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# Evaluation of phenolic profile, antioxidant and anticholinesterase effects of *Fuscoporia torulosa*

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**Abstract**: In this study, we investigated antioxidant and anticholinesterase activities of the hexane, chloroform, acetone, methanol and water extracts of *F. torulosa* mushroom with total phenolic contents. Also, HPLC-DAD was used to identify phenolic profile of *F. torulosa*. The acetone and methanol extracts of *F. torulosa* with the highest total phenolic contents showed the highest antioxidant activity in all assays except metal chelating assay. Furthermore, antioxidant activities of the acetone and methanol extract were found to be higher than α-tocopherol and BHA used as standards in DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and CUPRAC assays. When *F. torulosa* hexane extract (41.34±1.50 %) showed moderate AChE inhibitory activity, the acetone (40.78±0.30 %) and methanol (45.39±0.65 %) extracts of *F. torulosa* indicated moderate BChE inhibitory activity. Major phenolic compounds were identified as *trans*-2-hydroxy cinnamic acid (10.05 µg/g), gallic acid (5.01 µg/g) and *p*-coumaric acid (3.04 µg/g). These results suggest that *F. torulosa* mushroom could be used as a valuable natural antioxidant source for pharmaceutical industry.

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## 1. INTRODUCTION

Free radicals are produced from oxygen during aerobic respiration and excessive amount of formation and accumulation causes oxidative stress [1]. The formation of oxidative stress in living organisms, in particular damages the biomolecules such as DNA, proteins and lipids, resulting in many diseases such as hypertension, ischemia, neurodegenerative diseases and rheumatoid arthritis [2]. Antioxidants prevent or reduce the harmful effects of oxidative stress. The use of synthetic antioxidants is an old practice and the safety of these substances is questioned by consumers. At present, interest in alternative natural compounds with high antioxidant effect is increasing [3].

Alzheimer's disease (AD) eliminates neurons in the cortex and limbic structure in the brain, causing learning and memory loss and behavioral disorders in humans. AD is characterized by a reduction of acetylcholine (ACh) due to damage to cholinergic neurons in some specific parts of the brain, such as the hippocampus and cortex (cholinergic hypothesis) [4]. One effective approach in the treatment of AD is the inhibition of acetylcholinesterase

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(AChE), which is responsible for the hydrolysis of ACh [5,6]. During aging, a gradual decrease in antioxidant defense mechanism and increased oxidative stress cause neuronal injury and death, which is another neurotoxic pathway causing AD [7]. Previous studies have demonstrated that antioxidant therapy is successful in improving cognitive function and behavioral deficits in patients with mild to moderate AD [8].

It is well known that mushrooms have therapeutic properties since ancient times. Up to know, many bioactive compounds such as lectins, polysaccharides, terpenoids, alkaloids, sterols and phenolics having anticancer, antioxidant, antitumor, antiinflammatory, antifungal, antibacterial, antiviral, anti-immunomodulatory activities have been isolated from mushrooms. When literature studies are examined, it is seen that mushrooms are used especially due to anticancer activity [9-15]. Mushrooms show beneficial effects on cancer, either directly as antioxidants or preventing genetic factors that cause cancer [16].

Recently, studies on the discovery of bioactive compounds from mushrooms have become more important because of their functional and therapeutic properties. Therefore, in this report, we focused to evaluate antioxidant and anticholinesterase activities of the hexane, chloroform, acetone, methanol and water extracts of *F. torulosa* mushroom with total phenolic contents. Also, phenolic profile of *F. torulosa* were determined by HPLC-DAD.

## 2. MATERIAL and METHODS

### 2.1. Mushroom Material

*F. torulosa* (Pers.) T. Wagner & M. Fisch. was collected from Muğla, Turkey, in November-December, 2014 and January, 2015. The voucher specimen has been deposited at the herbarium of Natural Products Laboratory of Muğla Sıtkı Koçman University with Fungarium No AT-2436.

### 2.2. Instruments and Chemicals

Bioactivity measurements were carried out on a 96-well microplate reader, SpectraMax 340PC384 (Molecular Devices, Silicon Valley, CA). The measurements and calculations of the activity results were evaluated by using Softmax PRO v5.2 software (Molecular Devices, Silicon Valley, CA).

