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Nutritional composition, phytochemical and antioxidant properties of *Trametes versicolor* from Central Nepal

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Abstract

Medicinal mushrooms are highly valued due to their high levels of vital bioactive constituents with potential health benefits. *Trametes versicolor* is a widely known medicinal mushroom utilized as a nutrient source and for therapeutic purposes. Our study was carried out to examine the nutritional content, phytochemicals, antioxidants and antimicrobial activity of *T. versicolor*. The mushroom, collected from decaying wood at Phulchowki, Godawari, Lalitpur, was identified as *T. versicolor* based on morphological features and sequence analysis using the NCBI BLAST database. Nutritional analysis indicated that *T. versicolor* contains carbohydrates (59.64%), protein (11.56%), fat (1.13%), moisture (7.1%), dietary fiber (8.6%), and ash (6.01%). The samples demonstrated notable levels of total phenolics (151.05 ± 0.002 mg GAE/100g), flavonoids (124.48 ± 0.03 mg QAE/100g), tannins (725 ± 0.03 TAE mg/100g), lycopene (0.46 ± 0.015 mg carotenoids/g), *β*-carotene (1.11 ± 0.02 mg carotenoids/g), and terpenoids (2.78%). The antioxidant activity (IC_{50}) was 198.4 µg/ml. *Trametes versicolor* also showed the presence of various minerals, including Cu (0.049 ± 0.0027 mg/g), Fe (0.105 ± 0.005 mg/g), Mn (0.035 ± 0.002 mg/g), and Zn (0.03 ± 0.001 mg/g). The mineral constituents and rich nutritional and phytochemical profiles highlight its potential as a functional and nutraceutical food. This study supports its potential long-term utilization in healthcare and food industries, while establishing a basis for additional studies into its therapeutic implications.

Keywords – Bioactive constituents – Carbohydrate – Medicinal mushroom – Minerals – *Trametes versicolor*

Introduction

Mushrooms have long been important in cultures worldwide, not only for their edible purposes but also for their nutritional and therapeutic characteristics (Dawadi et al. 2023, Srivastava et al. 2024). The metabolites produced by mushrooms exhibit a broad range of bioactive properties, including immunomodulatory effects, antibacterial, antiviral, anti-aging, anti-inflammatory, anti-cancer, anti-hypoglycaemic and neuro-protective actions (Srivastava et al. 2024). Medicinal mushrooms have also demonstrated antiparasitic activity against pathogens such as *Leishmania* spp.

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and *Toxoplasma gondii* (Abugri et al. 2019). These features make mushrooms an interesting area of research for developing functional foods and pharmaceutical applications (Sharma et al. 2023).

Trametes versicolor, a member of the family Polyporaceae and phylum Basidiomycota, is one of the intensively studied mushrooms due to its vast range of applications in the pharmaceutical and food sectors (Bhambri et al. 2022, Barreras-Urbina et al. 2023). It grows on decaying wood and is found in temperate Asia, North America, and Europe (Bhambri et al. 2022, Lowenthal et al. 2023). The name 'Trametes' refers to flesh or fabric typically growing in overlapping layers or semicircular rosettes (Kuo 2017). This species is easily recognizable by its striking concentric bands of colour, resembling wild turkey's tail feathers, with hues ranging from brown and tan to blue, green, and reddish-orange (Darshan et al. 2024).

Studies on *T. versicolor* have revealed a rich phytochemical profile that includes amino acids, polysaccharides, and other bioactive substances that are thought to support its medicinal properties (Michalak et al. 2023). This species contains 18 different amino acids, along with polysaccharopeptides and polysaccharide-K, that have demonstrated significant immunomodulatory, anti-inflammatory, and antibacterial properties, highlighting their potential as a valuable natural resource in improving health (Bains & Chawla 2020). Traditionally, Turkey tail is consumed in various forms, including teas, decoctions, powdered extracts, and fermented substrates, all of which are believed to enhance vitality, promote longevity, and support immune function (Benson et al. 2019, Ajibola et al. 2024).

Although the health-enhancing properties of Turkey tail mushrooms have been known for a long time, there has been very little research on this medicinal mushroom in Nepal. The *Trametes versicolor* fungus was cultivated successfully for the first time in Nepal in 2023 (Mushroom Seed Nepal and Research Center 2023). However, no further research has yet been conducted on this mushroom. Thus, our study's main goals were to determine the Turkey tail mushrooms' nutritional, mineral, phytochemical, and antimicrobial qualities and to establish a foundation for further research on their usefulness.

Materials & Methods

Sample collection, tissue isolation and morphological identification

The fruiting bodies of Turkey Tail mushrooms were gathered from rotten tree branches in Phulchowki, Lalitpur (27.5711°N, 85.4056°E), Nepal, on 10th August 2021. The samples were transported in sealed plastic bags to the Laboratory. Dr. Jay Kant Raut and Dr. Mahesh Kumar Adhikari identified the collected mushroom species by comparison with reference strains. The mature fruiting body of the collected mushroom had a subcircular-shaped basidiocarp. The upper surface of the mushroom was yellowish brown with radial furrows, fine velvety hair, and had no stipe (Fig. 1).

