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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Comparative Assessment of Nutritional Composition, Polyphenol Content and Antioxidative Properties of Edible and Medicinal Mushroom: *Coriolus versicolor*

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Abstract: In recent years, edible and medicinal mushrooms have a very important position in functional food and bioactive components. Mushrooms attract the attention of the medical industry with their nutritional properties as well as their benefits for consumer health. The objectives of this research were to to reveal the nutritional composition, antioxidant potential, phenolic and flavonoid content of the commercially edible and medicinal mushroom *Coriolus versicolor* and to evaluate its anti-cancer effect against HT-29 cells. In this context, the nutrient composition was analyzed in accordance with the Association of Official Analytical Chemists (AOAC) procedure. *C. versicolor* showed a high nutritional value with protein, carbohydrate, dietary fiber and glucan content. The total phenolic (TPC) and total flavonoid contents (TFC) of the water, ethanol and methanol extracts of the specified mushroom were determined, and it was found that the ethanol extract had the highest TPC (172.80±2.35 mg GAE/g dw) and TFC (48.72±2.89 mg QE/g dw) values among the three extract types. In addition, the antioxidant capacity of extracts was compared with different methods (DPPH, ABTS, FRAP, and CUPRAC). The ethanol extract showed the highest DPPH (39.16±0.82 µM TE/g dw), ABTS (29.19±1.30 µM TE/g dw) and CUPRAC (37.17±0.79 µM TE/g dw) activities among the other extracts, while FRAP (21.01±1.62 µM TE/g dw) activity for water extract was determined to be the highest. Finally, when the anti-cancer effects of these extracts were evaluated against HT-29 cells, it was observed that ethanol, methanol and water extract inhibited 82.43%, 79.15% and 65.56%, respectively, at the end of 48 hours.

Keywords: Anti-cancer activity, antioxidative, *Coriolus versicolor*, mushroom extract, nutritional composition, polyphenol content.

Yenilebilir ve Tıbbi Mantar *Coriolus versicolor*'un Besin Bileşimi, Polifenol İçeriği ve Antioksidatif Özelliklerinin Karşılaştırmalı Değerlendirmesi

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Öz: Son yıllarda, yenilebilir ve tıbbi mantarlar, fonksiyonel gıda ve biyoaktif bileşen kaynakları olarak daha fazla dikkat çekmektedir. Besleyici özelliklerinin yanı sıra mantarlar, tüketici sağlığına olan faydaları ile de medikal endüstrinin ilgisini çekmektedir. Bu çalışma, ticari olarak satın alınan, yenilebilir ve tıbbi mantar *Coriolus versicolor*'un besin bileşimini, antioksidan potansiyelini, fenolik ve flavonoid içeriğini belirleyerek HT-29 hücrelerine karşı anti-kanser etkisini değerlendirmeyi amaçlamıştır. Bu kapsamda, ilk olarak, besin bileşimi Resmi Analitik Kimyacılar Birliği (AOAC) prosedürüne göre belirlenmiştir. *C. versicolor* mantarı, sahip olduğu protein, karbonhidrat, diyet lifi ve glukan içeriği ile yüksek bir besin değeri göstermektedir. Belirtilen mantarın su, etanol ve metanol ekstrelerinin toplam fenolik (TPC) ve toplam flavonoid içerikleri (TFC) belirlenmiş ve etanol ekstresinin üç ekstre türü içerisinde en yüksek TPC (172,80±2,35 mg GAE/g ekstre) ve TFC (48,72±2,89 mg QE/g ekstre) değerlerine sahip olduğu bulunmuştur. Ayrıca, bu ekstrelerin antioksidan kapasitesi farklı yöntemlerle (DPPH, ABTS, FRAP ve CUPRAC) karşılaştırılmıştır. Etanol ekstresi diğer ekstreler arasında en yüksek DPPH (39,16±0,82 µM TE/g ekstre), ABTS (29,19±1,30 µM TE/g ekstre) ve CUPRAC (37,17±0,79 µM TE/g ekstre) aktivitelerini gösterirken, su ekstresinin FRAP (21,01±1,62 µM TE/g ekstre) aktivitesi en yüksek olarak belirlenmiştir. Son olarak, bu ekstrelerin HT-29 hücreleri üzerindeki anti-kanser etkileri değerlendirildiğinde, 48 saat sonunda etanol, metanol ve su ekstrelerinin sırasıyla %82,43, %79,15 ve %65,56 oranında inhibe ettiği gözlenmiştir.

Anahtar kelimeler: Antikanser aktivite, antioksidatif, besin bileşimi, *Coriolus versicolor*, mantar ekstresi, polifenol bileşimi.

