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ORIGINAL PAPER

Phenolic profile, antioxidant and cholinesterase inhibitory activities of four *Trametes* species: *T. bicolor*, *T. pubescens*, *T. suaveolens*, and *T. versicolor*

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Abstract

Trametes genus is one of the most important medicinal mushroom species in the world. The present study focused on phenolic profile, cholinesterase inhibitory and antioxidant activities of four *Trametes* species (*T. bicolor, T. pubescens, T. suaveolens* and *T. versicolor*). Phenolic profiles of the mushrooms were characterized by HPLC–DAD. $ABTS^{\cdot+}$ scavenging, β-carotene-linoleic acid, Cupric-reducing antioxidant capacity (CUPRAC), $DPPH^{\cdot}$ scavenging, and metal chelating assays were performed to evaluate antioxidant activities of the extracts. Ellman method was used to test butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) inhibitory activities of the extracts. The most abundant compound was fumaric acid (4.51 ± 0.10 µg/g) in *T. bicolor*, *trans*-cinnamic acid (0.49 ± 0.05 µg/g) in *T. pubescens,* catechin hydrate in *T. suaveolens* (0.92 ± 0.16 µg/g) and *T. versicolor* (0.96 ± 0.19 µg/g). *T. pubescens* acetone extract showed the highest antioxidant activity in CUPRAC ($A_{0.50}$: 19.26 ± 0.21 µg/mL), $ABTS^{\cdot+}$ (IC_{50}: 3.55 ± 0.16 µg/mL), $DPPH^{\cdot}$ (85.12 ± 0.44%), β-carotene-linoleic acid (IC_{50}: 1.12 ± 0.41 µg/mL) assays. The best metal chelating activity was found in *T. versicolor* hexane extract (56.78 ± 0.63%). It was determined that *T. pubescens* hexane extract (IC_{50}: 7.37 ± 0.55, 15.24 ± 0.98 µg/mL, respectively) showed higher AChE and BChE inhibitory activities. The results of this study support the potential use of *Trametes* species to design new functional drug formulations.

Keywords *Trametes* species · Anticholinesterase activity · Phenolic compounds · Antioxidant activity

Introduction

Mushrooms are considered as nutraceutical food all over the world due to their important role in the prevention of several diseases [1]. Many cultures in the world, especially in the East, have approved that extracts from mushrooms may have health-improving benefits and become essential components of traditional folk medicine [2]. Also, a large number of literature studies have reported that mushrooms accumulate secondary metabolites, such as phenolics, polysaccharides, steroids and terpenes exhibiting immunomodulating, antiviral, antidiabetic, antitumor, anticancer, antioxidant and antibacterial effects [3–5]. Among various secondary metabolites reported from mushrooms, phenolic compounds have been gained more attention due to their potential advantages for human health such as antioxidant, antitumor, anti-inflammatory, anti-hyperglycemic, anti-osteoporotic, antimicrobial and anti-tyrosinase activities [6, 7]. On the basis of these valuable biological activities, the use of mushroom phenolics for nutraceutical, cosmetic and industrial applications have become a new trend appreciated in recent years [8].

Lentinus, Auricularia. Hericium, Grifola, Flammulina, Pleurotus, Tremella, Ganoderma, and *Trametes* mushroom species are appreciated as medicinal and functional mushrooms [9]. *Trametes* species belong to the Polyporaceae family and there are about 60 species of *Trametes* in the world, especially in warm climates. *T. versicolor* (Turkey tail), one of the most precious medicinal mushrooms, has been the most actively studied mushroom in the last twenty years and

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is the source and producer of polysaccharide krestin (PSK) and polysaccharide peptide (PSP). Also, *T. versicolor* has been reported to possess a great number of bioactive properties such as anticancer, antitumor, hepatoprotective, immunomodulating, antioxidant, antibacterial, anticholinesterase, and cardiovascular effects [10]. *T. pubescens* is an important mushroom used in Asian countries to treat gastrointestinal diseases and cancer in folk medicine [11]. *T. gibbosa* known as lumpy bracket has been reported to have antitumor, antimicrobial, antioxidant, antifungal, antineurodegenerative, cytotoxic, α-amylase, tyrosinase, and α-glucosidase inhibitory effects [12, 13]. Immunomodulatory, antioxidant, anticholinesterase, α-glucosidase, tyrosinase, and α-amylase inhibitory effects of *T. hirsuta*, called hairy bracket, have been stated [12–14].

Recent studies have shown that reactive oxygen and nitrogen-induced oxidative stress have an important effect in various inflammatory and degenerative diseases such as Alzheimer's disease (AD), rheumatoid arthritis, cancer, cataract, diabetes, and aging. Live systems have a balance between free radicals produced in extreme amounts for various reasons and exogenous and endogenous antioxidants have mission for detoxification of these radicals. The disruption of this balance towards oxidants is described as oxidative stress that plays a significant role in the etiopathogenesis of many diseases [15, 16]. In this context, when the endogenous antioxidants are inadequate, they must be supplemented to avoid the formation of oxidative stress. To support the antioxidant system, exogenous antioxidants can be taken with foods or some preparations and usually directly or indirectly. AD is a neurodegenerative disease that is common in the elderly, decreasing their mental abilities and cognitive processes [17]. The key therapeutic approach adopted in the AD therapy is the inhibition of acetylcholinesterase (AChE), which causes the breakdown of acetylcholine (ACh) in the brain, thereby increasing the amount of AChE [18]. It has been reported that many plants and bioactive substances derived from them have traditionally been used by the public to treat various neurodegenerative diseases. Galantamine, physostigmine, rivastigmine, huperzin A, etc., are natural or semi-synthetic derived AChE inhibitors specified for AD [19]. In addition, AD was well described to be associated with a decrease in the defence mechanism of the brain and plasma antioxidants [20]. Toxic substances such as peroxides, alcohols, aldehydes, free carbonyls, ketones caused by the effects of reactive oxygen species on high levels of proteins, lipids, enhanced DNA oxidation and glycoxidation end products, lead oxidative modifications in nuclear and mitochondrial DNA. These manifestations have been identified as the main signs of oxidative stress or damage that occurs during AD [21, 22].