Fungal isolation

For fungal isolation, the caps of the *T. versicolor* fruiting bodies were sterilized using 70% ethanol. A small piece of tissue from the inner stipe was excised and transferred aseptically onto Potato Dextrose Agar. The plates were incubated at 30 °C for 5 to 7 days, and pure cultures were obtained *via* repeated sub-culture.

Molecular identification

PCR and sequencing

The DNA extraction was performed using Cetyltrimethylammonium bromide (CTAB) method (Doyle & Doyle 1987). Polymerase Chain Reaction (PCR) was carried out by the amplification of the internal transcribed spacer (ITS) region. The primer sets used were ITS U1 - 5′ TCCGTAGGTGAACCTGCGG 3′ and ITS U4 - 5′ TCCTCCGCTTATTGATATGC 3′. The reaction was performed in Veriti™ Thermal Cycler (Applied Biosystems, USA) under the following conditions: initial denaturation at 94 °C for 4 minutes, followed by 35 cycles of denaturation at 94

°C for 40 seconds, annealing at 56 °C for 1 minute, and extension at 72 °C for 70 seconds. The final extension was done at 72 °C for 7 minutes. Sequencing was done using the Sanger Dideoxy Chain Termination method. The PCR-positive product was first purified using SAP-Exo Kit (Jena Bioscience, Germany). BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) was used for the sequencing reaction. After subsequent purification using 3M Sodium Acetate, the sample was loaded in 3500xL Genetic Analyzer (Applied Biosystems, USA).