INTRODUCTION

Recently, mushrooms have attracted great interest in food science and pharmacology due to their potential nutritional value and medicinal properties. They play an important role in our health with their use as food supplements (Assemie & Abaya, 2022; Lopez-Hortas et al., 2022). Edible mushrooms, are mainly consumed as fresh mushrooms with fruiting bodies or dried products. Medicinal mushrooms are mostly used in biopharmaceutical applications in powdered, loose, or liquid extract forms (Elkhateeb et ak., 2019). The chemical composition of mushrooms, which usually consists of 90% water and 10% dry matter, is important in terms of nutritional value. Mushrooms are good sources of protein, especially with their amino acid content (aspartate, glutamate, etc.) and are generally analogous to animal proteins. In this context, they are considered an ideal supplement for vegetarian diets (Assemie & Abaya, 2022; Mwangi et al., 2022; Sganzerla et al., 2022). The protein and total carbohydrate content of mushrooms differ between 15%-35% and 35%-70% of dry weight, respectively, according to the species and cultivation process. Furthermore, mushrooms are characterized as healthy food sources thanks to their high fiber content (chitin and β-glucans) and low-fat content. In addition to the valuable nutritional composition of mushrooms, their bioactive compounds provide consumers with both a good food source and positive medical effects on health and medicinal mushrooms have been used worldwide in folk medicine for centuries. (Stojanova et al., 2021; Wu et al., 2021). Mushrooms are foods with high antioxidant value owing to their polysaccharides and phenolic compounds. Antioxidants have an important value in human nutrition and protect cells from the negative effects of free radicals. Phenolics are aromatic hydroxyl compounds known for their high antioxidant potential. Flavonoids are the compounds responsible for the antioxidant activity specific to polyphenolic compounds, both as hydrogen donors in stabilized radical formation and due to their ability to chelate transition metal ions (Raseta et al., 2020; Stojanova et al., 2021).

Mushrooms and mushroom extracts have gained important biological activities such as antioxidant, antimicrobial, anti-cancer, anti-inflammatory, antiobesity, hypocholesterolemic, hypoglycaemic and immunomodulatory thanks to their secondary metabolites (polysaccharides, proteins, peptides, terpenoids, polyphenols, vitamins, and mineral elements). The chemical profile of medicinal mushrooms varies according to species, strain, cultivation conditions, the degree of maturity and the proportion of individual anatomical parts in the total mass of the mushroom (Barros et al., 2007; Safin et al., 2022). In virtue of their nutritional composition and bioactive compounds as well as their biological properties, mushrooms are attracting more attention as functional foods and nutraceutical agents to provide better health conditions (Matijasevic et al., 2016; Abdelshafy et al., 2022). The potential of several mushroom species as antitumor agents has been observed and it has been proposed to be used as a biological factor in the control of many types of cancer. Many studies have shown that *Auricularia* species against ACHN, MCF-7 and CoLo-205 cancers (Arora et al., 2013), *Ganoderma lucidum* against human lung cancer (A549, H441 and H661) (Chen et al., 2016), *Coprinus* species against human colon cancer (Colo-205) (Khan et al., 2016), *Lentinus edodes* species against colorectal carcinoma cells (HCT-116, SW-480) (Seklic et al., 2021). Some species of mushrooms known to be beneficial for health are used as a supply of bioactive compounds in the development of functional foods and new pharmaceuticals (Abdelshafy et al., 2022). In this context, *Coriolus versicolor*, an important medicinal mushroom, will be evaluated in this study.

C. versicolor, also known as *Trametes versicolor* or *Polyporus versicolor* in the literature, belongs to the family Polyporaceae and is included in the Basidiomycotina section (Cruz et al., 2016). Polysaccharides of *C. versicolor* [(polysaccharopeptide (PSP) and polysaccharopeptide Krestin (PSK)] have antioxidant effects and especially have blood sugar lowering effect. It is also used in the treatment and prevention of tumor diseases such as hepatitis B, liver, breast and stomach cancer and some immune deficiency diseases. These polysaccharides with therapeutic properties are the best known commercially from *C. versicolor* extracts. Both polysaccharides (PSP and PSK) consist of β-glucans, D-glucose polymers with β-1,3 and α-1,4 glycosidic bonds, but some may also contain ut some may contain six-carbon sugars (Knezevic et al., 2018).

In this context, the objective of this research was to reveal the nutritional composition of the medicinal and edible mushroom *C. versicolor* and to compare the antioxidant activities and phenolic contents of three different extracts (aqueous, ethanolic and methanolic) obtained from this mushroom species. In addition, the anti-cancer activities of these extracts on HT-29 human colon adenocarcinoma cells were evaluated.

