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Cholinesterase and tyrosinase inhibitory, and antioxidant potential of randomly selected Umbelliferous plant species and the chromatographic profile of *Heracleum platytaenium* Boiss. and *Angelica sylvestris* L. var. *sylvestris*

ILKAY ERDOGAN ORHAN¹, FATMA TOSUN¹
and KRYSZYNA SKALICKA-WOŹNIAK^{2*}

¹Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey and ²Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, 1 Chodzki Str, 20–093 Lublin, Poland

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Abstract. The neurobiological activity of the methanol extracts of thirteen Umbelliferae (Apiaceae) plants was tested against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and tyrosinase (TYR) using a high-throughput screening technique. Although the extracts displayed no to a low inhibition profile against the enzymes, the highest cholinesterase inhibition was observed with *Heracleum platytaenium* (32.52±3.27 % for AChE and 46.16±1.42 % for BChE) at 100 µg mL⁻¹. Since neurodegeneration is linked to oxidative damage, the antioxidant potential of the extracts were examined through radical scavenging, metal-chelating capacity, and reducing power experiments and they exerted modest levels of activity varying according to the method. The extracts had a better ability to scavenge the nitric oxide radical (19.47±2.09 to 54.91±1.98 %). Since these species are known to be rich in coumarins, quantitative high-performance liquid chromatography (HPLC) analysis indicated the presence of xanthotoxin, angelicin, isopimpinellin, bergapten, and pimpinellin in *Heracleum platytaenium* and angelicin and imperatorin in *Angelica sylvestris* var. *sylvestris*.

Keywords: neurobiological activity; coumarins; Apiaceae; HPLC.

INTRODUCTION

Alzheimer's disease (AD), the most common form of dementia, is a progressive neurodegenerative disorder affecting the elderly population, particularly over the age of 60. The cholinesterase (ChE) enzyme family, consisting of two sister enzymes, *i.e.*, acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholine-

* Corresponding author. E-mail: kskalicka@pharmacognosy.org
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sterase (BChE, EC 3.1.1.8), catalyzes the hydrolysis of acetylcholine (ACh), which has been proved to be in lower amounts in the brains of AD patients than usual. Consequently, ChE inhibitors, such as tacrine, rivastigmine, donepezil, and galanthamine, have been the most frequently prescribed drug class for the modern treatment of AD.¹ After AD, Parkinson's disease (PD) is another common neurodegenerative disorder worldwide with clear motor symptoms instigated by the degeneration of nigrostriatal dopaminergic neurons, often accompanied with cognitive conditions. It was stated that since enhancement of the cholinergic system by AChE inhibitors may cause a reduction in apathy and falls observed during PD, they might also be helpful for therapy of PD.² On the other hand, transcriptional induction of tyrosinase (TYR, EC 1.14.18.1) is known to initiate a neurotoxic production of cellular dopamine and its oxidative metabolites in excess amounts and, therefore, inhibition of TYR may also be a promising therapeutic approach toward PD.³ Nevertheless, as the current ChE inhibitors are only available for the symptomatic treatment of AD and PD, new therapeutic targets for these diseases still remain to be developed.

It is also worth mentioning that age-associated disorders with a neurodegenerative character, such as AD and PD, are usually linked to oxidative damage and, thus, a neuroprotective effect is correlated with prevention of the oxidative stress involved in the over production of reactive oxygen species along with metal dysregulation.⁴ Based on all the relevant data reported hitherto, it has become quite rational to imply a multi-target approach for the treatment of AD and PD.