At this point, it is very important to identify phenolic compounds in biological materials, mushrooms and foods and evaluate the antioxidant and anticholinesterase activities. In the current research, antioxidant and cholinesterase inhibitory activities of the methanol, hexane, and acetone extracts of four different *Trametes* species (*T. bicolor, T. pubescens, T. suaveolens*, and *T. versicolor*) which is accepted as one of the most important medicinal mushroom species in the world, were evaluated. Also, phenolic compositions of the *Trametes* species were characterized by HPLC–DAD.

Materials and methods

Mushroom materials and extraction

Trametes species were harvested from Fethiye, Muğla, Turkey in 2018 and identified by Cansu Korkmaz, Department of Biology, Muğla Sıtkı Koçman University. The specimens with voucher numbers have been deposited at the Natural Products Laboratory of Muğla Sıtkı Koçman University Fungarium at Muğla, Turkey.

After the harvesting of the mushroom samples, they were air-dried in the darkness and powdered. The dried and powdered fruit bodies of *T. bicolor, T. pubescens, T. suaveolens,* and *T. versicolor* were first extracted with *n*-hexane at room temperature. The acetone was used for the re-extraction of the mushroom residue and the mushroom residue was then re-extracted with methanol at room temperature. The solvent was evaporated under vacuum by an evaporator to give the extracts. All extracts were kept at + 4 °C for further tests.

Instruments

The phenolic compounds analysis was performed using a Shimadzu 20 AT series high-performance liquid chromatography-diode array detector (HPLC–DAD), (Shimadzu Cooperation, Kyoto, Japan). Bioactivity measurements were carried out on a 96-well microplate reader, SpectraMax 340PC384 (Molecular Devices, Silicon Valley, California, USA). The calculations and measurements of the bioactivity results were estimated by using Softmax PRO v5.2 software (Molecular Devices, Silicon Valley, California, USA).

Analysis of phenolic compounds

The phenolic compounds of the *Trametes* species were identified by using HPLC–DAD according to the described method of Barros et al. [23] with slight modifications [24]. Photodiode array detector (PDA) using 280 nm as the preferred wavelength was used for the detection. Retention times and UV data were confirmed by comparison with commercial standards for characterization of phenolic compounds. Calibration curve was drawn via the injection of standard compounds (fumaric acid, gallic acid,

catechin hydrate, protocatechuic acid, 6,7-dihydroxy coumarin, *p*-hydroxybenzoic acid, vanillin, caffeic acid, *p*-coumaric acid, 2,4-dihydroxybenzoic acid, coumarin, ferulic acid, ellagic acid, *trans*-2-hydroxy cinnamic acid, *trans*-cinnamic acid, rosmarinic acid) at a concentration range of 0.0–1.0 ppm to identify and quantify of the phenolic compounds. The results were given as µg per g of dry weight (dw).

Determination of antioxidant activity

$ABTS^{\cdot+}$ scavenging, β-carotene-linoleic acid, Cupric-reducing antioxidant capacity (CUPRAC), $DPPH^{\cdot}$ scavenging, and metal chelating activity on Fe^{2+} assays were used to perform antioxidant activities of the all extracts of *Trametes* species with slight modifications [25]. α-Tocopherol, EDTA and BHA were used as standards. The graph of the inhibition percentage (%) versus the concentration (µg/mL) was used to calculate the IC_{50} values of the extracts. The graph of the absorbance versus the concentration (µg/mL) was used to calculate 0.50 absorbance ($A_{0.50}$) values of the extracts. Results were stated as inhibition percentage (%) at 400 µg/mL concentration, 50% inhibition concentration (IC_{50}) and $A_{0.50}$ which corresponds to the concentration producing 0.50 absorbance for CUPRAC assay.

Determination of cholinesterase inhibitory activity

Butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) inhibitory activities of *Trametes* extracts were determined according to Ellman method with slight modifications [26]. Galantamine was used as standard. The graph of the inhibition percentage (%) versus the concentration (µg/mL) was used to calculate the IC_{50} values of the extracts. The results were expressed as inhibition percentage (%) of the enzyme at 200 µg/mL concentration of *Trametes* species extracts and 50% inhibition concentration (IC_{50}).

Statistical analysis

All data on phenolic profile, cholinesterase inhibitory, and antioxidant activity tests were the mean of three parallel sample measurements. Data were recorded as average ± S.E.M. (standard error of the mean). Significant differences between means were determined by student's test, p values < 0.05 were regarded as significant. The correlations between phytochemical composition and biological activities were performed by Pearson correlation test by using SPSS v22.0 software.

Results and discussion

Phenolic compounds

Phenolic compounds are a group of aromatic secondary metabolites commonly found among mushrooms and plants with numerous biological properties such as antibacterial, anticancer, and antioxidant activities. It has been shown in previous studies that these compounds are useful in the treatment of many diseases associated with stress such as inflammatory bowel syndrome, AD and cardiovascular disease [27, 28]. Therefore, the identification of biologically important phenolic compounds from natural sources is a serious requirement.