Pyrocatechol, ethylenediaminetetraacetic acid (EDTA), ethanol, methanol, chloroform, acetone, hexane, ferrous chloride, copper (II) chloride and ammonium acetate were obtained from E. Merck (Darmstadt, Germany). Polyoxyethylene sorbitan monopalmitate (Tween-40), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin–Ciocalteu's reagent (FCR), 3-(2-pyridyl)-5,6-di(2-furyl)-1,2,4-triazine-5′,5″-disulfonic acid disodium salt (ferene), β-carotene, linoleic acid, 2,2′-azino bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), α-tocopherol, neocuproine, butylated hydroxyl anisole (BHA), acetylcholinesterase from electric eels (AChE, Type-VI-S, EC 3.1.1.7, 425.84 U/mg, Sigma), acetylthiocholine iodide, butyrylcholinesterase from horse serum (BChE, EC 3.1.1.8, 11.4 U/mg, Sigma), butyrylthiocholine chloride, 5,5′-dithiobis (2-nitrobenzoic) acid (DTNB), galantamine, fumaric acid, gallic acid, protocatechuic acid, *p*-hydroxy benzoic acid, catechin hydrate, 6,7-dihydroxy coumarin, 2,4-dihydroxy benzoic acid, caffeic acid, vanillin, *p*-coumaric acid, ferulic acid, coumarin, *trans*-2-hydroxy cinnamic acid, ellagic acid, rosmarinic acid, *trans*-cinnamic acid were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals and solvents were in analytical grade.

### 2.3. Extraction

The aerial parts of *F. torulosa* (2.8 kg) were extracted separately with different solvents according to their increasing polarity: hexane, chloroform, acetone, methanol at room

temperature for 24 h and four times. Solvents were evaporated on a rotary evaporator at 40°C. The hexane (10.5 g), chloroform (29.8 g), acetone (38.4 g) and methanol (55.6 g) extracts were obtained. The remaining mushroom part was allowed to stand for one day with water at 80°C. The water extract of (17.6 g) were obtained by lyophilisation using a freeze-drier. All extracts were stored at +4°C until analysis.

### 2.4. Determination of Total Phenolic Content

The phenolic content of extracts was stated as microgram of pyrocatechol equivalents (PEs) [17]. The phenolic contents were calculated according to the following equation that was obtained from standard pyrocatechol graph:

$$\text{Absorbance}=0.0176[\text{pyrocatechol } (\mu g)] - 0.355 \ (r^2, 0.9992)$$

### 2.5. Analysis of Phenolic Profile

The phenolic compounds analysis was performed by our recent publication [18] with slight modification. The mushroom sample (3 g) was extracted with acetone: water (80:20 v/v; 30 mL) at –18 °C for 24 h. After ultrasonic bath for 15 min, the mushroom extract was centrifuged at 4000 rpm for 10 min and filtered through Whatman no. 4 paper. The residue was then re-extracted by two additional 30 mL of the acetone: water. The combined extracts were evaporated at 40 °C under reduced pressure to remove acetone. The obtained extract was solved in water: methanol (80:20) and filtered through a 0.20 µm disposable LC filter disk for HPLC-DAD. Separation was achieved on an Intertsil ODS-3 reverse phase C18 column (5 µm, 150 mm×4.6 mm i.d) thermostatted at 40 °C. The solvent flow rate was 1.5 mL/ min. The sample volume injection was 20 µL. The mobile phases used were: (A) 0.5 % acetic acid in water, (B) 0.5 % acetic acid in methanol. The elution gradient was as follows: 0–20 % B (0–0.01 min); 20–60 % B (0.01–2 min); 60–80 % B (2–15 min); 100 % B (15–30 min); 100–10 % B (3–35 min); 10–0 % B (35–40 min). Detection was carried out photodiode array detector (PDA) using 280 nm as the preferred wavelength. The phenolic compounds were characterized according to their retention times, and UV data were compared with commercial standards. Three parallel analyses were performed. For the quantitative analysis of phenolic compounds, calibration curves were obtained via the injection of known concentrations (0.0, 0.00782, 0.01563, 0.03125, 0.0625, 0.125, 0.25, 0.5 and 1.0 ppm) of different standards compounds i.e. gallic acid, fumaric acid, protocatechuic acid, catechin hydrate, *p*-hydroxy benzoic acid, 6,7-dihydroxy coumarin, caffeic acid, vanilin, 2,4-dihydroxy benzoic acid, *p*-coumaric acid, ferulic acid, coumarin, *trans*-2-hydroxy cinnamic acid, ellagic acid, rosmarinic acid, *trans*-cinnamic acid. The results were expressed as µg per g of dry weight (dw).