MATERIAL AND METHOD

Mushroom material: The commercial *Coriolus versicolor* mushroom was purchased from AGROMA Food Agriculture Livestock Industry Trade Limited Company (Denizli, Turkey). The *Coriolus versicolor* mushroom used in this study was harvested in Denizli prefecture in Turkey in 2022.

Evaluation of mushroom extracts nutritional value: The proximate nutritional composition, including ash, total protein, total carbohydrates, fat, total dietary fiber (TDF) and glucan content was carried out according to the Association of Official Analytical Chemists (AOAC) procedure for the dried and milled *Coriolus versicolor*.

Ash content was analyzed by burning of the sample in a muffle furnace (930.22 method, AOAC 2012). *Total protein content* of the sample was measured over the total nitrogen content determined by Kjeldal method (AOAC, 2007). *Total carbohydrate content* was determined using the method described by DuBois et al., (1956) and glucose was used as a standard. *Fat content* was analyzed by extraction in the soxhlet system (Method 920.39, AOAC 2012). *Dietary fiber content* was determined as total dietary fiber (TDF), soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) content by K-TDFR-100A (Megazyme Int., Dublin, Ireland) according to AOAC (method 991.43, AOAC, 2007). The results were expressed as g/100 g dried and milled mushrooms. *Glucan contents* were determined as total, α- and β-glucan contents using the β-glucan Assay Kit (Mushroom and Yeast) (Megazyme Int., Dublin, Ireland) according to the kit instructions. Results were expressed as g/100 g of dried and milled mushroom.

Preparation of mushroom extracts: The dried *C. versicolor* (Cv) mushrooms were powdered by grinding with a laboratory blender. Powdered materials were mixed in liquid (water, 50% aqueous ethanol, and 50% aqueous methanol) ratio of 1:20 (g/mL), and the blends were ultrasonication at room temperature for 30 min. Next, they were subject to solid-liquid extraction for 24 h at room temperature, in dark conditions, on a magnetic stirrer (540 rpm). At the end of the period, the extracts were centrifuged (5000 g for 15 min) and then filtered Whatman No:1. Solvents were completely evaporated with a rotary evaporator (Heidolph Laborota 4000) and then samples were lyophilized. Finally, *C. versicolor* water extract (Cv-dH_2O), 50% (v/v) aqueous ethanol extract (Cv-EtOH) and 50% v/v methanol extract (Cv-MeOH) were obtained and stored at +4 °C further experimental assays.

Evaluation of total phenolic and flavonoid content: The total phenolic content (TPC) of the *C. versicolor* extracts (Cv-dH_2O, Cv-EtOH, and Cv-MeOH) was measured by using a Folin-Ciocalteu colorimetric assay with minor changes (Gamez-Meza et al., 1999). Gallic acid as a standard (R^2=0.9974) and expressed as mg gallic acid equivalents (GAE) per g of extract (mg GAE/g dw). The total phenolic content (TFC) of the extracts was analyzed with the $AlCl_3$ colorimetric method (Chang et al., 2002), and Quercetin as a standard (R^2=0.9967). The findings were expressed as milligrams of Quercetin equivalent per gram of dry extract (mg QE/g dw)

In vitro antioxidant capacity

DPPH radical scavenging activity: DPPH radical scavenging activity of *C. versicolor* extracts (Cv-dH_2O, Cv-EtOH and Cv-MeOH) was detected using the method described by Brand-Williams et al., (1995) with minor changes. In brief, freshly prepared methanol DPPH solution with a concentration of 4×10^{-4} M was mixed with each extract at a ratio of 4:1 (v/v) and the absorbance was measured at a wavelength of 517 nm after incubation. DPPH radical scavenging activity was given as a function of Trolox equivalent antioxidant capacity (TEAC) (µM TE/g dw).

ABTS radical scavenging activity: ABTS radical cation scavenging activity of *C. versicolor* extracts (Cv-dH_2O, Cv-EtOH and Cv-MeOH) was evaluated by slightly modifying the method reported by Re et al., (1999). Firstly, ABTS stock solution was reacted with $K_2S_2O_8$ to obtain $ABTS^+$. The mixture was kept in the dark and overnight at room temperature. Each extract was mixed with $ABTS^+$ solution in a 1:10 v/v ratio. The resulting reaction mixture was incubated at room temperature in the dark and the absorbance was measured at a wavelength of 734 nm. ABTS radical cation scavenging activity was expressed as a function of TEAC (µM TE/g dw).

Ferric-reducing antioxidant power activity (FRAP): Ferric reducing antioxidant power activity of *C. versicolor* extracts (Cv-dH_2O, Cv-EtOH and Cv-MeOH) was measured by the method according to Benzie and Strain, (1996). Each extract was mixed with FRAP solution in a 1:9 v/v ratio. The mixture was incubated at 37 °C under dark conditions and the absorbance was measured at 593 nm wavelength. Results were expressed as a function of TEAC (µM TE/g dw).