The phenolic compounds of *Trametes* species were identified by HPLC–DAD and the results are given in Table 1. The HPLC–DAD chromatogram of standards can be seen in Fig. 1 and the HPLC–DAD chromatograms of *Trametes* species in Fig. 2. Sixteen phenolic compounds were analysed and five phenolic compounds were detected in *T. bicolor*, five in *T. pubescens*, six in *T. suaveolens*, four in *T. versicolor* mushrooms. The major compound was found as fumaric acid (4.51 ± 0.10 µg/g) in *T. bicolor*, *trans*-cinnamic acid (0.49 ± 0.05 µg/g) in *T. pubescens,* catechin hydrate in *T. suaveolens* (0.92 ± 0.16 µg/g) and *T. versicolor* (0.96 ± 0.19 µg/g) (Table 1).

In an earlier study, Im et al. [11] investigated the composition of phenolic contents of *T. pubescens* collected from Incheon, Korea by using HPLC. The major phenolic compounds were found as gallic acid (18.8 µg/g), (−)-epigallocatechin gallate (14.8 µg/g) and naringin (11.09 µg/g). In the study of Sheikh et al. [29], ascorbic acid (4.0%), catechol (0.05%) and cinnamic acid (0.21%) were detected in *T. hirsuta* (from Madhya Pradesh, Central India) ethanol extract. HPLC/MS–MS was employed to determine a total of 38 phenolic compounds in the water, ethanol, and methanol extracts of *T. versicolor* collected from Iriski Venac, Serbia and *p*-hydroxybenzoic acid was detected as the most abundant phenolic compound in *T. versicolor* methanol (184.00 µg/g), ethanol (465 µg/g) and water (141.00 µg/g) extracts [30]. *p*-Hydroxybenzoic acid was identified as the main phenolic compounds in the ethanol (154.30 µg/g) and water (42.00 µg/g) extracts of *T. versicolor* collected from Serbia by Raseta et al. [31]. Phenolic contents of the water and methanol extracts of *T. gibbosa* and *T. hirsuta* collected in Düzce, Turkey were identified by using RP-HPLC. (+)-Catechin was detected as the major phenolic compound in *T. gibbosa* (84.75 ± 2.40 µg/g) and *T. hirsuta* (128.47 ± 2.93 µg/g) methanol extracts while (+)-catechin and protocatechuic acid were identified as the major phenolic compounds in *T. gibbosa* (125.48 ± 2.48 and 143.27 ± 6.20 µg/g,

Table 1 Phenolic compounds of *Trametes* species

Phenolic compounds	RT (min)	*T. bicolor* (µg/g)	*T. pubescens* (µg/g)	*T. suaveolens* (µg/g)	*T. versicolor* (µg/g)
Fumaric acid	3.86	4.51 ± 0.10	nd	nd	nd
Gallic acid	5.66	0.13 ± 0.02	nd	0.24 ± 0.06	0.01 ± 0.01
Protocatechuic acid	8.88	0.19 ± 0.03	nd	0.16 ± 0.04	0.22 ± 0.05
p-Hydroxybenzoic acid	12.18	nd[a]	0.45 ± 0.02	0.34 ± 0.08	nd
Catechin hydrate	13.22	nd	nd	0.92 ± 0.16	0.96 ± 0.19
6,7-Dihydroxy coumarin	14.60	nd	nd	nd	nd
2,4-Dihydroxybenzoic acid	15.09	nd	nd	nd	nd
Caffeic acid	15.42	nd	0.11 ± 0.01	nd	nd
Vanillin	16.33	0.11 ± 0.01	0.09 ± 0.03	nd	nd
p-Coumaric acid	18.39	nd	0.08 ± 0.01	nd	nd
Ferulic acid	19.21	nd	nd	nd	0.01 ± 0.01
Coumarin	19.78	nd	nd	0.02 ± 0.01	nd
trans-2-Hydroxycinnamic acid	20.67	nd	nd	nd	nd
Ellagic acid	21.41	nd	nd	nd	nd
Rosmarinic acid	22.12	nd	nd	nd	nd
trans-Cinnamic acid	22.78	0.07 ± 0.01	0.49 ± 0.05	0.01 ± 0.01	nd

Values represent the means ± S.E.M. of three parallel measurements ($p < 0.05$)

