	5.72±0.12	31.52±1.3	54.5±1.6
3	Aqueous	14.8±1.2	21.8±1.5	2.6±0.7	3.83±0.15	31.78±1.5	57.3±1.5

Mean ±SD (n=5)

Examination of many samples of *P. versicolor* revealed the presence of significant amounts of phenol, flavonoids, and tannins (Table 2). The measurement of phytochemicals was performed by determining the maximum concentration in methanol, then ethanol, and finally aqueous extractions of *P. versicolor*. The results indicate that *P. versicolor* has elevated concentrations of flavonoids (14.53±2.45 mg/g), followed by total phenol (7.26±1.5 mg/g) and tannins (4.36±1.23 mg/g). Furthermore a comparison of various solvents for phytochemical is shown in figure 1. The results obtained in this investigation were in line with the findings reported earlier [18].

Table 2: Phytochemical analysis of *Polyporus versicolor*

Constituents (mg/g)	Aqueous	Ethanol	Methanol
Total phenol	2.06±0.27	4.28±0.72	7.26±1.52
Total flavonoids	4.13±0.16	5.15±1.38	14.53±2.45
Total tannins	1.33±0.25	2.87±0.54	4.36±1.23

Mean ±SD (n=5)

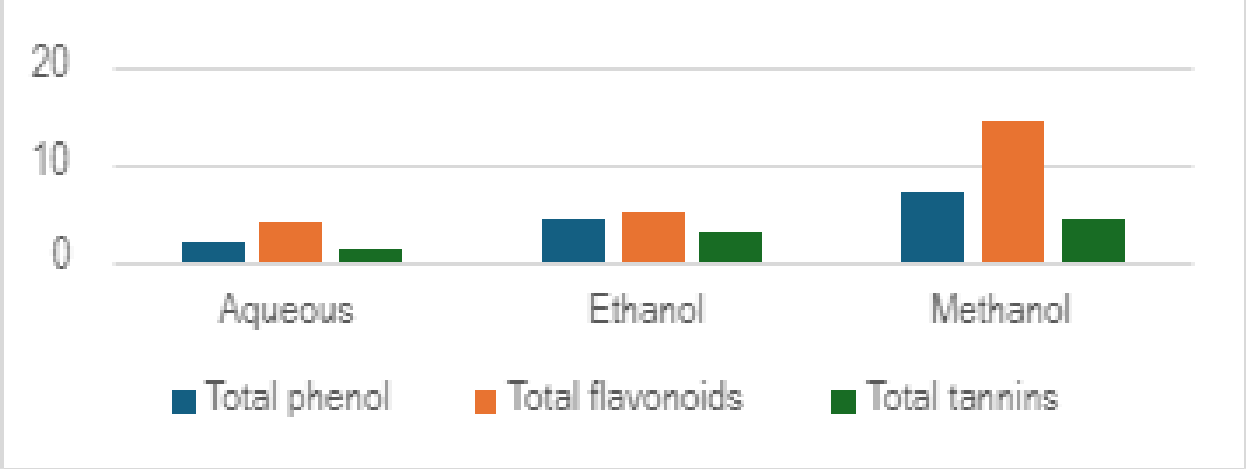

Figure 1 : Phytochemical analysis of mushroom samples

The HPLC analysis of the methanol extract of *P. versicolor* revealed the presence of flavonoids, specifically Quercetin, at a substantial level. This was measured at 750 nm, as shown in Figure 3. The presents of HPLC analysis of flavonoids, specifically Quercetin, was found in current study, however earlier similar results were reported in different mushroom species showing, a significant peak of Quercetin [19].

