Original Research Article

Investigation of Antioxidant, Antimicrobial, Antityrosinase, and Anticholinesterase Activities of *Psephellus hubar-morathii*

Running Title: Biological Activity of ***Psephellus hubar-morathii***

Abstract

The aim of this study was to determine of antioxidant, antimicrobial, anticholinesterase activities, and phenolic composition of *Psephellus hubar-morathii*. Ferric reducing antioxidant power (FRAP), cupric reducing antioxidant capacity (CUPRAC), and 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging activity were used to assess the antioxidant activities of extracts. Phenolic constituents were measured using reverse phase-high performance liquid chromatography (RP-HPLC), while antimicrobial activity was investigated using the agar well diffusion method. Better total phenolic content, FRAP, and CUPRAC results were determined in aqueous extract, while DPPH activity was greater in methanolic extract. Benzoic acid, and *p*-coumaric acid were detected as major phenolic compounds. Methanolic extract was especially effective against all microorganisms tested except for *Yersinia pseudotuberculosis*. The methanolic extract had a higher half-maximal inhibitory concentration (IC_{50}) on tyrosinase compared to kojic acid. The extract exhibited lower acetylcholinesterase, and butyrylcholinesterase inhibitor activities than galantamine. *Psephellus hubar-morathii* can be considered in the food, and drug industries due to their rich antioxidant, antimicrobial, anticholinesterase, and antityrosinase activities. It can be effective as anti-browning agents for explore of novel tyrosinase inhibitors in cosmetic.

Keywords: Antioxidant, Antimicrobial, anticholinesterase, *Psephellus hubar-morathii*

UNDER PEER REVIEW

Introduction

Free radicals, particularly oxygen free radicals (OFRs) or reactive oxygen species (ROS) (such as superoxide, hydroxyl and hydrogen peroxide), are active oxygen compounds produced by the oxidation reactions of exogenous factors [1]. These reactive species are capable of oxidizing proteins, lipids and DNA, and of triggering various degenerative and chronic diseases, such as cancer, diabetes and cardiovascular disease [2,3]. Antioxidants can suppress or delay oxidation when present at lower levels than oxidizable substrates [4]. They therefore play a key role in preserving human health and averting free radical-induced disease. The health benefits of antioxidants are so great that foodstuffs and pharmaceutical products are routinely reinforced with synthetic antioxidant supplements, including BHA, BHT and PG. However, it has also been suggested that synthetic antioxidants may have carcinogenic and other toxic side-effects [5]. Natural antioxidants are for that reason currently preferred to synthetic equivalents, and limitations on the use of the latter have been recommended.

Alzheimer's disease (AD) is a common neurodegenerative disease characterized by memory disturbance. The most prominent biochemical change in the disease is a decrease in cerebral acetylcholine levels [6]. Raising acetylcholine levels, by means of suppression of the two principal form of cholinesterase, acetylcholinesterase (AChE) and butrylcholinesterase, can therefore be adopted as a therapeutic approach in AD (BChE) [7]. Agents used to inhibit cholinesterase in the treatment of AD include tacrine, rivastigmine and galantamine. However, side-effects have also been observed with these compounds, particularly hepatotoxicity and gastrointestinal disturbances [8,9]. There has therefore been growing focus on safe and effective AChE inhibitors obtained from natural products.

Psephellus huber-morathii (Wagenitz) Wagenitz, otherwise known as *Centaurea huber-morathii* Wagenitz, is a member of the Asteraceae family. The genus *Centaurea* (Asteraceae) consists of some 500 species distributed in the Old World [10]. On the Anatolian peninsula, the genus is represented by approximately 190 species, more than 100 of which are endemic [11]. Some Centaurea species are employed as herbal therapies for fever, diabetes, hemorrhoid, and peptic ulcer in traditional Anatolian folk medicine [12,13]. Pharmacological and phytochemical studies of various different Centaurea species have identified antioxidant, antimicrobial and antipyretic properties [14-17].

UNDER PEER REVIEW

The aims of this work were firstly, the gain of more information about total phenolic quantity, the study of the potential natural antioxidant, antimicrobial, antityrosinase, anti acetylcholinesterase, antibutyrylcholinesterase effect of extracts of *Psephellus hubar-morathii,* secondly to carry on the relationships between total phenolic content and studied activities.

