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Investigation of the Biological and Chemical Properties of *Trametes versicolor* L. and Evaluation of Different Methods for Glucans Extraction

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ABSTRACT

Trametes versicolor L. possesses high potential for applications in pharmaceutical and food industries due to its biological and chemical properties. This study aimed to analyze the chemical composition, evaluate antibacterial activity, and compare three different glucan extraction methods (hot water extraction, acidic extraction, and acid-alkaline extraction) from *T. versicolor* L. According to chemical analysis, the protein, fiber, ash, and carbohydrate contents of the mushroom were determined to be 8.3%, 54.05%, 1.43%, and 30.09%, respectively. Additionally, the mineral content including iron, zinc, and magnesium was measured at 59.9, 25.9, and 300 mg/kg, respectively, while the total phenolic content was calculated to be 230 mg per 100 g. The diameter of the inhibition zone of the mushroom extract against *Staphylococcus aureus* was measured at 40 mm; however, no antimicrobial activity was observed against *Escherichia coli*. The extraction results showed that the highest total glucan and alpha-glucan content were obtained by the hot water extraction method with values of 66.35% and 10%, respectively, while the acid-alkaline method possessed the highest beta-glucan content (57.53%). This study demonstrates the high potential of *T. versicolor* L. as a rich source of bioactive compounds and minerals for application in the food and pharmaceutical industries.

1- Introduction

Medicinal mushrooms have attracted significant attention from researchers in recent years due to their bioactive compounds and extensive therapeutic properties. *Trametes versicolor* L., one of the well-known medicinal mushrooms, exhibits antioxidant, antibacterial, and immune-modulating properties, mainly attributed to its polysaccharides, polyphenols, and essential minerals elements. This mushroom is found in warm and humid regions such as North America, Europe, and parts of Asia, including Iran, Turkey, China, and Japan; primarily growing on the trunks of trees such as pine, beech, elm, and oak, as well as other angiosperms. Of the approximately 60 identified species of *T. versicolor*, around 12 have been reported in Iran, particularly in humid provinces such as Mazandaran, Gilan, Golestan, and East Azerbaijan [1]. *T. versicolor L.* is recognized as valuable medicinal source due to its rich content of nutrients including proteins, fibers, minerals, and polyphenolic compounds. In addition to being nutritionally rich, this mushroom can absorb mineral elements from compost or soil during its growth and store them in the structure of its bioactive compounds. This characteristic allows *T. versicolor* L. to meet the body's needs for essential minerals such as iron, zinc, calcium, and magnesium, which are vital for maintaining cellular functions at biological, chemical, and molecular levels [2]. Moreover, the antioxidant and polyphenolic compounds present in this mushroom enhance its nutritional and medicinal value. Therefore, a thorough investigation of its polyphenol content, antioxidant properties, and mineral composition is a crucial step toward utilizing its medicinal and industrial potential [3]. One of the most important bioactive compounds in *T. versicolor* L. is glucans, which are formed as bioactive polysaccharides in the cell walls of fungi. The fungal cell wall is composed of chitin (a polymer of β-1,4-N-acetylglucosamine), and polysaccharides such as α- and β-D-glucans and mannans. These components are recognized as polysaccharides resistant to human digestive enzymes and can be considered a type of dietary fiber. Glucans are the most common insoluble dietary fibers found in the fruiting body or mycelium of mushrooms. Their content varies depending on the species, environment, maturity level, and cultivation techniques, generally ranging from 1.3% to 46.5% [4]. Glucans play a fundamental role in enhancing immune system performance, reducing oxidative stress, and combating bacterial infections [5]; for example, their positive therapeutic effects on the respiratory system, including the prevention of recurrent respiratory infections in children and alleviation of symptoms, have been proven [6].The most commonly isolated glucans from mushrooms are β-D-glucans, which are known for their wide range of beneficial effects, such as immunomodulatory activities, anticancer properties, antimicrobial effects, and lipid-lowering capabilities. In contrast, α-D-glucans and α/β-D-glucan mixtures have been less extensively studied, although they are also considered to possess functional properties, particularly antioxidant and anticancer activities [7]. Beta-glucans are a heterogeneous group of glucose polymers linked by glycosidic bonds and are found in the cell walls of fungi, yeasts,

oats, barley, and some bacteria [8]. Fungal β-glucans typically consist of β-(1,3) linkages in the main chain and β-(1,6) linkages in their branches. The structure of these compounds, including the degree of branching and chain distribution pattern, varies among different fungal species [9]. Various extraction methods can influence the quality and quantity of the extracted glucans. Common methods for glucan extraction include hot water extraction, acid-alkaline extraction, and acidic extraction [10].