[a]nd: not detected

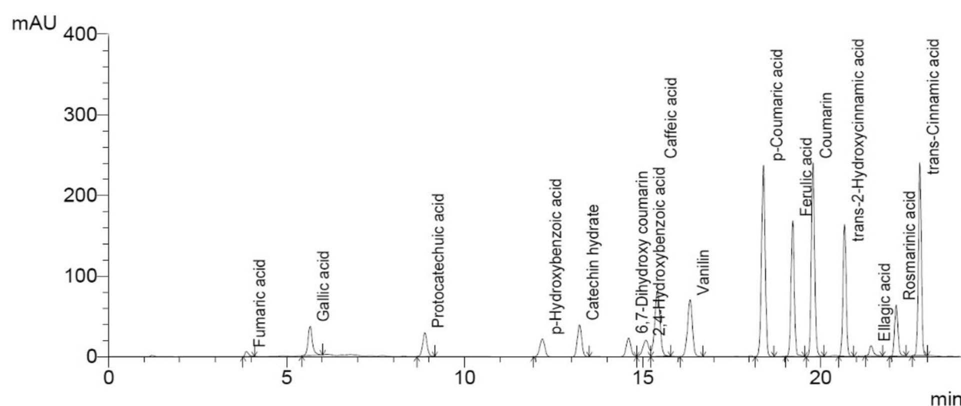

Fig. 1 The HPLC–DAD chromatogram of standard compounds

respectively) and *T. hirsuta* (564.91 ± 22.33 and 238.60 ± 7.44 µg/g, respectively) water extracts [13]. In parallel with our results, investigations on the phenolic profiles of *Trametes* species reported previously, *p*-hydroxybenzoic acid, gallic acid, cinnamic acid and catechin have been identified as the most abundant compounds. The observed differences can be attributed to the literature studies suggesting that phenolic compounds can differ depending on various factors including extraction solvent, ecological factors, mushroom species, collection localities and climate [32–34]. Also, phenolic compounds of *T. suaveolens* and *T. bicolor* were investigated for the first time in this study.

Antioxidant activity

Oxidative stress plays an important role during aging and increases the risk of chronic disease. The key to a long and healthy life is associated with the prevention of oxidative stress. The toxic effects of synthetic antioxidants on health are well known. Hence, requirements to explore natural alternative antioxidant food and ingredient sources are increasing [35, 36].

Antioxidant activities of *Trametes* extracts were analysed using the assays $ABTS^{\cdot+}$ scavenging, β-carotene-linoleic acid, CUPRAC, $DPPH^{\cdot}$ scavenging, and metal chelating activity. IC_{50} values, inhibition percentage (%)

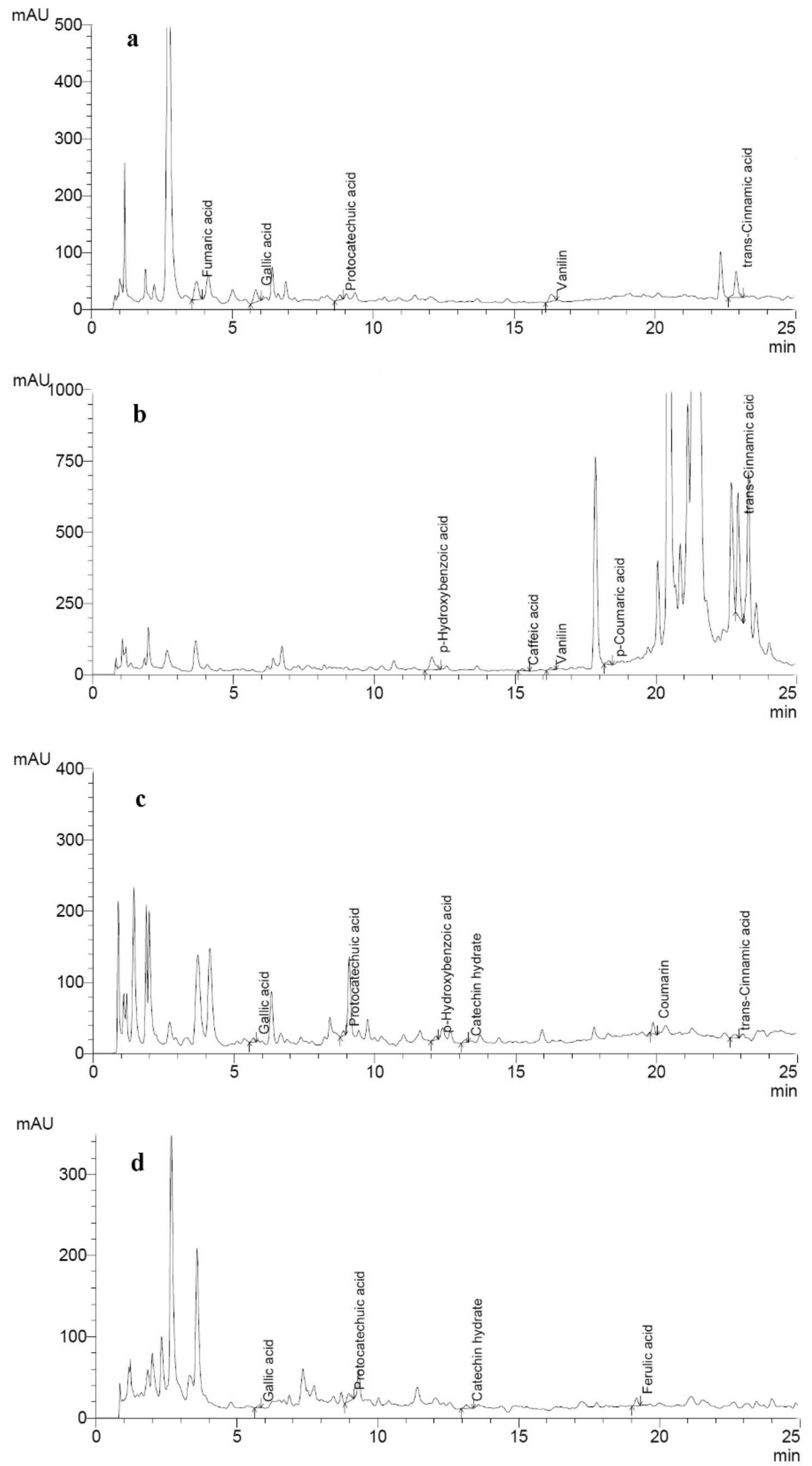

Fig. 2 The HPLC–DAD chromatograms of *Trametes species* **a** *T. bicolor*, **b** *T. pubescens*, **c** *T. suaveolens*, **d** *T. versicolor*