Cupric ion reducing antioxidant capacity (CUPRAC): The cupric ion-reducing antioxidant capacity of *C. versicolor* extracts (Cv-dH_2O, Cv-EtOH and Cv-MeOH) was evaluated the method described by Apak et al., (2004) with minor modifications. One mL each of $CuCl_2$ solution (1.0×10^{-2} M), neocuproin solution (7.5×10^{-3} M) and NH_4OOCCH_3 buffer solution (1M) (pH: 7.0) were mixed in a test tube. Each extract was added to the reaction mixture obtained by mixing with water in a 1:10 v/v ratio. After incubation, absorbance was measured at 450 nm wavelength. The results were expressed as a function of TEAC (µM TE/g dw).

In vitro anti-cancer activity

Cell culture and cell line: HT-29 (ATCC HTB-38) (human colon adenocarcinoma) cells were used in in vitro studies. Cells were kept in Dulbecco's Modified Eagle Medium (DMEM) consisting of high glucose and supplemented with 10% fetal bovine serum and 1% antibiotic solution.

MTT cytotoxicity assay: The cytotoxic property of *C. versicolor* extracts was evaluated in HT-29 cells by MTT

assay reported by Khodavirdipour et al., (2021). Concisely, cells at 70-80% confluency were trypsinized and seeded in a 96-well plate at a density of 1×10^4 cells/well. After overnight attachment, the medium was removed from the wells and a serial twofold dilution (1000-62.5 µg/mL) was added from the stock solution prepared at a concentration of 1000 µg/mL. Plates were incubated 5% CO_2 at 37 °C for 24 and 48 hours. After incubation, the medium was removed from each well, MTT reagent (Sigma-Aldrich®, Germany) was added and incubated for two hours at 37 °C in 5% CO_2. After incubation, the MTT reagent was aspirated, DMSO was added to dissolve formazan crystals and the plate was shaken for 5 min at room temperature. Absorbance was measured with an ELISA multifunctional microplate reader (Thermo Scientific™ Multiskan Go™, USA) at a wavelength of 570 nm. Untreated cells (without mushroom extract) were used as negative control, while DMSO-treated cells were used as vehicle control. Each experiment was performed in eight replicates (n=8). The inhibition (%) of the mushroom extracts was determined by Eq. 1. The Ic50 was calculated and analyzed by GraphPad Prism v. 8.

$$Inhibition\ (\%) = [A570\ nm\ of\ treated\ cells \div A570\ nm\ of\ control\ cells] \times 100 \quad \text{Eq.1}$$

Statistical analysis: The results were expressed as Mean±SD. *In vitro* test data were statistically analyzed with One-Way ANOVA and Tukey's multiple comparisons tests. The data were presented as a mean with a 95% confidence interval (CI). P-values less than 0.05 were deemed statistically significant.

RESULTS

Nutritional composition: The nutrient contents of the *C. versicolor* mushroom were given in Figure 1a. This edible and medicinal mushroom was determined to have approximately 9.07% ash, 29.22% protein, 56.16% carbohydrate and 2.03% fat content by the AOAC procedures. According to Assemie and Abaya, (2022) most the edible mushroom contain (35-70%) carbohydrates, (15-34.7%) protein, (10%) fat and (6-10.9%) minerals, also Cui and Chisti, (2003) reported that the content of PSP (polysaccharopeptide) and PSK (polysaccharopeptide Krestin) extracted from *C. versicolor* typically contain (34-35%) soluble carbohydrates, (28-35%) protein, (~7%) moisture, (6-7%) ash and the remainder are free sugars and amino acids. So, the present findings content of *C. versicolor* were in agreement with the reported values. Recently, dietary fibers of edible plant and macrofungal origin have come to the forefront with various health effects in obesity, diabetes, cancer, and intestinal diseases (He et al., 2022). Dietary fiber and glucan which are resistant to digestive enzymes are among the important components of edible mushrooms. Dietary fiber (TDF, IDF, and SDF) and glucan content (total, α, and β) of *Coriolus versicolor* mushroom are given in Figure 1b. IDF and SDF contents were determined as 31.42±1.04 g/100 g dw and 2.87±0.83 g/100 g dw, respectively. Total glucan content was determined as 21.04±0.77 g/100 g dw and this content was formed by β-glucans (16.82±1.41 g/100 g dw) and α-glucans (3.95±1.41 g/100 g dw) (Figure 1c). The dietary fiber and glucan content of *C. versicolor* has been reported previously. For example, *T. versicolor* NBIMCC 8939 contained 39.53±0.61 g/100g dw TDF, 36.21±0.45 g/100g dw IDF and 1.99±0.15 g/100g dw SDF (Angelova et al., 2022). Also in another study, selenium-enriched crude exopolysaccharides of *C. versicolor* were determined to have 31.12±1.76 mg/g total glucan, 8.33±0.02 mg/g α-glucan and 19.48±1.6 mg/g β-glucan contents (Miletic et al., 2021). Wild-type *C. versicolor* collected from North Rhine Westphalia, Germany, was found to contain 61.194±11.611 g/100g dw total glucans, 0.406±0.232 g/100g dw α-glucan and 60.788±11.795 g/100g dw β-glucan (Sari et al., 2017). When the studies in the literature are evaluated, in general, the nutrient composition of mushrooms varies from species to species, but it depends largely on the environment and cultivation techniques. According to the results obtained in our study, the composition of *C. versicolor* mushroom showed a high nutritional value with high protein, carbohydrate, dietary fiber, and glucan content.