In this study, in addition to evaluating the chemical composition and assessing the antioxidant and antibacterial properties of *T. versicolor* L., the extraction yield of β-glucans using three different extraction methods was compared. The ultimate aim of this research is to provide more detailed information about the active compounds of *T. versicolor* L., which could clear the way for its use in the pharmaceutical and food industries.

2- Materials and Methods

2. Materials and Methods

2.1 Preparation of Mushroom Sample

The cultivated strain M9911 of *T. versicolor* L. mushroom was obtained from Mycelia Co. (Belgium). After harvest, the mushroom bodies were dried in an oven at 50°C. The dried samples were then ground into powder and passed through a 40-mesh sieve.

2.2 Chemical Analysis

The total of protein, carbohydrate, fat, dietary fiber, and ash content of the mushroom powder were determined according to AOAC methods [11]. Total ash content was measured by heating the samples in a muffle furnace at 550–600°C until constant weight was achieved. Total protein content was determined using the Kjeldahl method (Kjeltec™ 2300 Analyzer Unit, Foss, Denmark), which involves digestion of the sample with sulfuric acid followed by measurement of total nitrogen to calculate protein content. Total fat was extracted using the Soxhlet extraction system (Gerhardt, Germany) with diethyl ether as a non-polar solvent. Total dietary fiber was measured using an enzymatic–chemical method that involves the removal of starch and protein with specific enzymes, followed by filtration and drying of the residue. Total carbohydrate content was calculated by subtracting the amounts of protein, fat, ash, and moisture contents from 100.

2.3 Determination of Mineral Elements

To measure the zinc, magnesium, and iron, atomic absorption spectroscopy (Perkin Elmer, USA) was used. Samples were prepared by acid digestion with nitric acid and were diluted to a specific volume after filtration. Calibration curves were prepared using standard solutions of the respective elements at different concentrations. The Samples were injected into a flame or graphite furnace, and the absorbance of monochromatic light by the free atoms of the element was recorded. The concentrations were calculated by comparing the absorbance values to the calibration curves. The limits of detection (LOD) and the accuracy were also evaluated [12].

2.4 Determination of Total Phenolic Content

To determine total phenolic content, 2 mg of mushroom powder was mixed with 1 mL of 50% methanol. Then, 20 µL of this mixture was combined with 1.58 mL of water and 100 µL of Folin-Ciocalteu reagent. After vortexing for 3 minutes, the mixture was allowed to stand at room temperature and then mixed with 300 µL of 20% sodium bicarbonate solution (w/v) and incubated at 40°C for 30 minutes. The absorbance was measured at a wavelength of 765 nm. The total phenolic content was expressed as gallic acid equivalents (GAE) [13].

2.5 Evaluation of Antibacterial Activity

The antibacterial activity of the mushroom powder was examined using the agar well diffusion method. First, the hot water extraction of *T. versicolor* L. was performed based on the method by Hwang et al. (2019) with slight modifications. For this purpose, 10 g of mushroom powder was mixed with 400 mL of hot water and stirred for 2 hours. The mixture was centrifuged at 5000 rpm for 10 minutes, and the supernatant was collected and stored at 4°C until use [14]. For the antibacterial activity assessment, Standard strains of *E. coli* and *S. aureus* were used. The microorganisms were cultured in nutrient broth at 37°C for 18 hours. The suspensions of approximately $10^{\wedge 8}$ CFU/mL, equivalent to 0.5 McFarland (OD= 0.1) was prepared using a spectrophotometer at a wavelength of 600 nm. Then, 100 µL of the suspension was spread on agar plates. Wells were created in the agar plate, and 40 µL of ethanolic mushroom extract at 0.05 g/mL concentration was placed in to the wells. The Plates were incubated at 37°C for 24 hours. Finally, the diameter of the inhibition zone was measured with a caliper [15].

2.6 Extraction of Glucans

Mushroom powder was used to investigate three methods of glucan extraction.