2. MATERIALS AND METHODS

2.1. Chemicals and Instrumentation

The following chemicals and reagents were used: 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich); butylated hydroxytoluene (BHT) (Supelco); galanthamine (Sigma); methanol, ethanol, acetic acid, dimethyl sulfoxide, and acetonitrile (Merck); 6-hydroxy–2,5,7,8-tetramethylchroman–2-carboxylic acid (Trolox), 2,4,6-tripyridyl-s-triazine (TPTZ), and Folin–Ciocalteau reagent (Fluka); polytetrafluoroethylene membranes (Sartorius).

Absorbance was calculated using a Spectro UV-Vis Double PC–8 auto cell spectrophotometer (Labomed Inc.). All solutions were prepared with deionized water purified in an Elgacan® C114 Ultra Pure Water System Deioniser (The Elga Group, Buckinghamshire, England).

Evaporation procedures were conducted using the IKA® RV 05 Basic (IKA®, Werke, USA) rotary evaporator system, while extraction was carried out with a heidolp promax 2020 shaker. All dissolution procedures ınvolved the use of a Heidolph Reax top vortex and Elma® Transsonic Digital ultrasonic water bath (Germany). A Hanna Instruments microprocessor pH meter was employed where appropriate.

2.2. Plant Material and Preparation of Extracts

Psephellus hubar-morathii were collected in 2016 from the Erzincan (Turkey), and identified by one of the authors (Ali Kandemir). The voucher specimens were kept in the herbarium of Erzincan University, Faculty of Science (herbarium number: 10862). Fifty grams of dried plant powder was weighed before mixing with 500 mL methanol. The resulting mixture was stirred for 24-h at room temperature, and then filtered. The resulting filtrate was evaporated with a rotary evaporator (IKA-Werke RV05 Basic, Staufen, Germany). The extract elicited was employed for antioxidant, antimicrobial, cytotoxicity, anticholinesterase, and antityrosinase activity investigation. The extract for use in HPLC analysis was further dissolved in HPLC grade methanol and passed through 0.45-µm membranes for additional filtration.

2.3. HPLC Conditions

The standards employed for HPLC analysis included vanillic acid, *p*-hydroxybenzoic acid, syringaldehyde, *p*-coumaric acid, sinapic acid, benzoic acid and quercetin. Stock solutions of the mixed standards were diluted over a concentration range of 5-100 µg mL^{-1} to produce the calibration curve. HPLC analysis of phenolic compounds was conducted on a reverse phase column (150 × 4.6 mm i.d, 5 µm) (Waters Spherisorb, Milfort, MA, USA), on a gradient program with the assistance of a two-solvents system [A: 100% methanol; B: 2% acetic acid in water (pH 2.8)], and a constant solvent flow rate set to 1.5 mL min^{-1} on a HPLC system (Shimadzu Corporation, LC 20 AT, Kyoto, Japan) (Table 1). The injection volume was adjusted to 20 µL. Signals were identified at 232, 246, 260, 270, 280, 290, 308, and 328 using DAD detection at a column temperature of 25ºC.

UNDER PEER REVIEW

2.4. Determination of Antioxidant Capacity

The Folin-Ciocalteu procedure was adopted in order to calculate total phenolic quantities in the extract [18]. Gallic acid was used as a standard, with the total phenolic content being expressed as mg of gallic acid equivalents per gram of 100 g sample. Briefly, 0.01, 0.02, 0.03, 0.04 and 0.05 mg/mL concentrations of gallic acid were prepared in methanol, while 0.1 and 1 mg/mL concentrations were also prepared in methanol. Next, 0.5 mL of each sample was placed into test tubes before being combined with 0.5 mL of 0.2 N Folin-Ciocalteu reagent and 1.5 mL of 2% sodium carbonate. The test tubes were then sealed with parafilm and incubated for 2 h at 20 °C, after which the absorbance was read spectrophotometrically at 760 nm. All measurements were conducted in triplicate.

The ferric reducing antioxidant power (FRAP) assay depends on calculating the iron reducing capacities of a given extract [19]. When exposed to 2,4,6-tripyridyl-S-triazine (TPTZ), the Fe^{2+}-TPTZ complex exhibits a blue color which is read at 593 nm. Briefly, 3.0 mL of working FRAP reagent was added to an appropriate volume/concentration of extract. This mixture was incubated for 4 min at 37 ^{0}C, after which the absorbance was measured against a ferrous sulfate standard at 593 nm. Trolox was also measured under identical conditions as a standard antioxidant compound for purposes of comparison. The results were expressed as μM Trolox equivalent of g sample.