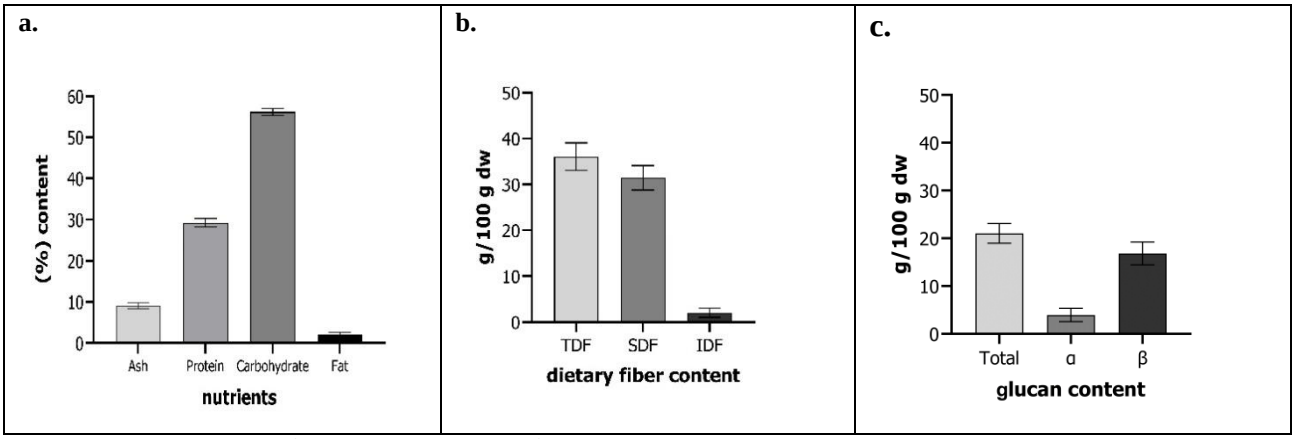

Figure 1. a. Nutrients **b.** dietary fiber and **c.** glucan content of *C. versicolor*

Total phenolic and flavonoid contents

Phenolic and flavonoid compounds are important metabolites generally found in edible and medicinal mushroom species. Thanks to these metabolites, mushrooms provide many benefits for human health (as well as anti-allergenic, anti-inflammatory, antioxidant, antimicrobial, and anti-tumor effects) and are also of interest as functional foods and nutraceutical agents (Abdelshafy et al., 2022; Silva et al., 2023).

Three different extracts (water, 50% aqueous ethanol, and 50% aqueous methanol) were obtained to evaluate TPC and TFC of *C. versicolor*. TPC ranged from 91.30±1.07 to 172.80±2.35 mg GAE/g dw, while TFC ranged from 30.36±1.14 to 48.72±2.89 mg QE/g dw (Figure 2). TPC and TFC of ethanol extract were higher than the other extracts. In research carried out by Matijasevic et al., (2016), the TPC of the *C. versicolor* methanol extract was measured as 25.8±1.4 mg GAE/g dw and TFC as 4.3±0.2 mg CE/g dw. In another study, water and ethanol extracts were obtained from *C. versicolor* mushroom collected from the Republic of North Macedonia and it was determined that the water extract had a composition of TPC 12.88±5.25 mg GAE/g dw and TFC 5.95±1.37 mg QE/g dw (Stojanova et al., 2021). Our results are consistent with the TPC of *T. versicolor* water and ethanol extracts (110.2±0.8 and 163.5±0.8 mg GAE/100 g dw respectively), reported by Raseta et al., (2020). The same authors measured the TFC in the water and ethanol extract at 31.4±0.7 mg QE/100 g dw and 37.8±0.3 mg QE/100 g dw respectively, which is in accordance with the current results. In general, polyphenol content varies depending on the solvent used. In the literature, higher phenol yields were obtained in ethanol and methanol organic solvents. The results obtained in our research are in harmony with the literature; ethanol was found to be a good choice for the processing of *C. versicolor* mushroom when ethanol was used as a solvent.