2.6.1 Hot Water Extraction

First, 10 grams of mushroom powder was passed through a 40-mesh sieve, then suspended in 500 mL of distilled water and stirred at 100°C for 3 hours using a hot plate. The mixture was centrifuged at 5000 rpm for 15 minutes. The supernatant was collected, mixed with an equal volume of ethanol (1:1 v/v), and incubated at 4°C for 24 hours. The precipitate was collected by centrifugation at 7000 rpm for 20 minutes [15].

2.6.2 Acid Extraction

For acid extraction, acetic acid adjusted to pH 5 was used. 10 grams of mushroom powder, sieved through a 40-mesh sieve, is mixed with 500 mL of distilled water and stirred at 95°C for 5 hours at 120 rpm. After centrifugation at 7000 rpm for 20 minutes, the supernatant was mixed with an equal volume of ethanol and incubated at 4°C for 24 hours. The precipitate was collected by centrifugation [16].

2.6.3 Acid-Base Extraction

In this method, 10 g of mushroom powder passed through a 40-mesh sieve was

stirred in 2 M sodium hydroxide at 90°C for 2 hours, followed by acid extraction with acetic acid at 80°C for 5 hours. After centrifugation at 5000 rpm for 10 minutes, the supernatant was diluted 1:1 (v/v) with ethanol and incubated at 4°C for 24 hours. The precipitate was collected by centrifugation [17].

2.7 Determination of Glucan Content

Total glucan, α-glucan, and β-glucan contents in the extracts were measured using a commercial Megazyme kit (K-YBGL, Ireland) designed for yeast and fungal glucan analysis, according to the manufacturer's instructions. In this method, Initial hydrolysis was performed with 10 N hydrochloric acid to remove covalent linkages betweem glucans and proteins or other polysaccharides such as chitin .Then, using hydrolysing enzymes such as exo-1,3-beta-glucanase and beta-glucosidase, D-glucose units were obtained, which were quantified by absorbance measurement at 510 nm. Total glucans (α-glucan and β-glucan) and alpha-glucan contents were determined, and beta-glucan was calculated by subtraction. Pure glucose and beta-glucan from *Saccharomyces cerevisiae*, provided in the kit, were used as a standard and a positive control respectively. The percent beta-glucan was calculated using Megazyme software (www.megazyme.com).

2.8 Statistical Analysis

Data were analyzed using SPSS version 19. A completely randomized design (CRD) was applied, and Duncan's Multiple Range Test was used to compare mean values. All experiments were performed in triplicate, and results are reported as mean ± standard deviation. Statistical significance was considered at $\alpha = 0.05$.

3-Results and Discussion

3.1. Chemical Analysis

The results of the analysis of the main components of the dried mushroom powder are presented in Table 1. As shown, the major constituents of *T. versicolor* L. are fiber compounds. The total fiber content of this mushroom indicates its high potential as a dietary fiber source in healthy diets. Besides regulating the digestive system, fiber can contribute to cholesterol reduction and blood sugar management, highlighting the potential significance of this mushroom in food and pharmaceutical industries [18]. Following fiber, total carbohydrates and protein compounds are the prodominant components of *T. versicolor* L. Comparing the results of the present study with previous research on the *T. versicolor* L. species shows significant differences in the chemical composition of the mushroom fruiting body. For instance, the protein content reported by Kivrak et al. (2020) and Upadhyaya et al. (2018) was higher than the amount measured in this study [19, 20]. Mustafa et al. (2023) and Upadhyaya et al. (2018) reported total fiber content in *T. versicolor* L. as 9.59–14.30% and 20%, respectively, which is significantly higher than the amount found in the present study; however, the ash content measured in this study (1.43%) was lower than previous ones (42.2–48.3% and 18.94% respectively). The crude fat content of T. *versicolor* L.was estimated at 1.23% in this research [19,

20]. This amount was lower than the 2% crude fat achieved by Upadhyaya et al. (2018), but consistent with the 1.35% and 1.26% values reported by Kivrak et al. (2020) and Mustafa et al. (2023), respectively [19,20,21]. The variations in the composition of *T. versicolor* L. may be attributed to differences in subspecies, mushroom origin, environmental growth conditions, or sample preparation and analysis methods [22, 23].