Regarding our since the year of 2000 ongoing research on finding new inhibitors of ChE and TYR from herbal sources, some encouraging results were obtained with coumarin-rich plants from Umbelliferae (Apiaceae) family, such as *Angelica officinalis*, against ChEs,⁵ and taking this finding into account, the aim now was to investigate the neurobiological effects of methanol extracts prepared from thirteen randomly selected edible Umbelliferous plants, including *Apium graveolens* L. (AG), *Angelica sylvestris* L. var. *sylvestris* (ASS), *Artemisia aquatica* L. (AS), *Astrantia maxima* Pallas subsp. *maxima* (AMM), *Coriandrum sativum* L. (CS), *Foeniculum vulgare* Miller (FV), *Heracleum platytaenium* Boiss. (HP), *Ligusticum alatum* (Bieb.) Sprengel (LA), *Petroselinum crispum* (Miller) A. W. Hill (PC), *Pimpinella affinis* Ledeb (PAF), *Pimpinella anisum* L. (PAN), *Smyrniolum olusatrum* L. (SO), and *Tordylium apulum* L. (TA) through their ChE and TYR inhibitory activity using microtiter ELISA (enzyme-linked immunosorbent assay). Relevantly, antioxidant potential of the extracts was evaluated using six *in vitro* high-throughput screening assays based on radical scavenging, metal-chelating, and reducing power mechanisms. Quantitative coumarin analysis was performed on the extracts of HP and ASS using a high-performance liquid chromatography (HPLC) technique.

EXPERIMENTAL

Plant materials

Samples of the studied umbelliferous plants were collected throughout Turkey. The plants were identified by Prof. Dr. Mecit Vural from the Department of Biology, Faculty of Arts and Sciences, Gazi University (Ankara, Turkey) and voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Gazi University (Ankara, Turkey). The collection sites and herbarium numbers of the plants are listed in Table S-I of the Supplementary material to this paper.

Extraction procedure

The air-dried and powdered parts used for each plant species (the leaves for AG; the fruits for FV, CS, PAN; the aerial parts for the rest) were extracted with methanol and the macerates obtained were evaporated *in vacuo* to dryness. The extracts were kept in a freezer until the experiments were performed.

Phytochemical content of the extracts. Determination of total phenol and flavonoid contents in the extracts

Total phenol content of the extracts was determined using the Folin–Ciocalteu reagent (Sigma, St. Louis, MO, USA).⁶ In brief, a number of dilutions of gallic acid (50–500 $\mu\text{g mL}^{-1}$) were obtained to prepare a calibration curve. The extracts and gallic acid dilutions diluted in ethanol (75 %) were mixed with 750 μL of Folin–Ciocalteu reagent and 600 μL of sodium carbonate in test tubes. The tubes were then vortexed and incubated at 40 °C for 30 min. Subsequently, the absorption was measured at 760 nm on a Unico 4802 UV–visible double beam spectrophotometer (Dayton, NJ, USA). The total flavonoid content of the extracts was established by the aluminum chloride colorimetric method.⁷ To sum up, a number of dilutions of quercetin (50–500 $\mu\text{g mL}^{-1}$) were obtained to prepare a calibration curve. Then, the extracts and quercetin dilutions were mixed with ethanol (75 %), aluminum chloride reagent, 100 μL of sodium acetate and distilled water. Following incubation for 30 min at room temperature, the absorbance of the reaction mixtures was measured at a wavelength of 415 nm using a Unico 4802 UV–visible double beam spectrophotometer (Dayton, NJ, USA). The total phenol and flavonoid contents of the extracts are expressed as gallic acid and quercetin equivalents (mg g^{-1} extract), respectively.

Quantification of coumarin derivatives in the HP and ASS extracts

HPLC analysis was performed on a Shimadzu system (Shimadzu, Japan) equipped with an automatic degasser (DGU-20A 3R), a quaternary pump (LC-20AD), an autosampler (SIL-20A HT) and diode-array detector (DAD) (SPD-M20A). The chromatographic separation was realized on a Zorbax Eclipse XDB C18 column (Agilent) (250 mm \times 4.6 mm, 5 μm) at 20 °C. The flow rate of the mobile phase was maintained at 1 mL min^{-1} and the injection volume was 10 μL . The LC pumps, autosampler, column oven, and DAD were monitored and controlled by LabSolutions 5.51 software (Shimadzu). A gradient of methanol (A) and water (B) was used as follows: 20 % A as starting point, then 20–50 % for 10 min; 50–60 % for 5 min; 60 % kept for 25 min; 80–100 % for 2 min. Then, post run was set for 18 min.