Table 2 Antioxidant activities of the extracts of *Trametes* species

		Antioxidant Activity				
		β-Carotene-linoleic acid assay	DPPH˙ assay	ABTS˙+ assay	CUPRAC assay	Metal chelating assay
Mushrooms	Extracts	IC_{50} (μg /mL)[a]	Inhibition (%)[b]	IC_{50} (μg/mL)[a]	$A_{0.50}$ (μg/mL)[c]	Inhibition (%)[b]
T. bicolor	Hexane	193.54 ± 0.40	1.78 ± 0.95	> 400	307.66 ± 0.66	21.12 ± 1.01
	Acetone	15.87 ± 0.87	24.64 ± 0.48	397.35 ± 0.08	172.50 ± 0.49	NA[d]
	Methanol	8.50 ± 0.16	37.64 ± 0.55	189.50 ± 0.15	296.15 ± 0.93	26.09 ± 0.89
T. pubescens	Hexane	> 400	3.03 ± 0.95	> 400	169.35 ± 0.19	2.51 ± 0.06
	Acetone	1.12 ± 0.41	85.72 ± 0.44	3.55 ± 0.16	19.26 ± 0.21	11.67 ± 0.69
	Methanol	1.06 ± 0.13	81.33 ± 0.12	4.45 ± 0.23	72.68 ± 0.24	20.97 ± 042
T. suaveolens	Hexane	380.10 ± 0.28	5.76 ± 0.16	> 400	340.90 ± 0.73	1.63 ± 0.02
	Acetone	> 400	7.48 ± 1.09	> 400	357.69 ± 1.05	11.37 ± 0.84
	Methanol	19.59 ± 0.30	18.07 ± 0.55	350.46 ± 0.32	> 400	25.28 ± 0.08
T. versicolor	Hexane	> 400	2.52 ± 0.87	> 400	> 400	56.78 ± 0.63
	Acetone	20.63 ± 0.10	38.09 ± 0.67	237.31 ± 0.51	186.95 ± 0.65	3.05 ± 0.05
	Methanol	1.90 ± 0.20	81.76 ± 0.30	4.82 ± 0.21	84.06 ± 0.50	14.78 ± 0.88
Standards	α-Tocopherol	2.10 ± 0.08	97.79 ± 0.15	38.51 ± 0.54	66.72 ± 0.81	NT[e]
	BHA	1.34 ± 0.04	94.74 ± 0.15	11.82 ± 0.09	24.40 ± 0.69	NT[e]
	EDTA	NT[e]	NT[e]	NT[e]	NT[e]	95.20 ± 0.13

[a] IC_{50} values represent the means ± SEM of three parallel measurements ($p < 0.05$)

[b] Inhibition (%) of 400 μg/mL concentration of the mushroom extracts ($p < 0.05$)

[c] $A_{0.50}$ values represent the means ± SEM of three parallel measurements ($p < 0.05$)

[d] NA: not active

[e] NT: not tested

at 400 μg/mL concentration of the standard compounds and *Trametes* extracts are presented in Table 2.

β-carotene-linoleic acid assay was used to test the lipid peroxidation abilities of the extracts. *T. pubescens* methanol and acetone extracts showed higher activity than α-tocopherol (IC_{50}: 2.10 ± 0.08 μg/mL) and BHA (IC_{50}: 1.34 ± 0.04 μg/mL) in β-carotene-linoleic acid assay with IC_{50} values of 1.06 ± 0.13 and 1.12 ± 0.41 μg/mL, respectively. Also, *T. versicolor* methanol extract (IC_{50}: 1.90 ± 0.20 μg/mL) was observed to be more active than α-tocopherol.

DPPH˙ and ABTS˙+ radicals were used to determine the radical scavenging activities of the extracts. In ABTS˙+ assay, *T. pubescens* acetone (IC_{50}: 3.55 ± 0.16 μg/mL), *T. pubescens* methanol (IC_{50}: 4.45 ± 0.23 μg/mL) and *T. versicolor* methanol (IC_{50}: 4.82 ± 0.21 μg/mL) extract displayed higher activity than α-tocopherol (IC_{50}: 38.51 ± 0.54 μg/mL) and BHA (IC_{50}: 11.82 ± 0.09 μg/mL). Also, these extracts indicated a degree of activity to compete with the standards in DPPH˙ assay.

The reducing power of Cu^{2+} of the extracts was determined by CUPRAC assay. *T. pubescens* acetone extract ($A_{0.50}$: 19.26 ± 0.21 μg/mL) showed the highest reducing power than the standards, followed by *T. pubescens* methanol extract with $A_{0.50}$ value of 72.68 ± 0.24 μg/mL and *T. versicolor* methanol extract ($A_{0.50}$: 84.06 ± 0.50 μg/mL).

Iron Fe^{2+} was used to measure the metal chelating activity of the extracts. As presented in Table 2, the metal chelating activities were highest for *T. versicolor* hexane extract (56.78 ± 0.63%), *T. bicolor* methanol extract (26.09 ± 0.89%) and *T. suaveolens* methanol extract (25.28 ± 0.08%), respectively.