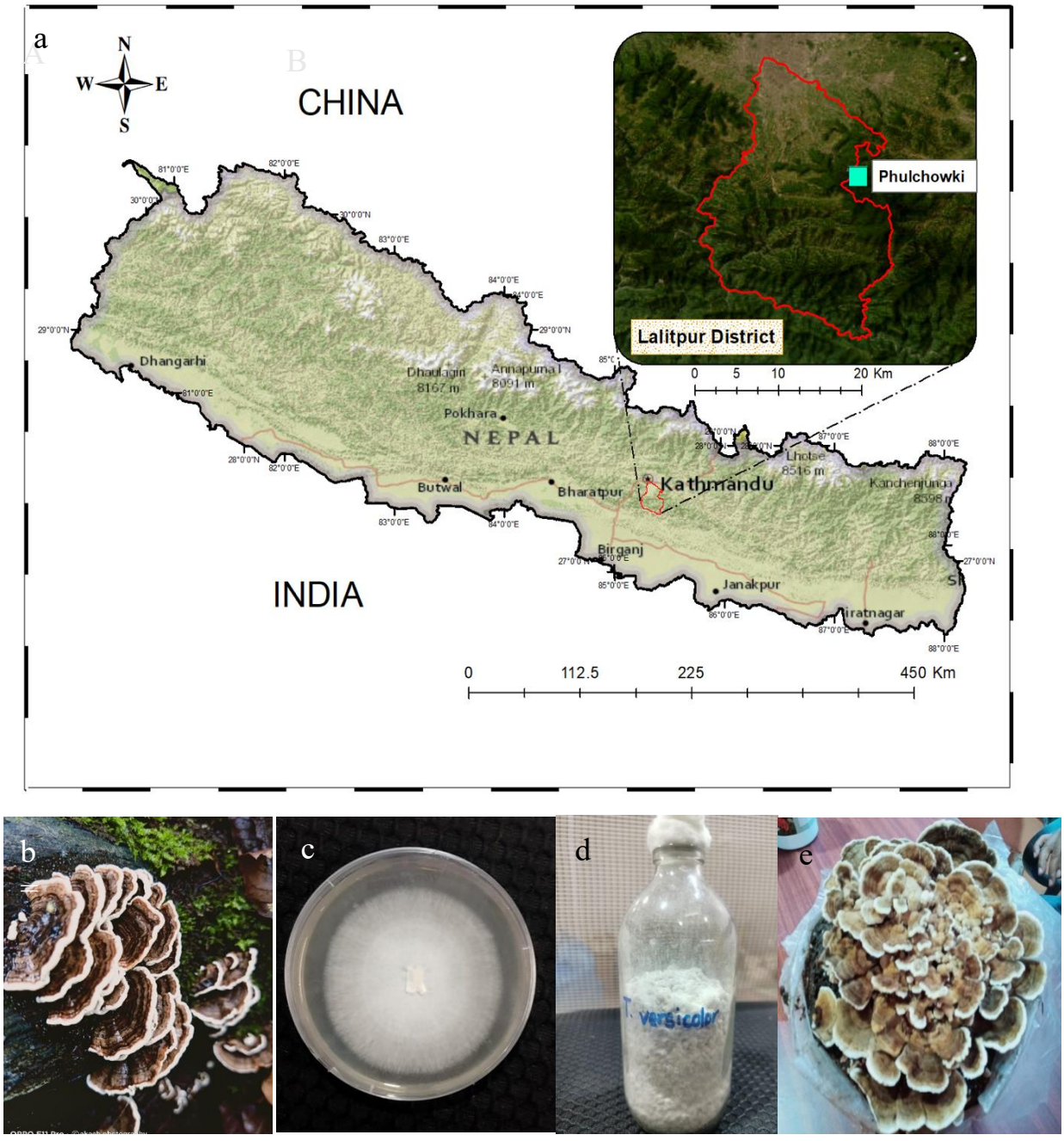


Fig. 1 – a. A map depicting the collection site of *T. versicolor* from Phulchowki, Nepal generated in ArcMap 10.8.2 using GPS coordinates collected during fieldwork, Source: (http://geoportal.ntnc.org.np/layers/ntnc:country/metadata_detail) b. *T. versicolor* fruiting bodies on natural habitat, c. Colony of *T. versicolor* on Potato Dextrose Agar, d. Mother spawn culture, e. Cultivated mushroom.

Phylogenetic analysis

The obtained sequences were analyzed and processed in Sequencher V 4.1.4. Sequence alignment was performed using MAFFT version 7 (Kuraku et al. 2013, Katoh et al. 2019). The validity of the model was tested using jModelTest 2 (Darriba et al. 2012). A phylogenetic tree was constructed with the help of Randomized Axelerated Maximum Likelihood (RAxML) (Stamatakis 2014), incorporating 1000 bootstrap repetitions and employing the HKY+GAMMA substitution model. The tree was visualized in the Interactive Tree of Life (ITOL) server (Letunic & Bork 2024) (Table1).

Table 1 Taxa included in the phylogenetic reconstruction

Species name	Strain/Isolate ID, voucher	Origin/Location	GenBank Accession No.
Trametes versicolor	TV1	Nepal	PX715929
Trametes versicolor	420526MF0062	China	MG719606
Trametes versicolor	CF99	China	KM979753
Trametes versicolor	SWFU000013	China	MK862242
Trametes versicolor	KUT 1002	Japan	AB733646
Trametes versicolor	Tr-11	India	PQ762165
Trametes elegans	420526MF0133	China	MH142005
Trametes elegans	SWFC 011542	China	MK937777
Trametes gibbosa	CLZhao 9818	China	PP094727
Trametes gibbosa	KMRB15090213	South Korea	ON402816
Trametes hirsuta	DMC333	Cameroon	KC589145
Trametes hirsuta	CIRM-BRFM 1902	Georgia	PV108803
Trametes pubescens	FP101554T	USA	JN164962
Trametes pubescens	KUC20121102-13	South Korea	KJ668443
Trametes sanguinea	100	Brazil	OP198494
Trametes sanguinea	20201102Trametes	China	OR198224
Agaricus bisporus	AG96313	-	FJ223226

Spawn media preparation

Spawn preparation was initiated once a pure culture of *T. versicolor* was obtained. A total of 200 g of wheat grains were boiled, rinsed thoroughly, and cooled. The grains were then mixed with 1% $CaCO_3$ and 1% gypsum, transferred into sterile polythene bags, sealed, and sterilized by autoclaving (121 °C for one hour). *Trametes versicolor* culture was inoculated into the bag, and incubation was done in a completely dark room until mycelia fully colonized the grains (Farimani & Farsi 2023).

Preparation of substrate and cultivation of *T. versicolor*

The sawdust (80%) was prepared with 1% $CaCO_3$, 1% gypsum, and 18% rice bran. The sawdust was packed in plastic bags (3 kg/bag) and was autoclaved. Next, 200g of mature spawn was added to the bags for inoculation. Then, it was shaken well to distribute the spawn evenly and incubated at room temperature in the dark for spawn run. Once the mycelium had extended to the bottom of the bag, it was opened and placed in the cultivation room to begin the fruiting process. The bags were supplied with water on a regular basis to keep the humidity at 75–85%. The mushrooms were gathered when the pilei were completely grown. The fruiting bodies were desiccated at room temperature, pulverized and kept in sealed plastic containers (Farimani & Farsi 2023).