### 2.6. Determination of Antioxidant Activity

Total antioxidant activity by β-carotene-linoleic acid test, DPPH free radical scavenging assay, ABTS cation radical scavenging assay, cupric reducing antioxidant capacity (CUPRAC) assay and metal chelating activity on $Fe^{2+}$ assays were carried out according to our earlier publication [19]. BHA, α-tocopherol and EDTA were used as antioxidant standards for comparison of the activities. The antioxidant activity results are expressed as 50 % inhibition concentration ($IC_{50}$) and inhibition percentage (%) at 200 µg/mL and $A_{0.50}$ which corresponds to the concentration producing 0.500 absorbance for CUPRAC assay.

### 2.7. Determination of Anticholinesterase Activity

Acetylcholinesterase and butyrylcholinesterase inhibitory activity were determined the spectrophotometric method developed by Ellman *et al.* [20]. AChE from electric eel and BChE from horse serum were used, acetylthiocholine iodide and butyrylthiocholine chloride were employed as substrates of the reaction. DTNB was used for the measurement of the

cholinesterase activity. Briefly, 150 µL of 100 mM sodium phosphate buffer (pH 8.0), 10 µL of the sample solution dissolved in ethanol at different concentrations and 20 µL AChE or BChE solution in buffer were mixed and incubated for 15 min at 25 °C, and 10 µL of 0.5 mM DTNB was added. The reaction was then initiated by the addition of 0.71 mM, 10 µL of acetylthiocholine iodide or 0.2 mM, 10 µL of butyrylthiocholine chloride. The hydrolysis of these substrates was monitored spectrophotometrically by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide or butyrylthiocholine chloride, respectively, at a wavelength of 412 nm utilizing a 96-well microplate reader. Galantamine was used as reference compounds. The results were given as inhibition percentage (%) of the enzyme at 100 µg/mL concentration of the extracts.

### 2.8. Statistical Analysis

All data on antioxidant and anticholinesterase tests were the average of three parallel sample measurements. Data were recorded as mean ± S.E.M. Significant differences between means were determined by student's test, $p$ values <0.05 were regarded as significant.

## 3. RESULTS and DISCUSSION

### 3.1. Total Phenolic Content

The calibration curve of pyrocatechol (0.0176[pyrocatechol (µg)] – 0.355; $r^2$, 0.9992) was used to determine the total phenolic content. Table 1 presents the total phenolic contents of the hexane, chloroform, acetone, methanol and water extracts of *F. torulosa.*

The methanol extract (131.35±0.29 µg PEs/mg) of *F. torulosa* has the highest level of the phenolic compounds among the other extracts. The total phenolic contents of the extracts were decreased in the order of methanol> acetone> water> hexane> chloroform. Total phenolic content of *Inonotus obliquus (Fuscoporia obliqua)* ethanol extract was found as 55.94±1.08 mg GAE/g extract by Zhang *et al.* [21]. The content of total phenols of 80 % ethanol, 80 % methanol and 95 % ethanol extracts of *Inonotus obliquus (Fuscoporia obliqua)* expressed as µg of gallic acid equivalents extracted from 100 mg extracts were found as 1388.505, 1404.907 and 662.184 respectively [22]. In the report of Seephonkai *et al.* [23], total phenolic contents of 50 % EtOH, 80 % EtOH, EtOH, EtOAc extracts of *F. torulosa* were studied and found as 43.80±0.78, 54.86±0.21, 62.51±0.65, 16.56±0.29 mg GA/100 mg of extract, respectively. The results obtained are consistent with the literature. Total phenolic contents of hexane, chloroform, acetone, methanol and water extracts of *F. torulosa* were studied for the first time in this research.

**Table 1.** Total phenolic contents of the extracts of *F. torulosa*[a]

| | | Total phenolic content (µg PEs/mg extract) |
|---|---|---|
| Extracts | Hexane | 42.67±0.36 |
| | Chloroform | 19.38±0.18 |
| | Acetone | 73.54±0.08 |
| | Methanol | 131.35±0.29 |
| | Water | 55.50±0.71 |

[a]Values expressed are means ± S.E.M. of three parallel measurements ($p < 0.05$).