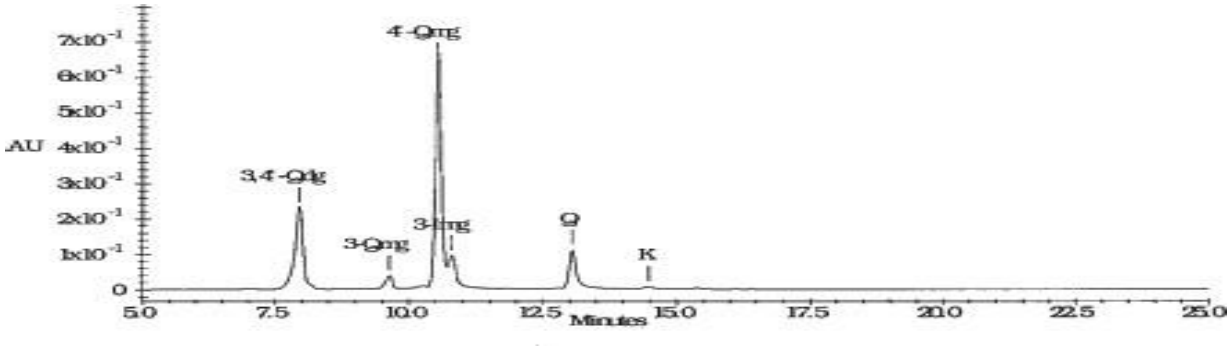

Figure 2: HPLC analysis of *Polyporus versicolor* higher consideration was obtained for quercetin and quercetin 3-O. rutinoside when extract was prepared in three different organic solvents further results are summarized in the following table 3.

Table 3: HPLC analysis of *Polyporus versicolor* for quercetin and quercetin 3-O. rutinoside, Extract were prepared in three different organic solvents

	Quercetin	**Quercetin 3-0 rutinoside**
Methanol	0.62 ± 0.04	0.75 ± 0.08
Ethanol	0.75 ± 0.05	0.82± 0.05
Chloroform	0.56 ± 0.03	0.45 ± 0.06

Data expressed as g/kg mean ± SD (n=5)

Fourier-transform infrared spectroscopy (FT-IR) is a technique that detects and analyzes chemical interactions inside a molecule [20]. It creates a unique fingerprint of the sample, which may be used to examine and identify various components in the sample. FT-IR is a very efficient analytical method used to identify functional groups and characterize covalent bonds. The functional group of quercetin represents as phenol with the hydrogen bonded O-H stretch from the range 3600-3100 cm-1 (Fig. 3).

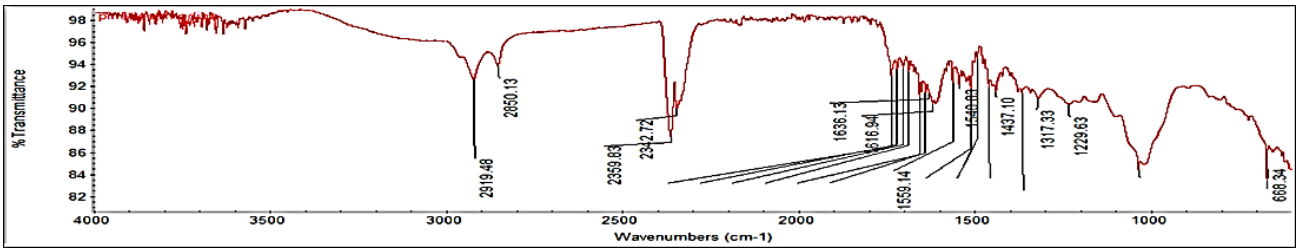

Figure 3: FT-IR analysis of *P. versicolor* showing functional groups at various wavelengths.

Discussion

The study was conducted for the phytochemical analysis of *P.versicolor*. The results confirm the presence of different biomolecules and phytochemicals which are potent antibacterial and antioxidant compounds, concentrations varying from extract to extract [21]. The therapeutic effects of a mushroom can vary greatly depending on its strain, locality, growing medium and conditions, section of the mushroom used, and processing stage, even under identical conditions. All of these substances change the mushroom's constitution, influencing its bioactivity. Phenolic compounds, found everywhere in mushrooms as well as in plants, are essential to the human diet and are appreciated for their antioxidant properties [22].

Results also showed that *P. versicolor* extracts had higher total phenolic content (TPC). Table 1's proximate properties indicate the extracts' feed or food potential. Table 2 shows that phytochemicals in mushrooms can be utilized as food and medication. Thus, the examined materials contain a variety of chemical compounds. The phytochemical investigation of this work identified various secondary metabolites, such as tannins, which are known for their antioxidant qualities and antimutagenic effects. These compounds have also been detected in other mushrooms [23].

Conclusions

Although research on therapeutic mushrooms has advanced rapidly in recent years. However, pharmacological characteristics of many species are still unknown or discovered. The major

focus of current study was on identifying the chemicals present in the extracts, as well as the metabolites responsible for various biological activities. Furthermore, it is crucial to fully understand the individual and collective behaviors of these acts, with a particular focus on the interactions occurring in a controlled laboratory environment and improving the planning of experiments conducted in living organisms and clinical settings. To ensure optimal quality standards, it is essential to standardize the production of mushroom supplements across the whole supply chain, including cultivation, extraction, and commercial formulation generation. Our study confirmed that mushrooms might be a good source of medicinal food for human populations.

Conflict of interest: The authors declared no conflict of interest for publication of this article.

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