Compounds were identified by comparison of their retention times and diode array detector (DAD) spectra with those of appropriate standards analyzed under the same conditions. The following standards were tested: simple coumarins (scopoletin, scoparone, decursin, umbelliferone, daphnetin, harniarin, esculetin (Sigma–Aldrich) and osthol (ChromaDex, USA) as well as furanocoumarins (angelicin, xanthotoxol, isopimpinellin, isoimper-

atorin (ChromaDex, USA), xanthotoxin, bergapten, imperatorin (Sigma Aldrich), byakangelicol, heraclenin, byakangelicin, and phellopterin (PhytoLab, Germany). Quantitative determination was performed at 254 and 320 nm. Quantitative analysis of pimpinellin was realized by calculation to angelicin (the lack of standard of pimpinellin). In order to confirm the identification of the coumarin derivatives, an HPLC coupled with an electrospray ionization (ESI) time-of-flight mass spectrometry (TOF-MS) was applied. An Agilent 1200 HPLC system equipped with 6210 MSD TOF mass spectrometer and Zorbax Stable Bond RP-18 (150 mm×2.1 mm, 3.5 μm) column was used. Analyses were performed using a gradient of 60 % acetonitrile in water (+ 0.005 mol L⁻¹ ammonium formate with 0.1 % formic acid) – solvent A, and 90 % acetonitrile in water (+0.005 mol L⁻¹ ammonium formate with 0.1 % formic acid) – solvent B, as described previously.⁸ Compounds were identified using the mass spectra of reference compounds, as well as MS data from the literature.⁹

Microtiter enzyme inhibition assays

Cholinesterase inhibition. AChE and BChE inhibitory activity of the samples was measured by a slightly modified spectrophotometric method of Ellman.¹⁰ Electric eel AChE (Type-VI-S; EC 3.1.1.7, Sigma, St. Louis, MO, USA) and horse serum BChE (EC 3.1.1.8, Sigma, St. Louis, MO, USA) were used, while acetylthiocholine iodide and butyrylthiocholine chloride (Sigma, St. Louis, MO, USA) were employed as the substrates of the reaction. 5,5'-Dithiobis(2-nitrobenzoic)acid (DTNB; Sigma, St. Louis, MO, USA) was used for the measurement of the anticholinesterase activity. All reagents and conditions were the same as described in a previous publication.¹¹ Briefly, in this method, 140 μL of sodium phosphate buffer (pH 8.0), 20 μL of DTNB, 20 μL of test solution and 20 μL of AChE/BChE solution were added *via* a multichannel automatic pipette (Gilson pipetman, Paris, France) in a 96-well microplate and incubated for 15 min at 25 °C. The reaction was then initiated with the addition of 10 μL of acetylthiocholine iodide/butyrylthiocholine chloride. Hydrolysis of acetylthiocholine iodide/butyrylthiocholine chloride was monitored by the formation of the yellow 5-mercapto-2-nitrobenzoate anion resulting from the reaction of DTNB with thiocholines, catalyzed by enzymes, at 412 nm utilizing a 96-well microplate reader (VersaMax Molecular Devices, Sunnyvale, CA, USA). Galanthamine (Sigma, St. Louis, MO, USA), an anticholinesterase alkaloid-type of drug obtained from the bulbs of snowdrop (*Galanthus* sp.), was used as the reference.

Tyrosinase inhibition

Inhibition of tyrosinase (TYR, EC 1.14.18.1; 30 U, mushroom tyrosinase, Sigma) by the samples was determined using a modified dopachrome method with L-3,4-dihydroxyphenylalanine (L-DOPA) as the substrate.¹² The assays were conducted in a 96-well microplate using an ELISA microplate reader (VersaMax Molecular Devices, USA) to measure the absorbance at 475 nm. An aliquot of the extracts dissolved in dimethyl sulfoxide (DMSO) with 80 μL of phosphate buffer (pH 6.8), 40 μL of tyrosinase, and 40 μL of L-DOPA were put into each well. The results were compared with the control (DMSO). *α*-Kojic acid (Sigma, St. Louis, MO, USA) was used as the reference.

Data processing for enzyme inhibition assays

The measurements and calculations were evaluated using Softmax PRO 4.3.2.LS software. The percentage inhibition of AChE/BChE was determined by comparison of the rates of reaction of the test samples relative to the blank sample (ethanol in phosphate buffer, pH 8). The extent of the enzymatic reaction was calculated based on Eq. (1):

$$E = 100 \frac{C - T}{C} \quad (1)$$

where E is the activity of the enzyme. The E value expresses the effect of the test sample or the positive control on the activity of AChE or BChE enzyme, articulated as the percentage of the remaining activity in the presence of test sample or positive control. C is the absorbance of the control solvent (blank) in the presence of enzyme and T is the absorbance of the tested sample (plant extract or positive control in the solvent) in the presence of enzyme.