Previously, DPPH˙ scavenging and metal chelating properties (at 0.125–2.0 mg/mL concentrations), reducing powers (at 0.5–4.0 mg/mL concentrations) of *T. pubescens* hot water and methanol extracts were studied. DPPH˙ scavenging activity of *T. pubescens* (from Incheon, Korea) hot water and methanol extracts ranged from 9.62 to 92.38% and from 41.91 to 93.45%, respectively, whereas; the metal chelating activity ranged from 59.23 to 96.85% and 67.66–91.62%, respectively. Reducing power of the hot water (2.83–2.92) and methanol (0.40–1.64) extracts of *T. pubescens* were very low [11]. Ferrous ion-chelating capacity (not active at 5000 μg/mL), ferric reducing antioxidant power (absorbance: 0.250 ± 0.02 at 500 μg/mL), DPPH˙ scavenging (5.14 ± 0.27% at 500 μg/mL) and β-carotene bleaching activities (not active at 5000 μg/

mL) of the ethanol extract of *T. versicolor* collected from Bolu, Turkey were investigated by Orhan and Üstün [37]. Janjušević et al. [30] reported DPPH˙ and OH˙ scavenging activities of the water, ethanol and methanol extracts of *T. versicolor* (from Iriski Venac, Serbia). In both activity methods, radical scavenging activity was reported to decrease in order of water > methanol > ethanol extracts. In a different study, antioxidant activity of the ethanol and water extracts of *T. versicolor* (from Serbia) was tested by using reducing power and DPPH˙, OH˙ and superoxide anion radical scavenging assays. When the best antioxidant activity was reported in the ethanol extract in DPPH˙ (IC_{50}: 5.6 ± 0.8 µg/mL), OH˙ (IC_{50}: 0.47 ± 0.01 µg/mL), and superoxide anion (IC_{50}: 5.0 ± 0.03 µg/mL) radical scavenging assays, water extract indicated the highest activity in reducing power assay (125.0 ± 0.3 mg AAE g^{-1} dw) [31].

Consequentially, when all mushroom species studied were compared, the acetone and methanol extracts showed higher antioxidant properties than the hexane extracts. It is concluded that the observed antioxidant ability may be due to the synergistic impact of the mycochemical components contained in mushrooms [38, 39]. Also, solvent polarity increased the dissolution of the compounds with antioxidant properties [40]. As far as we know, this is the first study on the antioxidant activities of *T. bicolor* and *T. suaveolens* mushrooms.

The Pearson correlation coefficient was employed to evaluate the correlation between the activities and phenolic content of extracts of four *Trametes* species. The correlation analysis concluded that both activities exhibited a strong correlation with phenolic content (Table 3). According to Table 3, it was revealed that catechin hydrate significantly contributed to both of the biological activities.

Cholinesterase inhibitory activity

Although the cause of AD has not been clearly established, AChE inhibitors (AChEI) are accepted as a way of treating the disease. AChE inhibitors (AChEI) are used to combat ACh loss resulting from the death of cholinergic neurons by reducing the rate of degradation of ACh [41, 42]. Traditional inhibitors used in the treatment of the disease are mostly derived from nature. Derivatives of synthetic and traditional inhibitors, compounds derived from nature and analogs of older drugs are among the newer inhibitors. For these reasons, studies on searching for more potent inhibitors of natural origin have increased in recent years [19].

Butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) inhibitory activities of *Trametes* extracts were measured according to the Ellman method. The results were illustrated in Table 4 as IC_{50} values and inhibition percentage (%) of the enzyme at 200 µg/mL concentration of the mushroom extracts and standard.

The best AChE and BChE inhibitory activities were observed in *T. pubescens* hexane and acetone extracts among other extracts. It was determined that *T. pubescens* hexane extract showed higher AChE and BChE inhibitory activities with IC_{50} values of 7.37 ± 0.55, 15.24 ± 0.98 µg/mL, respectively. Besides, *T. pubescens* acetone extract (77.79 ± 0.16%) indicated near-standard inhibitory activity in AChE assay, while *T. pubescens* acetone (74.88 ± 0.55%) and hexane (73.64 ± 0.15%) extracts exhibited near-standard activity in BChE assay at 200 µg/mL concentration. In an in vivo study of Chandra et al. [43], it has been reported that as a result of stimulation of lysosomal biogenesis by cinnamic acid, it has therapeutic effect for the AD treatment and other lysosomal disorders caused by the accumulation of toxic protein aggregates. Therefore, when evaluated the phenolic compounds

Table 3 Correlations among phenolic compounds and biological activity assays

	β-Carotene-linoleic acid	DPPH˙	ABTS˙⁺	CUPRAC	Metal chelating	AChE	BChE
β-Carotene-linoleic acid							
DPPH˙	0.746[a]						
ABTS˙⁺	0.887[a]	0.923[a]					
CUPRAC	0.399	0.783[a]	0.625[b]				
Metal chelating	−0.202	−0.128	−0.153	−0.250			
AChE	−0.499	−0.101	−0.290	0.460	−0.363		
BChE	−0.146	0.181	0.148	0.567	−0.473	0.722[a]	
Fumaric acid	−0.169	−0.357	−0.123	−0.406	0.556	−0.552	−0.141
Catechin hydrate	1.000[a]	1.000[a]	1.000[a]	1.000[a]	−1.000[a]	1.000[a]	1.000[a]
trans-Cinnamic acid	0.923	0.982	0.895	0.991	−0.966	0.983	−0.989

Data show Pearson Correlation Coefficients between the parameters

[a]Correlation is significant at 0.05 level ($p < 0.05$)

[b]Correlation is significant at 0.01 level ($p < 0.01$)

Table 4 Cholinesterase inhibitory activities of the extracts of *Trametes* species