### 3.2. Phenolic Profile

Phenolic profile of *F. torulosa* mushroom was determined by HPLC-DAD and results are expressed as µg per g of dry weight (dw) in Table 2. HPLC-DAD chromatograms of standards and *F. torulosa* were seen in Figs. 1-2. Totally 16 phenolic and organic acid compounds namely

fumaric acid, gallic acid, protocatechuic acid, *p* hydroxybenzoic acid, catechin hydrate, 6,7-dihydroxy coumarin, 2,4 dihydroxybenzoic acid, caffeic acid, vanillin, *p*-coumaric acid, ferulic acid, coumarins, *trans*-2-hydroxycinnamic acid, ellagic acid, rosmarinic acid and *trans*-cinnamic acid were identified in the mushroom.

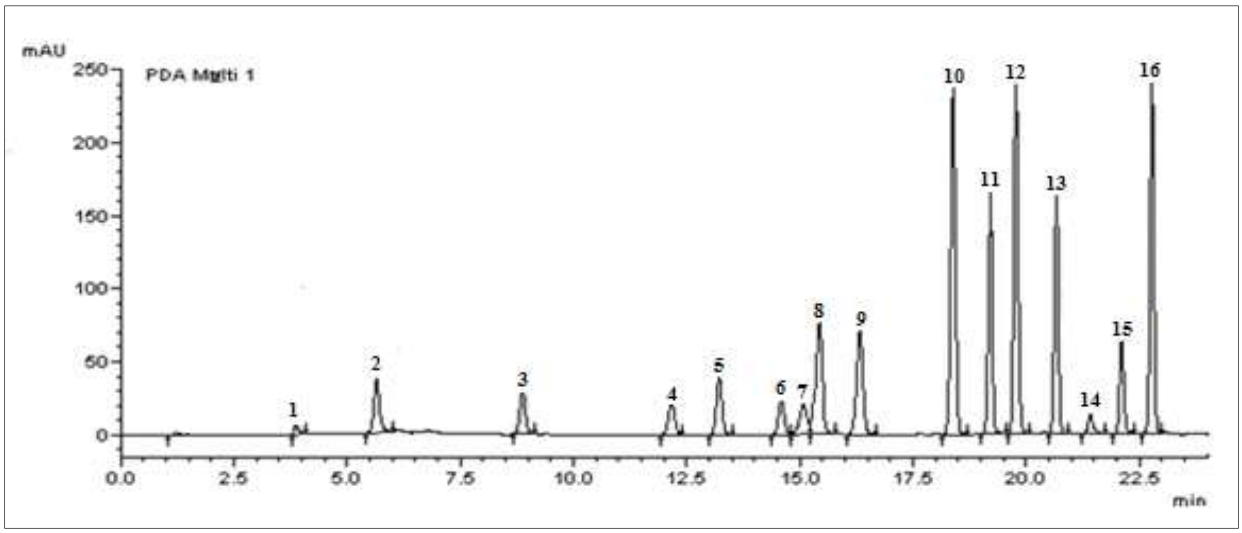


**Fig. 1. The HPLC-DAD chromatogram of standards**

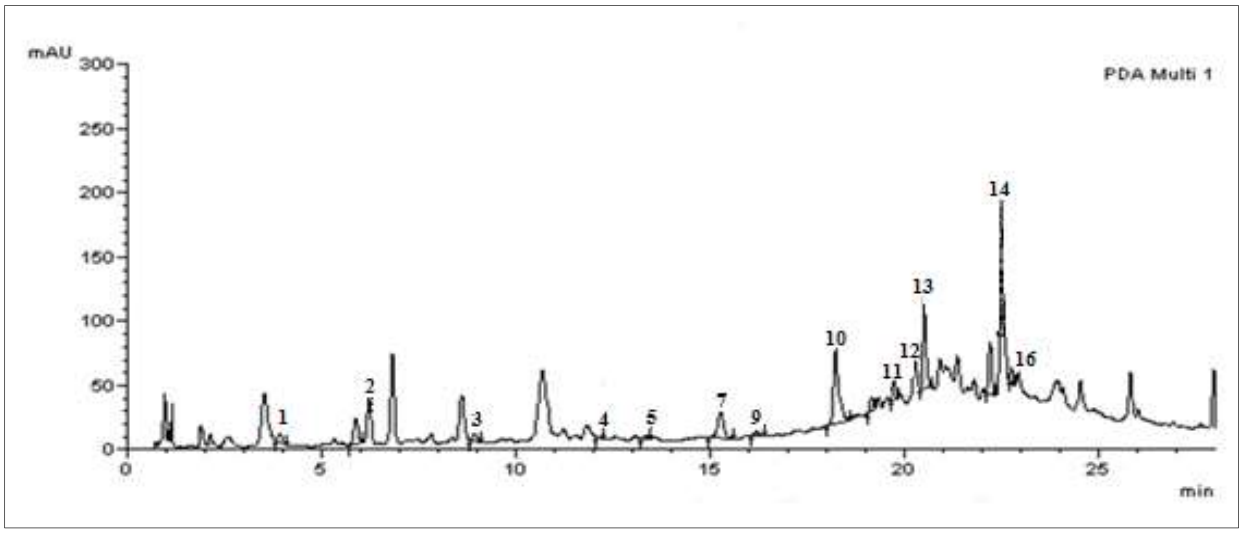


**Fig. 2. The HPLC-DAD chromatogram of *F. torulosa***

As it seen Table 2, *trans*-2-hydroxy cinnamic acid (10.05 µg/g), gallic acid (5.01 µg/g) and *p*-coumaric acid (3.04 µg/g) were identified as major phenolic compounds in *F. torulosa*. Cinnamic acid derivatives are known to exhibit a range of bioactivities including antibacterial, antiviral, antifungal and antioxidant properties [24]. Gallic acid, mostly found in plants, have been reported to have antioxidant, antimicrobial, anti-inflammatory, and anticancer effects [25]. *p*-Coumaric acid is widespread in plants and mushrooms, in free or bound form. It is an important phenolic compound because of its antioxidant, anticancer, antimicrobial, antivirus, anti-inflammatory, antiplatelet aggregation, anxiolytic, antipyretic, analgesic, and