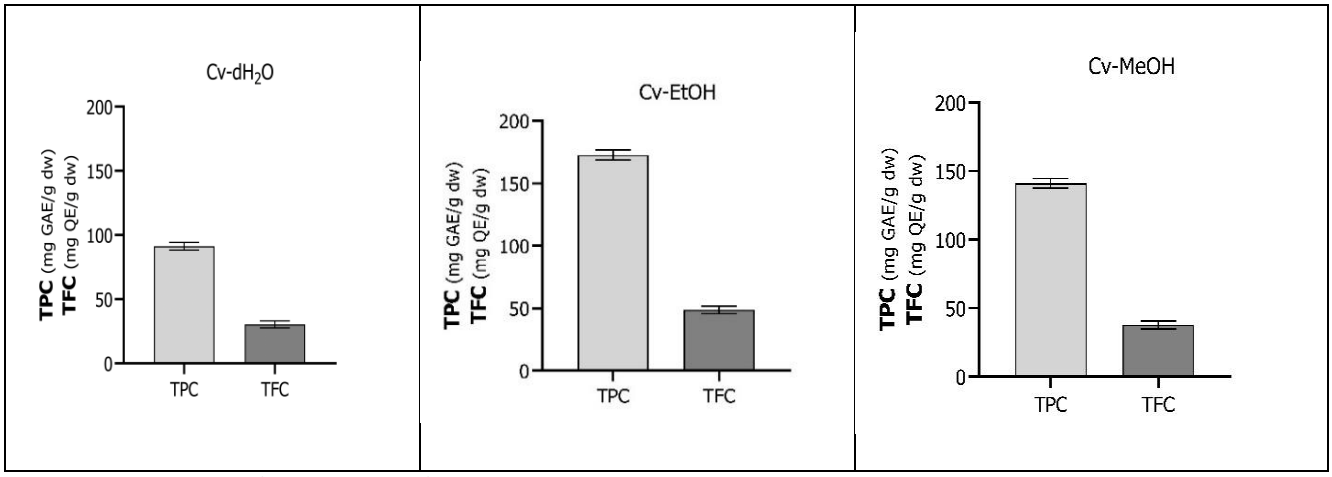

Figure 2. Total phenolic and flavonoid content of *C. versicolor* extracts

Antioxidant capacity

Antioxidant compounds of mushroom extracts have become an important resource in pharmaceutical and food industries to supply products with bioactive components, replacing synthetic antioxidant substances (Zielinski et al., 2016). Phenolic and flavonoid contents of edible and medicinal mushrooms are responsible for the antioxidant activity of mushroom extracts (Abd Razak et al., 2019; Aljadi & Kamaruddin, 2004). In addition, a strong correlation has been found between the polyphenol content and antioxidant activity of mushroom species thanks to its ability to scavenge hydroxyl groups (Smolskaite et al., 2015; Abd Razak et al., 2019; Contato et al., 2020). Four different antioxidant tests (DPPH, ABTS, FRAP, and CUPRAC) were applied to investigate the antioxidant capacity of *C. versicolor* extracts and the results obtained are indicated in Table 1.

DPPH radical scavenging activity: In the current study, all extracts were found to be efficient scavengers against DPPH radicals. EtOH extract showed the highest radical scavenging capacity, while water extract was the lowest radical scavenger.

ABTS radical scavenging activity: All extracts showed ABTS radical scavenging activity and ethanol extract was found to have the highest ABTS radical scavenging activity among all extracts.

Ferric reducing antioxidant power activity (FRAP): Among all extracts, water extract was determined to have the highest FRAP activity.

Cupric ion-reducing antioxidant capacity (CUPRAC): It was determined that all extracts were effective against cupric ions and, especially ethanol extract had the highest CUPRAC activity among all extracts.

The antioxidant activity of extracts obtained from *C. versicolor* has previously been reported. For example,

the dried and milled crude extract obtained from the *C. versicolor* mushroom showed 52.2±2.2 (µmol TE/g dw) DPPH scavenging ability, 69.5±2.0 (µmol TE/g dw) CUPRAC and 143.1±5.5 (µmol TE/g dw) ABTS scavenging ability (Kozarski et al., 2020). Another study used 80% ethanol, methanol, and water extracts from *T. versicolor* NBIMCC 8939 for DPPH, ABTS, FRAP, and CUPRAC activity. Among the three extracts, water extract was determined to have the highest DPPH (5.63±0.11 µM TE/g dw), ABTS (28.16±0.49 µM TE/g dw), CUPRAC (52.21±0.28 µM TE/g dw) activities and methanol extract was determined to have the highest FRAP (6.12±0.11 µM TE/g dw) activity (Angelova et al., 2022).