DPPH radical-scavenging activity is related to the antioxidant's DPPH cation radical scavenging capacity [20]. In brief, we added 0.75 mL of DPPH reagent (0.1 mM in methanol) to 0.75 mL of tea extract or standard, and subjected this to vigorous vortexing. We left this to stand in the dark for 30 mins at room temperature. Discoloration observed in DPPH was measured spectrophometrically at 517 nm. The percentage inhibition of the discoloration caused by the tea extract was measured using Trolox as standard, the values being expressed

UNDER PEER REVIEW

as SC_{50} (mg sample per mL). This represents the sample concentration resulting in 50% scavenging of DPPH radical.

The CUPRAC levels of extracts were studied using the spectrophotometric method [21]. To summarize, 1 mL of $CuCl_2$ solution ($1.0x10^{-2}$ M), 1 mL of neocuproine solution ($7.5x10^{-3}$ M) and 1 mL NH_4Ac buffer solution were mixed in a test tube. A range of different extract concentrations were added to the initial mixture to yield a final volume of 4.1 mL. The test tubes were then incubated for 30 mins. Absorbance was measured at 450 nm against a reagent blank. CUPRAC values were expressed as μM Trolox equivalent per gram of sample.

2.5. Antimicrobial Activity Assessment

Escherichia coli ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas auroginosa* ATCC 43288, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 43251, *Bacillus cereus* 709 ROMA, *Mycobacterium smegmatis* ATCC607, *Candida albicans* ATCC 60193, and *Saccharomyces cerevisiae* RSKK 251 were supplied by the Hifzissihha Institute of Refik Saydam (Ankara, Turkey). We dissolved the extract in dimethyl sulfoxide (DMSO) for material preparation in a stock solution of 178–256 mg/mL.

The agar-well diffusion method [22], with various modifications previously described elsewhere [23], was used for susceptibility screening. Each bacterium was suspended in Mueller Hinton (MH) (Difco, Detroit, MI) broth, while yeast-like fungi were suspended in yeast extracts broth. The micro-organisms were subsequently diluted to a level of approximately 106 colony-forming units (cfus) per mL. Sabouraud Dextrose Agar (SDA) (Difco, Detriot, MI) was used for yeast-like fungi, and brain heart infusion agar (BHA) was employed for *M. smegmatis* [24]. These were "flood-inoculated" onto the surface of MH and

UNDER PEER REVIEW

158 SD agars and then dried. In the following stage, 5-mm diameter wells were produced from the
159 agar with the help of a sterile cork-borer, after 8900- 12800 µg/50 µL of the extract
160 substances was placed into the wells. The plates were incubated for 18 h at 35 ^{o}C. *M.*
161 *smegmatis* was cultured for 3-5 days on BHA plates at 35 ^{o}C. The zone of inhibition was
162 measured against the test organism to determine antimicrobial activity. Ampicillin (10 µg),
163 streptomycin (10 µg), and fluconazole (5 µg) were employed as standard drugs, while
164 dimethylsulfoxide served as the control. Finally, minimal inhibition concentration (µg mL^{-1})
165 of *Psephellus hubar-morathii* were calculated.

166 **2.6. Antityrosinase Activity**

167 Tyrosinase inhibitory activity (TIA) (EC 1.14.1.8.1, 30 U, mushroom tyrosinase, Sigma) was
168 measured using different concentrations of kojic acid solutions as standard [25]. Reaction
169 mixture absorbance was read at 490 nm using the spectrophotometric method on a microplate
170 reader (VersaMax Molecular Devices, USA). The percentage of TIA was calculated using the
171 formula % inhibition = [[(A-B)-(C-D)] / (A-B)] x 100

172 **2.7. Acetylcholinesterase (AChE)/Butyrylcholinesterase (BChE) Inhibitory Activity**

173 The colorimetric Ellman method, with minor modifications, was used to investigate
174 acetylcholine esterase inhibitor (AChEI) and butyrylcholin esterase inhibitor (BChE)
175 activities [26]. AChE and BChE were employed as enzymes. Acetylthiocholine iodide and
176 butyrylthiocholine iodide served as substrates, while 5,5'-dithio-bis 2-nitrobenzoic acid
177 (DTNB) was employed as the coloring agent. The control and test compounds were dissolved
178 in sodium phosphate buffer (pH=8) prior to reduction to a concentration of 25-200 µg/mL.
179 Next, 130 µL of sodium phosphate buffer, 10 µL of the tested compound and 20 µL of the
180 enzyme were mixed in a 96-well plate and subjected to incubation for 15 min at 25 ºC. In the

UNDER PEER REVIEW

following procedure, 20 µL of DTNB and 20 µL of substrate (acetylthiocholine iodide or butyrylthiocholine iodide for each assay) were added to all wells. The resulting substrate was hydrolyzed to yield thiocholine. This reacts with 5,5-Dithiobis [2-nitrobenzoic acid] (DTNB) to give yellow-colored 2-nitro-5-thiobenzoate. Absorbance was measured spectrophotometrically at 412 nm. AChE and BChE inhibition fractions were calculated using the formula shown below and compared against galantamine used as standard.