anti-arthritis activities [26]. The high antioxidant activity exhibited by *F. torulosa* mushroom can be also influenced by the phenolic compounds it contains. Phenolic compounds of *Inonotus obliquus (Fuscoporia obliqua)* were analyzed using high performance liquid chromatography by Kim *et*

*al.* [27] and *p*-hydroxybenzoic acid (263 µg/g), kaempferol (53 µg/g), quercetin (52 µg/g), homogentisic acid (51 µg/g) and protocatechuic acid (50 µg/g) were identified as major phenolic compounds. In the literature, there is only one study about phenolic compounds of *F. torulosa.* In the report of Bal *et al.* [28], benzoic acid (170.6), chlorogenic acid (42.7), gallic acid (9.8) and catechin (2.7) were identified in *F. torulosa* ethanol extract.

**Table 2.** Phenolic compounds of *F. torulosa*

| No | Compounds | Retention time (min) | Composition (µg/g) |
|---|---|---|---|
| 1 | Fumaric acid | 3.86 | 0.33 |
| 2 | Gallic acid | 5.66 | 5.01 |
| 3 | Protocatechuic acid | 8.88 | 0.17 |
| 4 | *p*-Hydroxybenzoic acid | 12.18 | 0.01 |
| 5 | Catechin hydrate | 13.22 | 0.91 |
| 6 | 6,7-Dihydroxy coumarin | 14.60 | nd |
| 7 | 2,4-Dihydroxybenzoic acid | 15.08 | 0.48 |
| 8 | Caffeic acid | 15.42 | nd |
| 9 | Vanillin | 16.33 | 0.07 |
| 10 | *p*-Coumaric acid | 18.39 | 3.04 |
| 11 | Ferulic acid | 19.21 | 0.46 |
| 12 | Coumarin | 19.78 | 0.05 |
| 13 | *trans*-2-Hydroxy cinnamic acid | 20.67 | 10.05 |
| 14 | Ellagic acid | 21.41 | 0.39 |
| 15 | Rosmarinic acid | 22.11 | nd |
| 16 | *trans*-Cinnamic acid | 22.78 | 0.51 |

nd: Not detected.