Mushrooms	Extracts	Cholinesterase inhibitory activities			
		Acetylcholinesterase (AChE)		Butyrylcholinesterase (BChE)	
		Inhibition (%)[a]	IC_{50} (μg/mL)[b]	Inhibition (%)[a]	IC_{50} (μg/mL)[b]
T. bicolor	Hexane	27.15 ± 0.60	> 200	29.12 ± 0.94	> 200
	Acetone	37.23 ± 0.99	> 200	35.05 ± 0.96	> 200
	Methanol	2.94 ± 0.24	> 200	40.05 ± 0.66	> 200
T. pubescens	Hexane	82.38 ± 1.09	7.37 ± 0.55	73.64 ± 0.15	15.24 ± 0.98
	Acetone	77.79 ± 0.16	29.62 ± 0.62	74.88 ± 0.55	16.52 ± 0.84
	Methanol	27.63 ± 0.95	> 200	32.07 ± 0.52	> 200
T. suaveolens	Hexane	47.75 ± 0.89	> 200	34.21 ± 0.81	> 200
	Acetone	49.06 ± 0.27	> 200	57.38 ± 0.82	100.64 ± 0.82
	Methanol	4.87 ± 0.44	> 200	39.77 ± 0.53	> 200
T. versicolor	Hexane	41.32 ± 0.20	> 200	33.28 ± 0.75	> 200
	Acetone	34.48 ± 0.02	> 200	47.88 ± 0.44	> 200
	Methanol	13.48 ± 0.54	> 200	44.10 ± 0.97	> 200
Standard	Galantamine	80.41 ± 0.98	4.31 ± 0.03	82.23 ± 2.67	12.29 ± 0.06

[a]Inhibition % of 200 μg/mL concentration of *Trametes* extracts ($p < 0.05$)

[b]IC_{50} values represent the means ± SEM of three parallel measurements ($p < 0.05$)

results, it is thought that the high content of *trans*-cinnamic acid of *T. pubescens* is associated with higher anticholinesterase activity compared to other *Trametes* species.

Im et al. [11] reported anticholinesterase activities of hot water and methanol extracts of *T. pubescens* collected from Incheon, Korea. At concentrations in the range of 0.063–1.0 mg/mL, AChE inhibitory activities of the *T. pubescens* hot water and methanol extracts ranged from 47.21 to 83.15% and from 51.70 to 90.94%, whereas BChE inhibitory activities ranged between 47.93–63.58% and 49.49–75.73%, respectively. AChE inhibitory activities of the ethanol (44.35 ± 2.06%) and water (60.53 ± 2.12%) extracts of *T. versicolor* (from Iriski Venac, Serbia) at 100 μg/mL concentration were studied by Janjušević et al. [30]. AChE inhibitory properties (ranged from 24.7 ± 0.6 to 28.9 ± 0.8%) were noted for the ethanol extracts of the basidiocarp and mycelium of *T. hirsuta, T. gibbosa* and *T. versicolor* collected from Serbia [12]. In another study, the ethanol extract of *T. versicolor* collected from Bolu, Turkey displayed low AChE inhibitory activity (28.35 ± 0.57%) at 500 μg/mL concentration [37]. The obtained results of cholinesterase inhibitory activity show similarities with the literature. Cholinesterase inhibitory activities of *T. suaveolens* and *T. bicolor* mushrooms were revealed for the first time in this research.

Conclusion

The present study deals with four different *Trametes* mushroom species (*T. bicolor, T. pubescens, T. suaveolens* and *T. versicolor*) which served as natural sources of phenolic compounds, having significant cholinesterase inhibitory and antioxidant activities as well. HPLC–DAD results revealed the presence of some bioactive phenolic compounds could be responsible for the studied biological activities. According to the obtained results, *T. pubescens* methanol and acetone extracts showed higher or close activity than standards in CUPRAC, $ABTS^{·+}$, β-carotene-linoleic acid, $DPPH^{·}$ assays except for metal chelating assay. Also, *T. pubescens* acetone and hexane extracts were determined as potent AChE and BChE inhibitors. Because of the high antioxidant activities, *T. versicolor* methanol, *T. pubescens* acetone and methanol extracts can be classified as beneficial preventive and therapeutic agents for oxidative stress-related diseases. *T. pubescens* acetone and hexane extracts can be considered to be potent inhibitor agents in the therapy of Alzheimer's disease. So, the results of this study support the potential use of *Trametes* species to design new functional drug formulations.

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Declarations

Conflict of interest No potential conflict of interest was reported by the authors.