Table 1. Proximate Composition (%) of *T. versicolor* L.

Total protein	8.30
Total fiber	54.05
Crude fat	1.23
Total carbohydrate	30.09
ash	1.43
Moisture	4.90

3-2. Mineral Elements

Analysis of mineral elements revealed that *T. versicolor* L. contains significant amounts of essential elements such as iron, magnesium, and zinc (Table 2). These elements play vital roles in metabolic functions and maintaining overall health of body [24]. In current study, the levels of iron and magnesium were substantially higher than those reported by Bulam et al. (2022) for *T. versicolor* L. (iron 3.84 mg/kg and magnesium 6.77 mg/kg) and *Laetiporus. sulphureus* (iron 0.49 mg/kg and magnesium 4.59 mg/kg) [25]. Compared to Akgul et al. (2017), the iron content in the present study was lower while zinc content was higher than their reported values (154 and 15.68 mg/kg, respectively); additionally, the magnesium concentration exceeded that achivedd by Akgul et al. (2017) for *T. versicolor* L. (133 mg/kg) [26]. According to literature, the amount of iron, magnesium, and zinc in edible mushrooms is 14.6–835, 600–2500, and 8.29–158 mg/kg, respectively [27]. These discrepancies may be ralated to various factors such as growth location, environmental conditions, or differences in analytical methods. Since mushrooms play an important role in the decomposition of organic matter in nature, the substrate composition is also a critical factor influencing mineral content [28]. Iron is essential for oxygen transport in the body, and its deficiency can cause anemia.

Zinc is necessary for immune function, cellular growth, and wound healing. Calcium is also one of the most important elements for maintaining the health of bones and teeth, and plays a role in muscle contraction and nervous system function. The presence of these minerals in *T. versicolor* L. enhances its nutritional value for dietary and supplement applications [29].

Table 2. Concentration of Fe, Zn and Mg of *T. versicolor* L. (mg/kg).

Element	Concentration (mg/Kg)
Fe	59.90
Zn	25.90
Mg	300

3-3. Total Phenolic Content

In the present study, the total phenolic content of *T. versicolor* L. was measured at 230 mg per 100 g, which is significantly higher than previously reported values by Mostafa et al. (2023), Bulam et al. (2022), Bains et al. (2020), and Puia et al. (2018), who shown total phenol in the range of 8–77 mg per 100 g [14,25,30,31]. Phenolic compounds are known for their ability to scavenge free radicals, chelate metals, prevent unwanted reactive oxygen species, decompose peroxides, and reduce oxidative stress [32]. Studies have shown that *T. versicolor* L. is rich in various phenolic compounds such as gallic acid, caffeic acid, chlorogenic acid, ferulic acid, and flavonoids including quercetin and catechin [33]. These compounds play significant roles in antioxidant, anti-inflammatory, and cytoprotective activities. The high levels of these compounds indicate the strong potential of this mushroom as a natural source of bioactive compounds for use in food and pharmaceutical products [34].

3-4. Antibacterial Activity

As shown in Figure 1, the extract of *T. versicolor* L. had no inhibitory effect on the growth of *E. coli* (at concentration approximately $10^{\wedge 7}$ CFU/mL), but effectively inhibited the growth of *S. aureus* at the same concentration. The diameter of the inhibition zone against *S. aureus* was measured to be 15 mm. These findings are consistent with those of Mustafin et al. (2022), who also reported that methanolic extracts of *T. versicolor* L. had no effect on *E. coli* growth but exhibited inhibitory activity against *S. aureus* strains INA 00761 and FDA209P, with inhibition zones ranging from 12 to 15 mm after 21 days of culture [15]. Similarly, İnci et al. (2022) informed inhibition zones of 15.66 mm and 10.23 mm for methanolic and ethanolic extracts, respectively, against *S. aureus* at $10^{\wedge 6}$

CFU/mL; while For *E. coli* at the same concentration, methanolic and ethanolic extracts showed inhibition zones of 15.66 mm and 12.33 mm, respectively [35]. The differences in inhibition zones may result from variations in extraction methods, extract concentrations, or experimental conditions [36]. The antimicrobial properties of medicinal mushrooms have been widely studied, and many extracts show more pronounced effects against Gram-positive bacteria compared to Gram-negative ones, which aligns with the current findings [35–39].