Nutritional analysis

Moisture content (%)

About 10 g of the mushroom was weighed in a crucible, dried in a hot air oven at 105 ℃ and then left in a desiccator for cooling. The heating and cooling cycles were carried out repeatedly to get a stable reading (AOAC 2023). The following formula was used to calculate the mushroom moisture content (%):

$$\text{Moisture (\%)} = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Weight of sample taken}} * 100$$

Crude Fat content (%)

The crude fat was calculated using the Soxhlet extractor device (SM Scientific Instruments, India). About 2 g of pulverized samples were kept in a paper thimble and inserted into the extractor. 100 mL of petroleum ether were put into a round-bottom flask before weighing. The Soxhlet extractor equipment was set up with the flask at 60 °C, and the extraction was carried out for 6 hours. A rotary evaporator operating at 40 °C under reduced pressure was used to remove ether. The fat content was weighted and reported as g/100 g of sample (Chew et al. 2011).

Ash content (%)

The dry ashing method was used to measure the amount of ash (Kakoti et al. 2021). A crucible containing approximately one gram of the sample was heated to 550 °C in a muffle furnace until it turned into a white or grayish ash. After cooling, the crucible's ash was weighed. The following is an estimate of the total ash content (%):

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample taken}} * 100$$

Crude fiber content (%)

Approximately 1 g of the sample was taken into a conical flask with 100 mL of 1.25% sulfuric acid. The mixture was shaken every five minutes while it boiled for thirty minutes. Muslin cloth was used to filter the boiled contents. After thoroughly rinsing the residue with hot water, the acid was removed. Following that, 100 mL of a 1.25% sodium hydroxide solution was added to the residue in the same conical flask. The mixture was boiled for another 30 min, filtered again, and rinsed with hot water to remove any remaining alkali. The residue was transferred to a crucible and dried at 110 °C in a hot air oven to achieve constant weight (W1). Subsequently, dried residue was incinerated in a muffle furnace for three hours at 550 °C, cooled, and weighed again (W2). The reduced weight during the incineration process is the crude fiber content (Aina et al. 2012).

$$\text{Crude fiber (\%)} = \frac{(\text{Weight of residues (W1)} - \text{Weight of ash (W2)})}{\text{Weight of sample taken}} * 100$$

Mineral analysis

An atomic absorption spectrophotometer (AAS) (AAS Agilent Technologies 240 FSI Flame, USA) was used to analyze the minerals following a dry ashing digestion method. A porcelain crucible containing about 500 mg of the dried sample was heated to a furnace temperature of 500 °C. After about four hours of ashing, the sample was reduced to a white or grey ash residue. After combining the residue with 1 mL of concentrated nitric acid, an ash solution was created and left undisturbed for 24 hours. After further digestion with 1 mL of 25% HNO_3, the mixture was filtered through ashless filter paper. The filtrate was then adjusted to 50 ml with distilled water. The concentrations of copper (Cu), iron (Fe), zinc (Zn), lead (Pb) and manganese (Mn) were assessed using Atomic Absorption Spectrometry (Paul et al. 2014, Gebrelibanos et al. 2016).

Carbohydrate content

A modified anthrone reagent technique, as described by Osborne & Voogt (1978), was used to quantify the amount of carbohydrates. A total of 500 mg mushroom sample was mixed with 15 mL of 52% (v/v) perchloric acid and 10 mL of distilled water and incubated in the dark for 18 hours. Following incubation, the sample mixture was filtered, and the filtrate was diluted twenty times. A 200 µL of diluted filtrate was mixed with 500 µL of anthrone solution. The mixture was vortexed and heated in the boiling water for 12 min to facilitate the reaction. After cooling, the absorbance was measured at 630 nm. Carbohydrate concentration was determined based on a glucose calibration curve ranging from 0 to 500 µg/mL ($y = 0.0024x + 0.0025$, $R^2 = 0.9994$) and presented as g/100 g of the sample.

Protein content

A modified Bradford assay was used to determine the sample's protein content (Bradford 1976). A 0.2 gm sample was combined with distilled water of volume 20 mL, and the mixture was incubated for 24 hours at 50 °C and 100 rpm in a shaking incubator (Innovative Life Science Tools, USA). After incubation, it was filtered, and the resulting filtrate was used for protein estimation. For the assay, the filtrate was combined with Bradford reagent in a 1:10 ratio in microfuge tubes. After giving the tubes a thorough vortex, they were incubated at room temperature for at least five minutes. The absorbance was then measured at 595 nm. Protein content was estimated based on the standard curve and presented as Bovine Serum Albumin standards with concentrations ranging from 0 to 500 µg/mL ($y = 0.0014x - 0.0158$, $R^2 = 0.9909$). Using the calibration curve, the protein content was computed and displayed as g/100 g of the sample.

Phytochemical analysis

Preparation of the crude extracts

The phytochemical components of mushroom were analyzed using a methanolic extraction technique, in accordance with the procedure described by Ihayere & Okhuoya (2022). Five grams of powdered mushroom sample was added to 50 mL of methanol for extraction. The process was conducted at 37 °C for 48 hours with continuous agitation at 100 rpm. Following extraction, the mixture was passed through filter paper, and the resulting filtrate was collected and subsequently dried by evaporating methanol using a water bath at 40 °C (Thakur et al. 2022, Rana et al. 2024).