% Inhibition = 100 - [(A1 / A2) x 100] where

A1 = Absorbance of the sample solutions at 412 nm

A2 = Average absorbance of the control solutions at 412 nm.

3. RESULTS

3.1. HPLC Chromatograms

Chromatograms of the phenolic standards and methanolic extract are presented in Figures 1-2. The various quantities of phenolic compounds measured in the samples are shown in Table 1. The results indicated the presence in the methanolic extract of the plant of *p*-coumaric acid (2.21 mg/g) and benzoic acid (11.55 mg/g).

Table 1. Phenolic composition of the methanolic extract of *Psephellus hubar-morathii*

Phenolic compounds	Retention time (min)	Amount (mg g^{-1})
p-hydroxy benzoic acid	4.411	-
Vanillic acid	5.102	-
Syringaldehyde	6.383	-
p-coumaric acid	7.437	2.21
Sinapic acid	7.947	-
Benzoic acid	9.588	11.55
Quercetin	14.720	-

UNDER PEER REVIEW

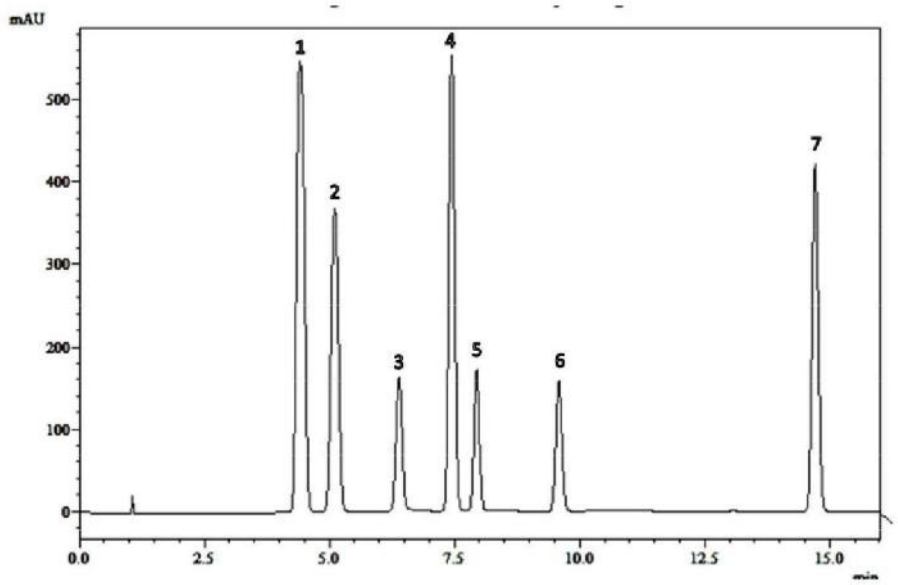

Fig. 1. RP-HPLC chromatogram of phenolic standards (50µM) searched in *Psephellus hubar-morathii* samples detected at 270 nm by DAD. Peak identification: (1) *p*-hydroxy benzoic acid, (2) vanillic acid, (3) syring aldehyde, (4) *p*-coumaric acid, (5) sinapic acid, (6) benzoic acid, (7) quercetin.