The type and concentration of phenolic compounds significantly influence the antimicrobial potency of mushroom extracts. Higher phenolic content correlating with stronger antimicrobial effects [40]. Additionally, the type of microorganism affects susceptibility, as Gram-positive bacteria are more sensitive to phenolic compounds than Gram-negative bacteria due to differences in cell wall structure. Gram-negative bacteria possess an outer membrane rich in lipopolysaccharides, which increases resistance by limiting the permeability of antimicrobial agents, whereas Gram-positive bacteria have a thick peptidoglycan layer that easily absorbs antimicrobial agents, making them generally more susceptible [41,42]. Phenolic compounds exert antimicrobial effects through multiple mechanisms, including interacting with cell wall proteins and subsequently changing the structure and function of the wall, denaturing microbial enzymes, and or making unavailability of some environmental nutrients (such as carbohydrates, proteins, vitamins, and minerals) through complex formation [43,44].

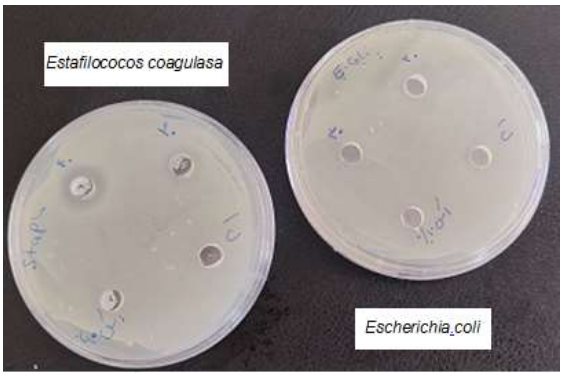

Figure 1 - Diameter of the inhibition zone of the ethanolic extract of *T. versicolor* L. against *S. aureus* and *E. coli*.

3-5. Glucan Extraction

The extraction of glucans involves two essential steps: the separation of the cell wall from the cytoplasm and the extraction of glucans from the insoluble cell wall. Quantitative determination of glucans and evaluation of the efficiency of three extraction methods on the glucan content of *T. versicolor* L. were performed. According to the results presented in Table 3, the extraction method significantly affected the glucan yield. The highest total glucan content (66.35%) was obtained using the hot water extraction method,

followed by the acid-alkali method. Meanwhile, the extraction efficiency of total glucans using the acid-based method was 32.72%.

Table 2. Average glucan yield based on extraction method of *T. versicolor* L.

Extraction method	Total glucans g/100g dm	α-glucans g/100g dm	β-glucan (g/100g)
Hot water	66.35±0.015[a]	10.04±0.045[a]	56.27±0.03[b]
Acid-based	32.72±0.025[c]	8.5±0.075[c]	26.85±0.05[c]
Acid-Alkali	66.07±0.015[b]	5.87±0.032[b]	57.53±0.03[a]

Angelova et al. (2022) reported that the total glucan, α-glucan, and β-glucan contents extracted by alcoholic extraction from *T. versicolor* L. were 30.39, 6.79, and 22.34 g/100 g, respectively. The lower amounts compared to the present study can be connected to differences in solvent type used for extraction [45]. In the study by Mirończuk-Chodakowska et al. (2020) on 18 wild mushroom species and three commercial species, β-glucan content ranged from 10.50 g/100 g dry matter in *Macrolepiota procera* to 34.97 g/100 g dry matter in *Tricholoma portentosum*. α-Glucan content in various species was generally below 10 g/100 g dry matter. Total glucan content ranged from 11.4 g/100 g (*Macrolepiota procera*) to 45.9 g/100 g (*Pleurotus ostreatus*) [46]. Compared to these findings, the current study showed significantly higher β-glucan content (26.85–57.53 g/100 g) in *T. versicolor* L., possibly due to species differences and extraction methods. α-Glucan levels (5.87–10.04 g/100 g) in this study were within the range reported by Mirończuk-Chodakowska et al. [46].