Phenolic content

A modified Folin-Ciocalteu method, as described by Singleton et al. (1999), was used to determine the protein content in the sample. A 150 µL of Folin-Ciocalteu phenol reagent and 50 µL of the extract were mixed, and the mixture was left to react for three minutes. To this content, 150 µL of saturated Na_2CO_3 solution was added, and diluted to a final volume of 1,500 µL using distilled water. After 90 min of dark incubation, its absorbance was measured at 725 nm. Gallic acid concentrations ranging from 0 to 500 µg/mL were used to create a standard curve, which produced the equation $y = 0.0021x + 0.0596y$ with an R^2 value of 0.9962. Milligrams of gallic acid were used to represent the phenolic content.

Flavonoid content

The flavonoid content was determined based on modified aluminum chloride assay, as described by Zhishen et al. (1999). About 250 µL of mushroom extract was combined with 1 mL of distilled water. Next, 75 µL of 5% sodium nitrite was added, and the mixture was left to react for 5 min. After this, 75 µL of 10% aluminum chloride was incorporated, followed by the addition of 0.5 mL of 1 M sodium hydroxide. After 5 min, the reaction mixture was adjusted to a volume of 2 mL with distilled water. The equation $y = 0.0019x + 0.0207$ with an R^2 value of 0.9982 was obtained by creating a calibration curve using standard solutions of quercetin, which ranged from 0 to 500 µg/mL. The absorbance of the quercetin standards and sample extracts was measured at 510 nm, and the flavonoid was presented as mg of quercetin equivalents (QAE) per 100 gm of extract.

Tannin content

The tannin amount was assessed according to the modified Folin-Ciocalteu technique as mentioned by Mythili et al. (2014). In this procedure, 7.5 mL of distilled water was mixed with 0.1 mL of extract, followed by 1 mL of a 35% sodium carbonate solution and 0.5 mL of the Folin-Ciocalteu phenol reagent. The mixture was adjusted to 10 mL with distilled water, thoroughly mixed, and incubated in darkness for 30 min. Absorbance readings for the sample and tannic acid standards were taken at 725 nm, with a reagent blank serving as the baseline. The curve was generated using tannic acid concentrations (0 to 500 µg/mL), resulting in the equation y = 0.0004x + 0.0032 with an R^2 value of 0.9973. Tannic acid (TAE) mg/100 gm of extract were used to indicate the tannin content.

***β*-carotene and lycopene content**

The *β*-carotene and lycopene contents were determined employing the technique described by Nagata & Yamashita (1992). Initially, 10 mL of an acetone-hexane solvent was combined with 0.1 g of the mushroom extract. The mixture was vigorously agitated for 1 min and then filtered. Three wavelengths were used to measure the absorbance: 453 nm, 505 nm, and 663 nm. The concentrations of β-carotene and lycopene were determined by the specific formulas for their respective absorbance values.

Lycopene (mg/100) = -0.0458 A663 + 0.372 A505 – 0.0806 A453

β-carotene (mg/100) = -0.216 A663 + 0.304 A505 + 0.452 A453

Terpenoid content

About 0.1 g of sample was placed in 9 mL of ethanol, left to stand for 24 hours and processed according to the procedure by Ferguson (1956). A common formula was used to determine the total terpenoids:

$$\text{Terpenoid (\%)} = \frac{(\text{Weight of residues})}{\text{Weight of sample taken}} * 100$$

Antioxidant activity

The activity of antioxidants was calculated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay with few modifications (Singh et al. 2002). A 500 µL aliquot of the extract prepared at various concentrations was combined with 500 µL of 0.1 mM DPPH solution. The mixture was left in the dark for half an hour to facilitate the reaction to proceed. Absorbance was read at 517 nm with ascorbic acid as the standard. The Radical Scavenging Activity (RSA) was determined as follows:

$$\text{RSA (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Weight of sample taken}} * 100$$

The antioxidant activity was also presented as the IC_{50} value, which denotes the concentration (in µg/mL) required for the extract to achieve 50% inhibition of DPPH radical.

Evaluation of antibacterial activity of extracts

The antibacterial potential of the extracts was tested against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 70062, *Enterobacter faecalis* ATCC 29212, and *Staphylococcus aureus* ATCC 25923, using the well diffusion method. Bacterial isolates were inoculated in nutrient broth and incubated at 37 °C for 24 hours.

To prepare the working samples, the dried mushroom extracts were dissolved in 5% dimethyl sulfoxide (DMSO) at concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL. A volume of 40 µL from each concentration was introduced into the wells. Chloramphenicol was used as a positive control, while 5% DMSO was used as a negative control. The Petri plates were incubated

overnight at 37 °C, and the zones of inhibition (mm) were measured to determine antibacterial efficacy (Mbwambo et al. 2007).