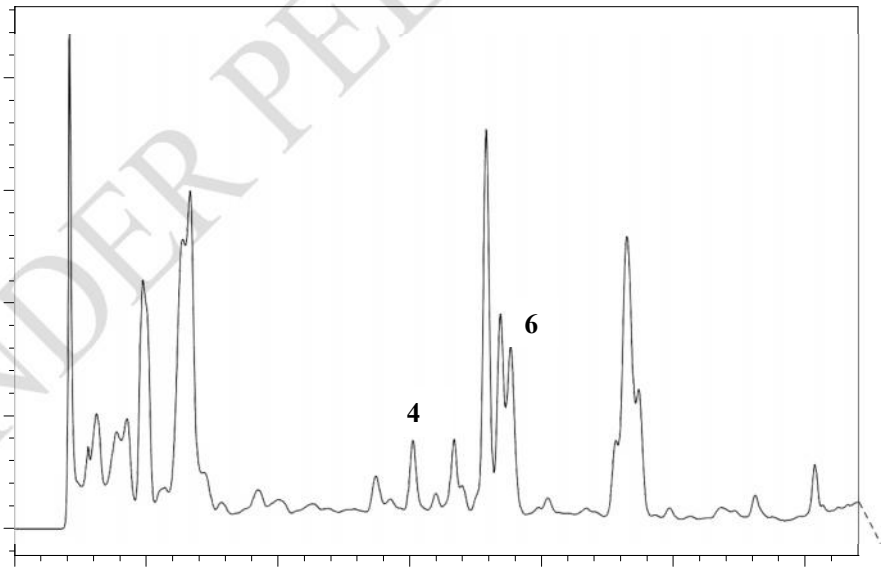

Fig. 2. RP-HPLC DAD chromatogram of *Psephellus hubar-morathii* methanol extract (50 mg/mL) at 270 nm. Peak identification: (4) *p*-coumaric acid, (6) benzoic acid.

UNDER PEER REVIEW

3.2. Antioxidant Activities of *Psephellus hubar-morathii* Extracts

TPC values determined for the aqueous and methanolic extracts were 13.9 ± 0.460 and 10 ± 0.268 mg of GAE/g, respectively (Table 2).

DPPH scavenging activity values determined for aqueous and methanolic extracts in this study were 0.3379 ± 0.0049, 0.2073 ± 0.0036 mg/mL, respectively (Table 2). The extracts exhibited a lower radical scavenging capacity than BHT (0.0031 ± 0.0002 mg/mL). FRAP values for aqueous and methanolic extracts were 841 ± 4.699, 666 ± 3.210 μM Trolox/g sample, respectively (Table 2). The CUPRAC activity of the aqueous and methanolic extracts in this study was determined as 1322 ± 8.940, 1230 ± 7.915 μM Trolox/g sample, respectively (Table 2).

Table 2. The antioxidant activities of *Psephellus hubar-morathii* extracts

Test Compounds	TPC[†]	FRAP [‡]	CUPRAC[§]	DPPH[¶2]
Aqueous extract	13.9 ± 0.460	841 ± 4.699	1322 ± 8.940	0.3379 ± 0.0049
Methanolic extract	10 ± 0.268	666 ± 3.210	1230 ± 7.915	0.2073 ± 0.0036
BHT				0.0031 ± 0.0002

[†]Total phenolic content expressed in mg of gallic acid equivalent (GAE) per gram of dry plant weight.
[‡] FRAP value expressed as μM trolox equivalents (TE) per gram of dry plant weight.
[§] Trolox equivalent antioxidant capacity (TEAC) value expressed in μM trolox equivalents (TE) per gram of dry plant weight.
[¶2]Concentration of test sample (mg/mL) required to produce 50% inhibition of the DPPH radical.

3.3. Antimicrobial Activities of *Psephellus hubar-morathii* Extracts

The antimicrobial activities of *Psephellus hubar-morathii* extracts against the bacteria tested was assessed in terms of the presence of minimal inhibition concentrations (Table 3). The methanolic extract exhibited antimicrobial effect against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis and Mycobacterium smegmatis,* but not *Yersinia pseudotuberculosis.*

UNDER PEER REVIEW

Table 3. Antimicrobial activities of *Psephellus hubar-morathii* extracts (µg/mL)

Tested Compounds	Quantity (µg/mL)	Microorganisms and Minimal Inhibition Concentration (µg/mL)									
		Gram negative			Gram positive				No gram	Yeast Like Fungi	
		Ec	Yp	Pa	Sa	Ef	Lm	Bc	Ms	Ca	Sc
Methanolic Extract	10000	125	-	250	250	350	700	350	62.25	350	350
Aqueous Extract	10000	-	-	-	-	-	-	-	-	-	-
Ampicillin	10	10	18	>128	35	10	10	15	-	-	-
Streptomycin	10								4		
Fluconazole	5									>8	>8

Ec: *Escherichia coli* ATCC 25922, Yp: *Yersinia pseudotuberculosis* ATCC 911, Pa: *Pseudomonas aeruginosa* ATCC 27853, Sa: *Staphylococcus aureus* ATCC 25923, Ef: *Enterococcus faecalis* ATCC 29212, Lm: *Listeria monocytogenes* ATCC 43251, Bc: *Bacillus cereus* 702 Roma, Ms: *Mycobacterium smegmatis* ATCC607, Ca: *Candida albicans* ATCC 60193, Sc: *Saccharomyces cerevisiae* RSKK 251, (-): no activity of test concentrations (10 000 µg/mL).

3.3. Antityrosinase Activity of *Psephellus hubar-morathii* Extract

We investigated the antityrosinase activity of *Psephellus hubar-morathii* extract. The half-maximal inhibitory concentration (IC_{50}) of the methanolic extract (575.44 µg mL^{-1}) on tyrosinase were higher than the kojic acid (63.0957 µg mL^{-1}).