Kozarski et al. (2012) extracted glucans from *Ganoderma applanatum*, *Ganoderma lucidum*, *Lentinus edodes*, and *T. versicolor* using hot water extraction. They reported total glucan content in *T. versicolor* L. as 36.3 g/100 g dry weight, with α- and β-glucan contents of 2.9 and 33.4 g/100 g dry weight, respectively, which are lower than those obtained by hot water extraction in the present study [47]. Conversely, Rajabzadeh Shandiz et al. (2020) compared acidic, hot water, and acid-alkali extraction methods for β-glucan from *Agaricus bisporus*, finding the acid-based method yielded higher total glucan extraction than hot water and acid-alkali methods [48]. This contrasts with the present findings, which could be explained by inherent differences in fungal cell wall structure, as well as experimental conditions such as acid concentration, temperature, extraction time, or equipment used.

In a study investigating the effects of two mineral acids (H_2SO_4 and HCl) on β-glucan extraction from *T. versicolor* L., β-glucan yields were 1.47 and 3.53 g/100 g mushroom, respectively. These values were significantly higher than amount of β-glucan extracted by citric acid in the

current research. Moreover, the combination of alkali with citric acid increased β-glucan yield from 26.85 to 57.53 g/100 g mushroom. These results demonstrate that combining alkali with citric acid enhances β-glucan extraction efficiency compared to citric acid alone, aligning with the current study's findings on the synergistic effect of extraction reagents in increasing glucan yield [49].

4-Conclusion

The results of the present study demonstrated that *T. versicolor* L. possesses valuable bioactive properties and chemical constituents, which make it a promising candidate for applications in the food and pharmaceutical industries. The presence of significant amounts of essential minerals such as iron, zinc, and magnesium further emphasizes the nutritional role of this mushroom. Moreover, different glucan extraction methods significantly influenced the yield and quality of the extracted glucans, with the acid-alkali method providing the highest β-glucan extraction efficiency and the hot water method yielding the greatest total glucan content. Additionally, the antibacterial activity of the mushroom against *S. aureus* and its high antioxidant content highlight its potential for use in the development of immune-enhancing and oxidative stress-reducing products. Based on the findings, further research is recommended to elucidate the underlying biological mechanisms related to its therapeutic properties and to optimize extraction methods. Furthermore, feasibility studies on the utilization of this mushroom in the production of functional products, such as dietary supplements, fortified foods, and herbal medicines, could pave the way for its commercial development.

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بررسی خواص زیستی و شیمیایی قارچ *Trametes versicolor* L و ارزیابی روش‌های مختلف استخراج گلوکان‌ها از آن

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چکیده

قارچ *Trametes versicolor* L. به دلیل خواص زیستی و شیمیایی خود، پتانسیل بالایی برای استفاده در صنایع دارویی و غذایی دارد. این مطالعه با هدف آنالیز ترکیبات تشکیل دهنده قارچ، ارزیابی خواص ضدباکتریایی و مقایسه سه روش مختلف استخراج گلوکان‌ها (شامل استخراج با آب داغ، اسیدی و اسید-قلیایی) از قارچ *T. versicolor* L. انجام شد. براساس آنالیز شیمیایی، مقدار پروتئین، فیبر، خاکستر وکربوهیدرات قارچ، به ترتیب ۸/۳، ۵۴/۰۵، ۱/۴۳، ۳۰/۰۹ درصد بدست آمد. همچنین محتوای عناصر معدنی شامل آهن، روی و منیزیم به ترتیب ۵۹/۹، ۲۵/۹ و ۳۰۰ میلی‌گرم بر کیلوگرم و میزان پلی‌فنلی قارچ؛ ۲۳۰ میلی‌گرم در ۱۰۰ گرم محاسبه گردید. قطر هاله عدم رشد عصاره قارچ علیه *Staphylococcus aureus*، ۴۰ میلی‌متر اندازه‌گیری شد؛ هرچند عصاره قارچ برعلیه *Escherichia coli* خاصیت ضدمیکروبی نداشت. همچنین نتایج استخراج و محاسبه میزان گلوکان‌های قارچ نشان داد که بالاترین محتوای گلوکان کل و آلفا گلوکان در روش آب داغ با مقادیر ۶۶/۳۵ و ۱۰ درصد حاصل شد. در حالی که روش اسید-قلیا بیشترین محتوای بتاگلوکان (۵۷/۵۳ درصد) را نشان داد. این پژوهش نشان‌دهنده پتانسیل بالای *T. versicolor* L. به‌عنوان منبعی غنی از ترکیبات زیستی و عناصر معدنی برای کاربرد در صنایع غذایی و دارویی است.