Statistical analysis

All measurements were conducted in triplicate, and the results were expressed as mean ± standard deviation (SD). The data sheets were prepared in Microsoft Excel 365.

Results and discussions

PCR and sequencing

After processing the sequence files and trimming the primer sequences, the final amplicon was determined to be 589 base pairs in length (Fig. 2). The BLAST query revealed 99.49% similarity to *T. versicolor* (GenBank: KM979753), covering 100% of the query sequence.

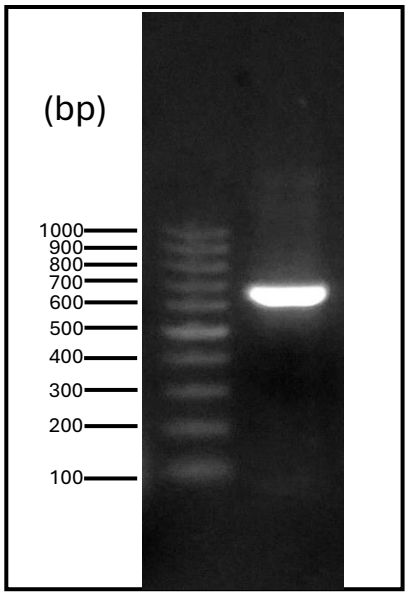


Fig. 2 – A distinct band observed on the 1.5% agarose gel following the PCR run, clearly contrasted against the 100 bp ladder.

The phylogenetic tree resolved various *Trametes* species, with *T. sanguinea* forming a distinct clade separate from the other *Trametes* species. It was revealed that our isolate, *T. versicolor* TV1 (GenBank: PX715929), forms a distinct clade apart from other *Trametes* species, with *T. pubescens* being the closest to *T. versicolor*. Notably, our isolate shows a close genetic affinity with the Chinese strains CF99, SWFU000013, and 420526MF0062, rather than with the Japanese and Indian strains KUT 1002 and Tr-11, respectively. The separation between *T. pubescens* and *T. versicolor* is strongly supported by a bootstrap value of 94, indicating a robust phylogenetic distinction. The close genetic similarity to the Chinese strain indicates a potential geographic link or recent common ancestry, which may be relevant for understanding regional distribution patterns (Fig. 3).

Nutritional Analysis

Biochemical and proximate constituent analysis is vital for assessing mushroom's quality, preservation, and processing, ensuring their effective use in health supplements and pharmaceuticals (Darshan et al. 2024). The nutrient analysis of the collected mushroom in our study revealed the following profile (Table 2).

As shown in Table 2, the observed carbohydrate content was 59.64%, which is lower than the level previously explained by Angelova et al. (2022). However, comparable studies have documented varying ranges: 48.75–66.75%, 38.33–41.94% and 54.55% (Upadhyaya et al. 2017, Mostafa et al.

2023, Desisa et al. 2024). Similarly, the total protein content of *T. versicolor* was found to be 11.56%, which aligns closely with findings from other studies that reported protein content as 11.07%, 7.46–14.65%, and 8.12–11.06% (Łysakowska et al. 2023, Mostafa et al. 2023, Desisa et al. 2024). These variations may result from differences in environmental conditions, cultivation methods, harvesting techniques, substrate type, climate, and geographic factors (Lu et al. 2020, Desisa et al. 2024).