3.4. Anticholinesterase Activities of *Psephellus hubar-morathii* Extract

We also investigated the AChE, and BChE inhibitor activities of *Psephellus hubar-morathii* extract. AChE inhibitor activity (% inhibition) of the aqueous extract was found 9.2 ± 0.5, 15.5 ± 0.4, 24.9 ± 0.2 and 30.4 ± 0.8 µg mL^{-1} for the 25, 50, 100 and 200 µg mL^{-1} , respectively (Table 4). AChE inhibitor activity (% inhibition) of the methanolic extract was found 3.4 ± 0.7, 8.3 ± 0.3, 27.8 ± 0.5 and 38.8± 0.7 µg mL^{-1} for the 25, 50, 100 and 200 µg mL^{-1} , respectively. AChE inhibitor activity (% inhibition) of galantamine was measured at

UNDER PEER REVIEW

64.5 ± 1.2, 72.2 ± 0.9, 78.6 ± 0.8 and 84.2 ± 0.3 µg mL^{-1} for 25, 50, 100 and 200 µg mL^{-1} (Table 4).

BChE inhibitor activity (% inhibition) of the aqueous extract was found 21.0 ± 0.2, 46.8 ± 0.4, 55.3 ± 0.8 and 68.3 ± 1.3 µg mL^{-1} for the 25, 50, 100 and 200 µg mL^{-1} , respectively. BChE inhibitor activity (% inhibition) of the methanolic extract was found 10.5 ± 0.4, 32.1 ± 0.6, 54.9 ± 0.9 and 72.6 ± 1.5 µg mL^{-1} for the 25, 50, 100 and 200 µg mL^{-1} , respectively, while BChE inhibitor activity (% inhibition) of galantamine was 41.3 ± 0.7, 56.6 ± 0.8, 68.7 ± 1.2 and 80.1 ± 0.4 µg mL^{-1} for 25, 50, 100 and 200 µg mL^{-1} (Table 4).

Table 4. Acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) inhibitor activities (% inhibition)

	Samples	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL
AChE Inhibitor Activity	AE	9.2 ± 0.5	15.5 ± 0.4	24.9 ± 0.2	30.4 ± 0.8
	ME	3.4 ± 0.7	8.3 ± 0.3	27.8 ± 0.5	38.8 ± 0.7
	Galantamin	64.5±1.2	72.2±0.9	78.6±0.8	84.2 ± 0.3
BChE Inhibitor Activity	AE	21.0 ± 0.2	46.8 ± 0.4	55.3 ± 0.8	68.3 ± 1.3
	ME	10.5 ± 0.4	32.1 ± 0.6	54.9 ± 0.9	72.6 ± 1.5
	Galantamin	41.3±0.7	56.6±0.8	68.7±1.2	80.1 ± 0.4

AE: Aqueous extract; **ME:** Methanolic extract

4. DISCUSSION

Various different chemical tests were employed in this study. These relied on the ability of antioxidants to function as reducing agents (ferric and cupric ions) and as radical scavengers (DPPH scavenging assay). There has been extensive previous research and discussion of the capacity of pure antioxidant compounds and plant extracts. Various methods and variations have been developed and applied to determine antioxidant capacity [27]. However, no single universal technique is capable of accurately assessing the antioxidant potential of plant

UNDER PEER REVIEW

products or antioxidant compounds. We therefore performed a number of antioxidant assays to achieve a better understanding of the extracts' antioxidant potential.

Phytochemicals are increasingly regarded as bestowing health benefits, and as agents concerned with maintaining and functions in cells, tissues, or the body as a whole. Phytochemicals commonly associated with human health include polyphenols, carotenoids and tocopherols. Polyphenols are the principal secondary metabolites in the majority of plants. They are reported to exhibit antioxidant and free radical scavenging activities. The administration of polyphenol in diet to reduce the risk of coronary heart disease has been investigated in previous studies [28]. These compounds are also employed as anticarcinogenic and anti-inflammatory agents [29,30]. The measurement of polyphenol content in plant materials has become an essential tools in determining their value to human health [31]. Reports have shown that, in addition to polyphenolics, plants containing vitamin C, carotenoids, tocopherols, and other such compounds are also beneficial to human health. The total phenolic contents in the *Psephellus hubar- morathii* extracts are summarized in Table 2. Total phenolic content was found as 13.9 ± 0.460 and 10 ± 0.268 (mg gallic acid / g sample) for aqueous and methanolic extract of the plant, respectively. Since the phenolic compounds are very important constituents of plants and known as powerful chain-breaking antioxidants, 7 total phenolic content of the extracts was investigated and expressed as milligram per gram of extract, as shown in Table 1. Benzoic acid, and *p*-coumaric acid were found as major phenolic constituents of *Psephellus hubar- morathii* extract. Karamenderes et al. (2007) determined total phenolic contents in methanolic extracts from eight different *Centaurea* species between 43.44 and 120.90 mg GAE/L [32]. Ttotal phenolic contents of hexane and chloroform extracts in their study were 17.33–42.11 and 25.66– 72.11 mg GAE/L, respectively [32]. Various extraction solvents were employed for the purpose of determining