In an additional study, the antioxidant activity of *C. versicolor* was evaluated. DPPH (773 mM TE/g dw), ABTS (2.930 mM TE/g dw), and FRAP (1.710 mM TE/g dw) tests revealed antioxidant activity (Maeng et al., 2017). When the antioxidant capacity values measured in the present study were compared with previous studies, it was observed that some antioxidant capacity types were higher or lower than others. The main reason for this situation may be due to the habitat of the mushroom, cultivation, collection time, the concentrations used in antioxidant activity analyses, extraction solvent and extraction methods, etc.

Table 1. *In vitro* antioxidant activity values of *C. versicolor* extracts.

	Mushroom Extracts		
	Cv-dH_2O	**Cv-EtOH**	**Cv-MeOH**
DPPH (µM TE/g dw)	12.02±1.75	39.16±0.82	30.05±1.48
ABTS (µM TE/g dw)	19.08±1.60	29.19±1.30	24.27±0.76
FRAP (µM TE/g dw)	21.01±1.62	15.63±1.24	12.45±0.80
CUPRAC(µM TE/g dw)	16.33±0.56	37.17±0.79	28.64±0.93

Anti-cancer activity: The anti-cancer effects of water, ethanol and methanol extracts of *C. versicolor* on HT-29 (ATCC HTB-38) (human colon adenocarcinoma) cells for 24 and 48 hours were evaluated. The results are given in Figure 3. During 24 and 48 hours, it was shown that the inhibition percentages increased with the increase in concentration in all three extract types and accordingly, the highest concentration (1000 µg/mL) in all three extracts was determined to have the highest anti-cancer activity against HT-29 cells. At the highest concentration of water extract 60.83% and 65.56%, at the highest concentration of methanol extract 71.27% and 79.15%, and finally, at the highest concentration of ethanol extract, 75.19% and 82.43% inhibition percentages against HT29 cells were obtained at 24 and 48 hours, respectively. The IC_{50} values of water, ethanol and methanol extracts were determined as 636.2 µg/mL, 363.8 µg/mL and 396.1 µg/mL after 48 h, respectively. As shown in Figure 3 it was determined that ethanol extract had the highest anti-cancer effect against HT-29 cells among the three extract types. Methanol and water extracts also have a potent anti-cancer effect on HT-29 cells. In some of the previous studies, the anti-cancer effect of *C. versicolor* was evaluated. For example, the anti-cancer effects of polysaccharide-rich extracts from *T. versicolor* were evaluated in HT-29 cells. This effect was seen even at 10 µg/mL, the lowest concentration tested for the extracts. These results suggest that *T. versicolor* extract reduces cell growth and thus reduces the oncogenic potential in colon cancer cells (Roca-Lema et al., 2019).

In a different study, the anti-cancer effect of selected *Trametes* species from Serbia against HeLa, LS174, and A549 cells was evaluated. The results showed that *T. versicolor* basidiocarp extract had the most effective anti-cancer activity against HeLa cells (IC50 42.40 ± 0.74µg/mL) among the indicated cancer cells (Knezevic et al., 2018). Moreover, the antiproliferative activity of H_2O and EtOH extracts of *T. versicolor* against the growth of MCF-7 cells was evaluated for 24 and 72-hour incubation periods. Notably, ethanol extracts of *T. versicolor* were found to have the strongest growth inhibitory activity (IC_{50}=327.0±0.7 µg/mL) after 24 h acute phase and the best chronic cytotoxic activity (IC_{50}=701.8±0.6 µg/mL) after 72 h (Raseta et al., 2020). Several studies have shown that compounds as well as extracts isolated from different species of the genus *Trametes* have considerable cytotoxic potential against different types of cancer (Ren et al., 2006; Harhaji et al., 2008; Shnyreva et al., 2018).

CONCLUSION

This study revealed the potential of crude macrofungal biomass as a dietary supplement due to the high protein, carbohydrate, dietary fiber, and glucan content of edible and medicinal mushroom *C. versicolor*. The crude *C. versicolor* mushroom may be an ideal approach to achieve natural dietary supplements with significant health benefits. Furthermore, among the *C. versicolor* extracts examined, the best results of antioxidant analyses were found in the ethanolic extract. Total flavonoid and total phenolic contents were found to have the highest value in ethanol extract and thus phenolic and flavonoid contents were found to be related to antioxidant activity. In this context, this extract has the potential to replace some synthetic antioxidants used for industrial purposes. With the evaluation of its effect on HT-29 cells, it was revealed that ethanol extract could be used in a new treatment approach for colon cancer patients. In the light of these promising results obtained, novel nanoformulations can be designed for the use of this mushroom extract both as a dietary supplement and as a therapeutic agent in anti-cancer therapies, thus maintaining their bioactivity, controlled release, and bioavailability as well as stability throughout storage, processing and consumption.