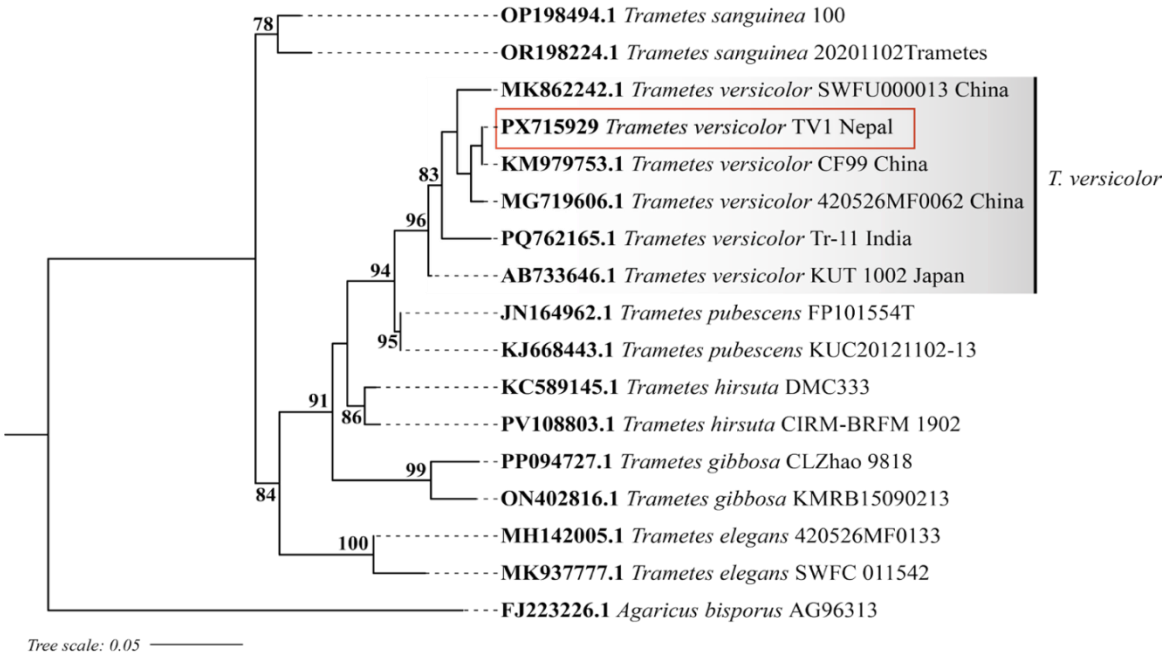


Fig. 3 – Maximum likelihood phylogenetic tree with bootstrap consensus values >70, detailing phylogenetic relationships among the *Trametes* species.

Table 2 Nutritional analysis of *T. versicolor.*

Components	Percentage (%)	Results from previous research (%)
Carbohydrate	59.64	48.75-66.75 (Upadhyaya et al. 2017), 54.55 (Desisa et al. 2024)
Protein	11.56	11.07 (Łysakowska et al. 2023), 8.12-11.06 (Desisa et al. 2024)
Crude fiber	8.6	12.89–18.38 (Upadhyaya et al. 2017)
Moisture	7.1	6.74-11.64 (Desisa et al. 2024)
Ash	6.01	2.42 and 3.48 (Mostafa et al. 2023)
Fat	1.13	1.35 (Łysakowska et al. 2023)

The crude fiber in *T. versicolor* from our study was 8.6%. Desisa et al. (2024), Upadhyaya et al. (2017) and Mostafa et al. (2023) reported fiber ranging from 12.89–18.38%, 9.59–14.30% and 20.89%, respectively. These differences can be ascribed to environmental factors, and substrate composition (Koutrotsios et al. 2014). The moisture content of *T. versicolor* from our study was 7.1%. Previous studies have also revealed moisture content ranging from 6.7 to 12.5% (Upadhyaya et al. 2017, Desisa et al. 2024). The lower moisture level of the mushrooms implies their lower perishability (Upadhyaya et al. 2017). This feature prolongs shelf life and enhances storage stability, which is beneficial for survival in both wild and cultivated environments (Giri & Prasad 2007).

The ash content of *T. versicolor* mushroom was found to be 6.01%. A study of wild *T. versicolor* from the Himalayas reported a lower ash content ranging between 2.42 and 3.48% (Mostafa et al. 2023). Other studies have reported ash content of 3.56–6.54% (Desisa et al. 2024) and

18.94% (Upadhyaya et al. 2017). The difference in ash content could be due to the presence of various metal elements in the substrate, which are absorbed by the mycelia and carried to the fruiting body of the mushroom (Mostafa et al. 2023). Environmental factors, including geographic location and soil type, also significantly influence the mineral content of mushrooms (Koutrotsios et al. 2014). The crude fat of Turkey Tail mushroom was found to be 1.13%, which is quite similar to values reported in previous studies, such as 1.35% (Łysakowska et al. 2023) and 0.93–1.26% (Mostafa et al. 2023). The consistent fat content may potentially indicate the metabolic adaptability of *T. versicolor* in different substrate environments (Koutrotsios et al. 2014).

Mineral content

In our research, the iron concentration was found to be the highest among the detected micronutrients, with a concentration of 0.105 ± 0.005 mg/g (Table 3). The detected level suggests that the consumption of such mushrooms could contribute to dietary iron intake, potentially aiding in the prevention of iron-deficiency anemia (Ogidi et al. 2021). The copper, manganese, and zinc concentrations were relatively lower, with values of 0.049 ± 0.0027 mg/g, 0.035 ± 0.002 mg/g, and 0.030 ± 0.001 mg/g, respectively. The lead content was negligible, indicating that the sample was free from significant contamination of this toxic metal. Very scarce study is available on the mineral constituents of Turkey Tail mushroom. A study by Mostafa et al. (2023) reported very high mineral constituents in *T. versicolor* in comparison to our study. This might result from the presence of different metals within the substrate.

Table 3 Mineral analysis of *T. versicolor.*

Micronutrients	Values (mg/g ± SD)
Copper	0.049 ± 0.0027
Lead	Negligible
Iron	0.105 ± 0.005
Manganese	0.035 ± 0.002
Zinc	0.03 ± 0.001

Phytochemical analysis

Mushrooms are significant macrofungi with bioactive substances and therapeutic properties (Bains et al. 2021, Shyaula et al. 2026). In our study, the bioactive metabolites like flavonoids, phenolics, tannins, β-carotene, lycopene and terpenoids were analyzed to study the phytochemical profile of *T. versicolor*. The obtained result is presented in Table 4.