UNDER PEER REVIEW

295 the total phenolic content of *C. ensiformis* extract, with values ranging from 16.01 to 64.61
296 mg pyrocatechol, depending on the solvent [33].

297 Stable organic radical DPPH have been widely employed for determining the antioxidant
298 activities of plant extracts or antioxidant compounds. This assay involves reduction of the
299 purple radical (picrylhydrazyl) by antioxidant compounds to the corresponding pale yellow
300 hydrazine (picrylhydrazine). Discoloration is correlated with the free radical scavenging
301 activity of the sample investigated. Table 2 shows the antioxidant activity of aqueous and
302 methanol extracts of *Psephellus hubar- morathii.* The antioxidant activities obtained by the
303 DPPH method for the *Psephellus hubar- morathii* extracts are compared with BHT used as
304 positive controls. In the DPPH assay, metanolic extract of *Psephellus hubar- morathii*
305 demonstrated the highest DPPH radical scavenging activity, which was close to that of
306 aqueous extract of *Psephellus hubar- morathii* (Table 2). Tepe et al. (2006) reported an IC_{50}
307 of 67.8 mg/mL in *C. mucronifera* [14]. Their results demonstrated that the methanolic extract
308 of *C. drabifolia* subsp. *detonsa* exhibits more potent free radical scavenging activity than *C.*
309 *mucronifera.* The IC_{50} values of 15 Asteraceae species ranged from 198 to 2009.7 mg/mL
310 [34]. *Centaurea drabifolia* subsp. *detonsa* exhibits more powerful free radical scavenging
311 abilities than these Asteraceae species. In another study, IC_{50} values of DPPH scavenging
312 activity in six Iranian Achiella species ranged between 32.92 and 118.90 mg/mL [35].

313 Previous reports indicate that reducing properties are usually associated with the presence of
314 reductones [36]. These are known to exhibit antioxidant activities by breaking the free radical
315 chain through the donation of a hydrogen atom. We therefore, measured the conversion of a
316 Cu^{2+} to the Cu^{+} form. Cupric reducing power increased in line with sample concentrations. In
317 the CUPRAC assay known as electron transfer-based assay, antioxidant capacity was more
318 effectively of aqueous extract of *Psephellus hubar- morathii.* The CUPRAC assay has been

UNDER PEER REVIEW

319 employed to measure the cupric-reducing power activities of plant extracts or antioxidant
320 compounds in a number of studies [37]. The assay relies on Cu^{2+} being reduced to Cu^{1+} by
321 antioxidant compounds when neocuproine is present. The advantages of this system include
322 simplicity, the transparency of the end point and mechanism, easily accessible
323 instrumentation, and excellent intra- and inter-assay reproducibility [38]. CUPRAC values
324 have been found as 1322±8,940 and 1230±7,915 (μM Trolox/g sample) for aqueous and
325 methanolic extract of the plant, respectively (Table 2). Zengin et al. (2012) reported that
326 cupric ion reducing power of ascorbic acid is stronger than *C. drabifolia* subsp. *Detonsa* [39].
327 Reducing power activities for *Centaurea* species including *C. solsititialis* [16], *C. drabifolia*
328 subsp. *Detonsa* [39] and *C. kotschyi* var. *persica* have been reported [15].

329 This assay is based the reduction of complex (Fe(III)–TPTZ) to (Fe(II)–TPTZ) by antioxidant
330 compounds. The Fe(II)–TPTZ complex yields a blue color, with peak absorbance at 593 nm.
331 The aqueous extract obtained from *Psephellus hubar- morathii* (841 ± 4.699 μM TE/g) had
332 highest activity followed by methanolic extract (666 ± 3.210 μM TE/g). Various studies have
333 used the FRAP assay to assess plants' antioxidant capacities [40-42]. However, no study on
334 the FRAP activities of *Psephellus* species have been documented.