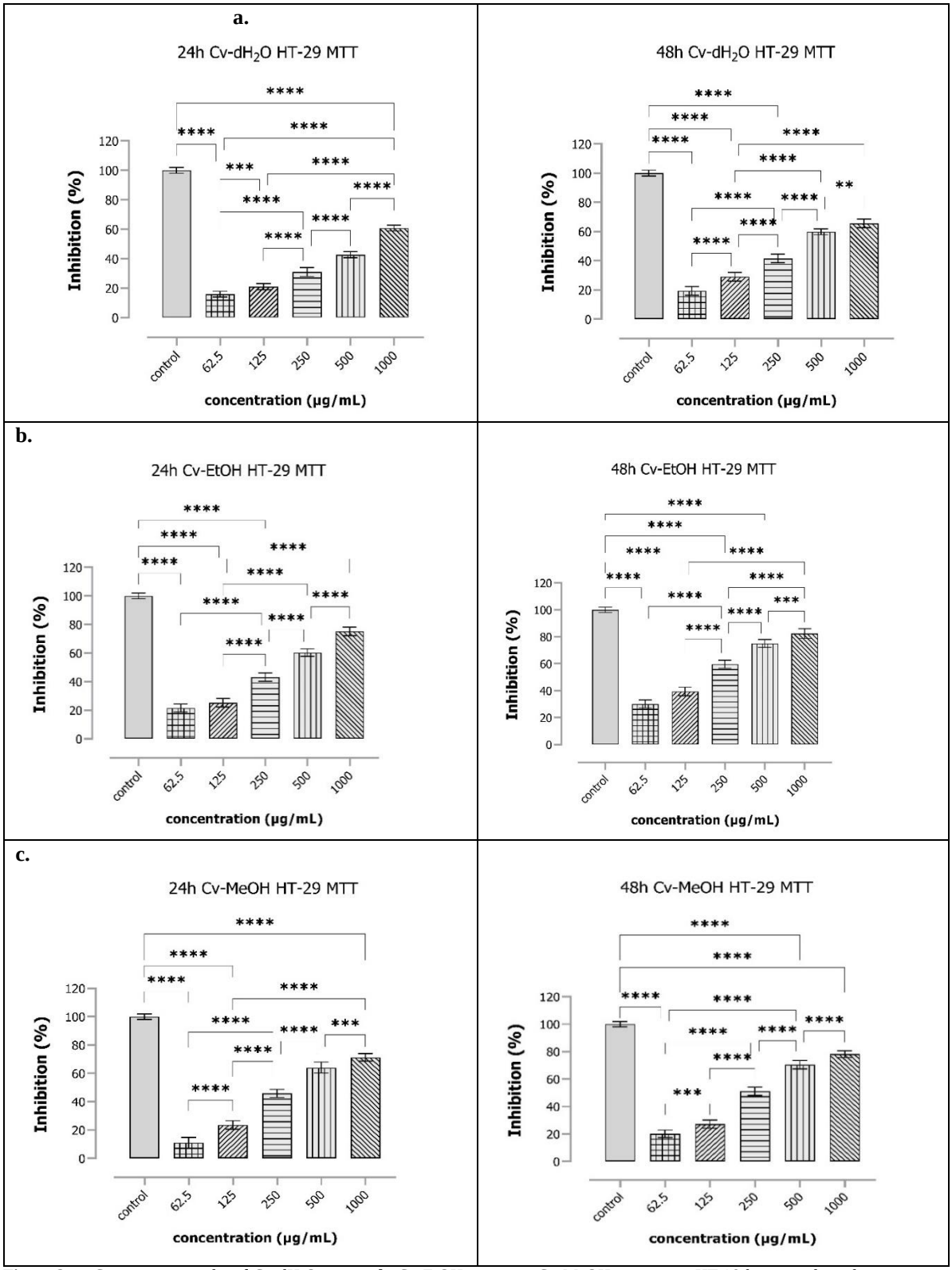

Figure 3. a. Cytotoxicity results of Cv-dH_2O extract, **b.** Cv-EtOH extract **c.** Cv-MeOH extract on HT-29 human colon adenocarcinoma cells (A, P value below 0,05 was considered as statistically significant).

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