The observed flavonoid content of Turkey Tail mushroom in our study was 124.48 ± 0.03 QAE mg/100g, which is lower than the value noted by Mostafa et al. (2023) and Saltarelli et al. (2019), where the observed flavonoid content was 9.02–14.01 mg QAE/g and 13.13 QAE/g, respectively. Upadhyaya et al. (2017) and Angelova et al. (2022) also reported lower flavonoid content in their study. Similarly, the phenolic content was found to be 151.05 ± 0.002 GAE mg/100g, which is different from other reported studies (Upadhyaya et al. 2017, Bains & Chawla 2020, Angelova et al. 2022, Mostafa et al. 2023). The differences in flavonoid and phenolic levels may have been the result of various factors, such as extraction temperature, solvent extraction, harvesting time, and the analytical techniques used across the study (Srivastava et al. 2024).

The total tannin of Turkey tail mushroom in this study was determined to be 725 ± 0.03 mg/100 g TAE. The significant tannin levels in *T. versicolor* demonstrate its strong antioxidant and antimicrobial potential, indicating its value for developing pharmaceutical and nutraceutical products. The terpenoid content of *T. versicolor* mushroom was determined to be 2.78%. This content could be a result of site-specific factors and analytical methods used in the study (Srivastava et al. 2024). The studied mushrooms contained 1.11 ± 0.02 mg/g *β*-carotene. Previous studies have reported lower *β*-carotene levels from wild *T. versicolor* ranging from 0.31–0.72 mg/g (Mostafa et al. 2023). Similarly, the lycopene content in this study was 0.46 ± 0.015 mg/g. Other studies have

reported the lycopene value from 0.02–6.85 mg/g (Bains & Chawla 2020, Mostafa et al. 2023). The variations probably arise from maturity stage, substrate composition and environmental conditions (Lin & Xu 2020).

Table 4 Phytochemical analysis of *T. versicolor.*

Phytochemical content	This study	Results from previous research
Flavonoid (QAE mg/100g)	124.48± 0.03	9.02–14.01 QAE/g (Mostafa et al. 2023), 13.13 QAE/g (Saltarelli et al. 2019)
Phenolic (GAE mg/100g)	151.05 ± 0.002	4.07 mg/ml (Abugri et al. 2019)
Tannin (TAE mg/100g)	725 ± 0.03	-
β-carotene (mg carotenoids/g)	1.11 ± 0.02	0.31–0.72 mg/g (Mostafa et al. 2023)
Lycopene (mg carotenoids/g)	0.46± 0.015	0.02–6.65 mg/g (Bains & Chawla 2020, Mostafa et al. 2023)
Terpenoid (%)	2.78	3.65–4.21 (Srivastava et al. 2024)

In our study, the IC_{50} of *T. versicolor* was found to be 198.4 µg/ml. This value differs from the previous study by Bulam et al. (2022) where the reported value was 18.10 ± 0.10 mg/ml. As per the studies by Bulam et al. (2022) and Chun et al. (2021), the antioxidant properties of mushrooms correlate strongly with their polyphenolic, flavonoid content and polysaccharides. These variations could be due to several factors, such as variations in growing conditions, substrate composition, and harvesting time, that may significantly impact the mushroom's bioactive compound production (Haukongo et al. 2021).

In this study, ATCC reference strains were tested to study the antimicrobial potency of mushroom extract, if present. None of the strains were inhibited by mushroom extract, indicating the lack of inhibitory potential of our mushroom against pathogenic microbes. Our finding is in contrast to the previous study by Camilleri et al. (2024) where inhibitory effects of methanolic extract of Turkey tail mushroom were reported against bacteria as well as fungal pathogens. These differences might be due to insufficient extract concentration, extraction inefficiencies lowering bioactive release and methanolic solvent selectivity favoring non-antimicrobial compounds over phenolics, and tannins (Canlı et al. 2019, İNci et al. 2022).

Conclusions

The results reveal that Turkey tail mushroom contain various micro and macro nutrients along with health beneficial phytochemicals such as phenolics, flavonoids, terpenoid, *β*-carotene, lycopene and minerals like Cu, Fe, Mn and Zn, suggesting its application potential as nutraceutical and functional food.

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Author's contribution

AB, MS and IS were involved in fieldwork and sample collection. MS and IS conducted laboratory experiments. MS analyzed and interpreted the data. MS, IS, NB, MAS, and CK drafted the manuscript. CK and MS edited the manuscript. JM reviewed the manuscript. LRB supervised and reviewed the manuscript. All authors revised and approved the final manuscript.

Accessibility of data

The article includes all data in tables. Nevertheless, any questions can be sent to the authors of record.

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