References

1. M.E. Valverde, T. Hernández-Pérez, O. Paredes-López, Int. J. Microbiol. **2015**, 14 (2015)
2. J.E. Smith, N.J. Rowan, R. Sullivan, Biotechnol. Lett. **24**, 1839–1845 (2002)
3. S.P. Wasser, A.L. Weis, Biotechnol. Lett. **19**, 65–96 (1999)
4. S.P. Wasser, Int. J. Med. Mushrooms **12**(1), 1–16 (2010)
5. S.P. Wasser, Int. J. Med. Mushrooms **19**(4), 279–317 (2017)
6. A. Podkowa, A. Kryczyk-Poprawa, W. Opoka, B. Muszyńska, Eur. Food Res. Technol. **247**, 513–533 (2021)
7. O. Taofiq, A.M. Gonzalez-Paramas, A. Martins, M.F. Barreiro, I.C.F.R. Ferreira, Ind. Crop. Prod. **90**, 38–48 (2016)
8. A.M. Abdelshafy, T. Belwal, Z. Liang, L. Wang, D. Li, Z. Luo, L. Li, Crit. Rev. Food Sci. Nutr. **17**, 1–21 (2021)
9. A. Ganeshpurkar, G. Rai, A.P. Jain, **4**(8), 127–135 (2010)
10. C.R. Hobbs, Int. J. Med. Mushrooms **6**, 195–218 (2004)
11. K.H. Im, T.K. Nguyen, J. Choi, T.S. Lee, Molecules **21**, 639 (2016)
12. A. Knezlevic, M. Stajic, I. Sofrenic, T. Stanojkovic, I. Milovanovic, V. Teslevic, J. Vukojevic, PLoS One **13**(8), e0203064 (2018)
13. G. Zengin, A. Karanfil, M.C. Uren, M.S. Kocak, C. Sarikurkcu, H. Gungor, C.M.N. Picot, M.F. Mahomoodally, RSC Adv. **6**, 73351–73357 (2016)
14. R. Ma, R. Yang, X. Liu, Z. Chen, C. Yang, S. Wang, Int. J. Med. Mushrooms **17**, 267–276 (2015)
15. E. Büyüktuncel, Marmara Pharm. J. **17**, 93–103 (2013)
16. L.K. MacDonald-Wicks, L.G. Wood, M.L. Garg, J. Sci. Food Agric. **86**, 2046–2056 (2006)
17. P.M. Kidd, Altern Med. Rev. **13**, 85–115 (2008)
18. P. Barai, N. Raval, S. Acharya, A. Borisa, H. Bhatt, N. Acharya, Behav. Brain Res. **356**, 18–40 (2019)
19. S. Shaikh, A. Verma, S. Siddiqui, S.S. Ahmad, S.M.D. Rizvi, S. Shakil, D. Biswas, D. Singh, M.H. Siddiqui, S. Shakil, S. Tabrez, M.A. Kamal, C.N.S. Neurol, Disord. Drug Targets **13**, 391–401 (2014)
20. A. Gella, N. Durany, Cell. Adh. Migr. **3**(1), 88–93 (2009)
21. M.A. Lovell, W.R. Markesbery, Nucleic Acids Res. **35**, 7497–7504 (2007)
22. Y. Feng, X. Wang, Oxid. Med. Cell. Long. **2012**, 17 (2012)
23. L. Barros, M. Duenas, I.C.F.R. Ferreira, P. Baptista, C. Santos-Buelga, Food Chem. Toxicol. **47**, 1076–1079 (2009)
24. G. Tel-Çayan, J. Food Biochem. **43**(4), e12790 (2019)
25. F. Çayan, G. Tel-Çayan, E. Deveci, M. Öztürk, M.E. Duru, Int. J. Med. Mushrooms **21**(11), 1075–1087 (2019)
26. G.L. Ellman, K.D. Courtney, V. Andres, R.M. Featherston, Biochem. Pharmacol. **7**, 88–95 (1961)
27. V. Payamnoor, M.R. Kavosi, J. Nazari, J. For. Res. **31**, 1381–1390 (2019)
28. C. Rice-Evans, Free Radic. Biol. Med. **36**, 827–828 (2004)
29. I.A. Sheikh, D. Vyas, M. Ganaie, K. Dehariya, V. Singh, Int. J. Pharm. Pharm. Sci. **6**, 679–684 (2014)
30. L. Janjušević, M. Karaman, F. Šibul, G. Tommonaro, C. Iodice, D. Jakovljević, B. Pejin, J. Enzyme Inhib. Med. Chem. **32**, 355–362 (2017)
31. M. Raseta, M. Popovic, P. Knezevic, F. Sibul, S. Kaisarevic, M. Karaman, Chem. Biodivers. **17**, e2000683 (2020)
32. D.A. Abugri, W.H. McElhenney, J. Nat. Prod. Plant Resour. **3**(3), 37–42 (2013)
33. Z. Zargoosh, M. Ghavam, G. Bacchetta, A. Tavili, Sci. Rep. **9**, 16021 (2009)
34. S. Kabtni, D. Sdouga, I.B. Rebey, M. Save, N. Trifi-Farah, M.L. Fauconnier, S. Marghali, Sci. Rep. **10**, 8293 (2020)
35. T. Finkel, N.K. Holbrook, Nature **408**, 239–247 (2000)
36. M. Kozarski, A. Klaus, D. Jakovljevic, N. Todorovic, J. Vunduk, P. Petrović, M. Niksic, M.M. Vrvic, L. van Griensven, Molecules **20**, 19489–19525 (2015)
37. I. Orhan, O. Üstün, J. Food Compost. Anal. **24**, 386–390 (2011)
38. K.S. Sonam, S. Guleria, Ann. Pharmacol. Pharm. **2**, 1086 (2017)
39. G. Zengin, M.Y. Paksoy, J. Glamocilja, M. Sokovic, A. Diuzheva, J. Jekő, Z. Cziáky, M.J. Rodrigues, L. Custodio, M.F. Mahomoodally, Food Res. Int. **123**, 414–424 (2019)
40. A. Barchan, M. Bakkali, A. Arakrak, R. Pagan, A. Laglaoui, Int. J. Curr. Microbiol. Appl. Sci. **3**(11), 399–412 (2014)
41. C. Guo, L. Sun, X. Chen, D. Zhang, Neural Regen. Res. **8**, 2003–2014 (2013)
42. T.K. Nguyen, K.H. Im, J. Choi, P.G. Shin, T.S. Lee, Mycobiology **44**(4), 291–301 (2016)
43. S. Chandra, A. Roya, M. Jana, K. Pahan, Neurobiol. Dis. **124**, 379–395 (2019)

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