### 3.3. Antioxidant Activity

β-carotene-linoleic acid, DPPH free radical scavenging, ABTS cation radical scavenging, cupric-reducing antioxidant capacity (CUPRAC) and metal chelating activity assays were used to determine antioxidant activities of the hexane, chloroform, acetone, methanol and water extracts of *F. torulosa.* All of the extracts showed antioxidant activities in a dose-dependent manner. Table 3 shows the $IC_{50}$ values and inhibition percentage (%) at 200 µg/mL concentration of the extracts and standard compounds (BHA, α-tocopherol, and EDTA).

The methanol extract of *F. torulosa* showed the highest antioxidant activity in all assays except metal chelating assay and followed by the acetone extract. Antioxidant activity of the methanol extract of *F. torulosa* was found to be higher than α-tocopherol and BHA used as standards in $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and CUPRAC assays with $IC_{50}$ value of 15.03±0.25, 10.06±0.87 and 17.43±0.29 µg/mL, respectively. When the acetone extract of *F. torulosa* showed higher antioxidant activity than α-tocopherol in $DPPH^{\bullet}$ assay ($IC_{50}$: 25.66±0.38 µg/mL), it showed higher antioxidant activity than α-tocopherol and BHA in $ABTS^{\bullet+}$ ($IC_{50}$: 11.53±0.41 µg/mL) and CUPRAC ($A_{0.50}$: 17.93±0.06 µg/mL) assays. The methanol extract with higher concentrations of phenolic contents showed the highest activity in all antioxidant activity assays except metal chelating assay. Szychowski *et al.* [22] investigated antiradical activity against $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and inhibition of xanthine oxidase of 80 % ethanol, 80 % methanol, 95 % ethanol and 95 % methanol extracts of *Inonotus obliquus* (*Fuscoporia obliqua*). For 80 % ethanol, 80 % methanol, 95 % ethanol and 95 % methanol $IC_{50}$ values were calculated as 279.60±81.17, 447.70±16.18, 412.30±52.12, 404.10±84.14 µg/mL for $DPPH^{\bullet}$ assay; 4.17±0.76, 4.60±2.52, 4.83±1.07, 4.73±0.13 µg/mL for $ABTS^{\bullet+}$ assay; 34.37±6.08, 44.36±5.44, 62.80±7.32, 58.89±6.03 µg/mL for inhibition of xanthine oxidase assay. Previously, Khadhri *et al.* [29] studied antioxidant activity of the ethanol extract of *F. torulosa* by using $DPPH^{\bullet}$ radical

scavenging, the reducing power of iron and the iron-chelating power assays and the ethanol extract showed high antioxidant activity in DPPH• and iron-chelating assays. In a different study, DPPH• radical scavenging activities of the hexane, chloroform, 50% methanol and water extracts of *F. torulosa* were determined and the water extract indicated higher antioxidant activity among the other extracts [30]. Bal *et al.* [28] investigated DPPH• radical scavenging activity of the ethanol extract of *F. torulosa* and the extract was found to have high activity. Antioxidant activity of crude extracts (water, 50 % EtOH, 80 % EtOH, EtOH, EtOAc) of *F. torulosa* was tested by using DPPH• assays and $IC_{50}$ values were calculated as 134.27±1.41, 18.88±0.38, 7.30±0.34, 19.23±0.42, 49.57±0.96 µg/mL, respectively [23]. Our results are in accordance with the literature. In this report, antioxidant activities of various extracts obtained from *F. torulosa* were investigated in details for the first time.

**Table 3.** Antioxidant activity of the extracts of *F. torulosa* by β-Carotene-linoleic acid, DPPH•, ABTS•+, CUPRAC and metal chelating assays[a]