335 The extracts obtained *Psephellus hubar- morathii* have rich antioxidant activity. *p*-coumaric
336 acid and benzoic acid that we found in the plant with HPLC studies have antioxidant effective
337 properties. FRAP activity of *Psephellus hubar- morathii* may be based on its phenolic
338 compounds.

339 The methanolic extract showed high antimicrobial activity on *Escherichia coli, Pseudomonas*
340 *aeruginosa, Staphylococcus aureus, Enterococcus faecalis and Mycobacterium smegmatis*
341 Micro-organisms can result in a wide range of diseases. If a plant exhibits an antimicrobial
342 effect on these micro-organisms, effective remedies can be developed from these. There has

UNDER PEER REVIEW

been considerable research into the antimicrobial activities of different *Psephellus* species. Güven et al. (2005) reported that five species of *Centaurea* L. exhibited a broad range of antimicrobial activity to varying degrees. Particularly, ethyl acetate extracts of *C. odyssei* and *C. kurdica* showed significant antibacterial and anticandidal activities [43]. Kumarasamy et al. (2003) found significant antimicrobial activity of serotonin conjugates from *C. nigra* against penicillin-resistant *E. coli* [44]. Cansaran et al. (2010) investigated the antimicrobial activities of different *C. cankiriense* extracts [45]. The ethyl acetate and methanol extracts both inhibited the growth of 13 bacteria. The MIC values of the ethyl acetate extracts were 250 µg/mL for *E. coli* and 62.5 µg/mL for *S. aureus*. All the Centaurea species in that study exhibited weaker antibacterial activity than the ethyl acetate extract of *C. cankiriense.* In addition, *C. bornmuelleri, C. hubermorathii* and *C. schiskinii* had no effect on *E. coli* or *B. cereus* in the micro-dilution assay [46].

Tyrosinase is a major enzyme in the synthesis of melanin in mammals. Melanin protects cutaneous tissues against ultraviolet (UV) damage by absorbing UV sunlight and eliminating reactive oxygen species. Overproduction or abnormal melanin pigmentation gives rise to cosmetic concerns in humans. Potent tyrosinase inhibitors therefore need to be developed [47]. Tyrosinase inhibition in this study involved the use of L-DOPA as substrate and kojic acid as a positive control. The half-maximal inhibitory concentration of the methanolic extract on tyrosinase was calculated at 575.44 (µg/mL). These findings appear to indicate that *Psephellus huber-morathii* extract may have the potential for use in the the design and development of novel tyrosinase inhibitors for employment as anti-browning agents.

AChE inhibitors are employed in the management of mild-moderate Alzheimer's disease. Recent studies have also looked for novel AChE inhibitors from herbal sources [48]. This study examined the anticholinesterase activity of *Psephellus hubar- morathii* for the first time.

UNDER PEER REVIEW

Aktumsek screened various *Centaurea* extracts for AChE inhibitory activity using Ellman's colorimetric method. Methanol extracts were observed to exhibit better activity against AChE than aqueus extracts. At 2 mg/ mL, water extracts of *C. pyrrhoblephara* and *C. antalyense* showed no activity against AChE. The methanolic extract of *C. polypodiifolia* var. *pseudobehen* (24.54%) emerged as the most active extract, followed by *C. antalyense* (21.25%) and *C. pyrrhoblephara* (14.31%). Galantamine equivalent values between 0.71 and 1.29 mg/g extract were also identified in the extract. In terms of BChE, all extracts apart from the methanolic extract of *C. pyrrhoblephara* exhibited activity inhibition. The methanolic extract of *C. polypodiifolia* var. *pseudobehen* exhibited the highest level of inhibition against both enzymes (45.50% inhibition). The aqueous extracts of *C. pyrrhoblephara* and *C. antalyense* exhibited no activity on AChE, but did display inhibition activity against BChE [49].

5. CONCLUSION

The *Psephellus hubar- morathii* extracts investigated in this study appear to possess antioxidant and antimicrobial properties. Extracts of *Psephellus hubar- morathii* also exhibited moderate inhibitory activities against AChE, BChE, and antityrosinase. This study represents the first report of anticholinesterase activity for members of the genus *Centaurea*. Novel plant-derived bioactive molecules are urgently needed, and these plant extracts may represent a natural source of antioxidants and anticholinesterase agents, particularly in foodstuffs and medicinal products. Further studies are now needed to confirm the bioactive compounds involved in the antioxidant, antimicrobial, and anticholinesterase activities observed in these extracts.

UNDER PEER REVIEW

Conflict of Interest

The authors declare that there are no conflicts of interest

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UNDER PEER REVIEW

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