| | | Antioxidant activity | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | β-Carotene-linoleic acid assay | | DPPH• assay | | ABTS•+ assay | | CUPRAC assay | | Metal chelating assay |
| | | Inhibition (%) (at 200 µg/mL) | $IC_{50}$ (µg/mL) | Inhibition (%) (at 200 µg/mL) | $IC_{50}$ (µg/mL) | Inhibition (%) (at 200 µg/mL) | $IC_{50}$ (µg/mL) | Absorbance (at 200 µg/mL) | $A_{0.50}$ (µg/mL) | Inhibition (%) (at 200 µg/mL) |
| Extracts | Hexane | 36.84±0.97 | >200 | 4.39±0.48 | >200 | 9.19±0.45 | >200 | 0.44±0.01 | >200 | 39.81±1.68 |
| | Chloroform | 82.09±0.76 | 23.42±0.23 | 5.70±0.39 | >200 | 15.66±0.71 | >200 | 0.45±0.01 | >200 | 3.51±0.20 |
| | Acetone | 95.68±0.03 | 3.59±0.27 | 78.92±0.92 | 25.66±0.38 | 90.52±0.07 | 11.53±0.41 | 3.25±0.04 | 17.93±0.06 | 15.21±0.35 |
| | Methanol | 97.60±0.70 | 2.57±0.01 | 79.20±0.13 | 15.03±0.25 | 91.62±0.15 | 10.06±0.87 | 3.74±0.04 | 17.43±0.29 | 33.67±0.37 |
| | Water | 93.94±0.79 | 8.23±0.31 | 39.07±0.33 | >200 | 37.58±0.71 | >200 | 0.58±0.01 | 151.41±0.12 | 27.16±0.74 |
| Std | α-Tocopherol | 90.51±0.18 | 2.10±0.08 | 87.14±0.28 | 37.20±0.41 | 85.83±0.12 | 38.51±0.54 | 0.85±0.02 | 66.72±0.81 | NT[b] |
| | BHA | 92.80±0.02 | 1.34±0.04 | 88.36±0.29 | 19.80±0.36 | 86.70±0.10 | 11.82±0.09 | 2.47±0.01 | 24.40±0.69 | NT[b] |
| | EDTA | NT[b] | NT[b] | NT[b] | NT[b] | NT[b] | NT[b] | NT[b] | NT[b] | 94.70±0.60 |

[a] Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$).
[b] NT: not tested.

### 3.4. Anticholinesterase Activity

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities of the hexane, chloroform, acetone, methanol and water extracts of *F. torulosa* were screened by using Ellman method. All of the extracts showed anticholinesterase activities in a dose-dependent manner. Inhibition percentage (%) at 100 µg/mL concentration of the extracts and standard compound (galantamine) are given in Table 4.

Among the extracts, the hexane extract (41.34±1.50 %) of *F. torulosa* was found as the most active against AChE enzyme. The chloroform, acetone and methanol extracts indicated moderate activity against BChE enzyme with inhibition value of 35.18±0.55, 40.78±0.30 and 45.39±0.65 %, respectively, when the hexane and water extracts were found to be inactive. According to our knowledge, there is no study about anticholinesterase activities of *Fuscoporia* species in the literature.

**Table 4.** Cholinesterase inhibitory activities of the extracts of *F. torulosa*[a]

| | | Cholinesterase Inhibitory Activity | |
|---|---|---|---|
| | | AChE assay | BChE assay |
| Extracts | Hexane | 41.34±1.50 | NA[b] |
| | Chloroform | 8.42±0.28 | 35.18±0.55 |
| | Acetone | 22.50±0.28 | 40.78±0.30 |
| | Methanol | 14.80±0.06 | 45.39±0.65 |
| | Water | NA[b] | NA[b] |
| Standard | Galantamine | 78.76±0.52 | 79.27±0.56 |

[a] Inhibition % of 100 µg/mL concentration of the extracts and compounds. Values represent the means ± S.E.M. of three parallel measurements ($p < 0.05$).
[b] NA: not active

## 4. CONCLUSION

In this research, antioxidant and anticholinesterase activities of various extracts of *F. torulosa* were determined with the total phenolic contents. Also, phenolic profile of the mushroom analyzed by HPLC-DAD. The acetone and methanol extracts with the highest content of total phenolic contents displayed higher antioxidant activity than standards in DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and CUPRAC assays. Totally, thirteen phenolic compounds were identified by using HPLC-DAD and *trans*-2-hydroxy cinnamic acid, gallic acid, *p*-coumaric acid were found as major phenolic compounds. In conclusion, this study reveals that extracts obtained from *F. torulosa* mushroom could be used as promising antioxidant and anticholinesterase agents. However, it is necessary to carry out isolation studies to discover the compounds responsible for these bioactivities.

### Conflict of Interest

The authors declare that there is no conflict of interests in this current study.

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