The data are expressed as the average inhibition \pm standard error mean (SEM) and the results were taken from at least three independent experiments performed in triplicate.

Microtiter assays for antioxidant activity by radical-formation mechanisms

DPPH radical scavenging activity. The stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined by modification of the method of Blois.¹³ The samples (30 μ L) and reference dissolved in ethanol (75 %) were mixed with 2700 μ L of DPPH solution (1.5×10^{-4} mol L⁻¹). The remaining amount of DPPH \cdot was measured at 520 nm using a Unico 4802 UV-visible double beam spectrophotometer (Dayton, NJ, USA). Gallic acid (Sigma, St. Louis, MO, USA) was employed as the reference.

DMPD radical scavenging activity. The assay is based on reduction of the purple-colored radical DMPD $^+$ (*N,N*-dimethyl-*p*-phenylendiamine). According to the method,¹⁴ a reagent comprising of 0.1 mol L⁻¹ DMPD, 0.1 mol L⁻¹ acetate buffer (pH 5.25), and 0.05 mol L⁻¹ ferric chloride solution, which led to formation of the DMPD radical, was freshly prepared and the reagent was equilibrated to an absorbance of 0.900 ± 0.100 at 505 nm. Then, the reagent was mixed with 50 μ L of the extract dilutions, as well as the reference, and the absorbance was taken at 505 nm using a Unico 4802 UV-visible double beam spectrophotometer (Dayton, NJ, USA). Quercetin was employed as the reference and the experiments were performed in triplicate.

Nitric oxide (NO) radical scavenging activity. The scavenging activity of the extracts and reference against NO was assessed by the method of Marcocci *et al.*¹⁵ Briefly, the extract dilutions were mixed with 0.005 mol L⁻¹ sodium nitroprusside and incubated for 2 h at 29 °C. An aliquot of the solution was removed and diluted with Griess reagent (1 % sulfanilamide in 5 % phosphoric acid and 0.1 % *N*-1-naphthylethylenediamine dihydrochloride). The absorbance of the formed chromophore was measured at 550 nm using a Unico 4802 UV-visible double beam spectrophotometer (USA).

Microtiter assays for antioxidant activity by metal-chelating and reducing power mechanisms

Metal-chelating capacity. The metal-chelating capacity of the extracts and reference through ferrous ion was estimated by the method of Chua *et al.*¹⁶ Briefly, dilutions of the extracts were incubated with 0.002 mol L⁻¹ iron(II) chloride solution. The reaction was initiated by the addition of 0.005 mol L⁻¹ ferrozine into the mixture and left standing at ambient temperature for 10 min. The absorbance of the reaction mixture was measured at 562 nm using a Unico 4802 UV-visible double beam spectrophotometer (Dayton, NJ, USA). The ratio of the inhibition of the ferrozine-Fe²⁺ complex formation was calculated. Ethylenediamine-tetraacetic acid (EDTA) was employed as the reference in this assay.

Ferric-reducing antioxidant power assay (FRAP). The FRAP values of the samples and the reference were determined using the assay of Oyaizu.¹⁷ Different concentrations of the extracts were mixed with 2500 μ L of phosphate buffer (pH 6.6) and 2500 μ L of potassium ferricyanide. Later, the mixture was incubated at 50 °C for 20 min and, then, trichloroacetic acid (10 %, 2.500 mL) was added. After the mixture had been shaken vigorously, the solution

was mixed with distilled water (2.500 mL) and ferric chloride (0.1 %, 0.500 mL). After incubating for 30 min, the absorbance was read at 700 nm using a Unico 4802 UV-visible double beam spectrophotometer (Dayton, NJ, USA) and compared to that of chlorogenic acid (Sigma, St. Louis, MO, USA) as the reference.

Phosphomolibdenum-reducing antioxidant power (PRAP) assay

In order to perform PRAP assays on the extracts, each dilution was mixed with 10 % phosphomolybdic acid solution + ethanol (1:1 volume ratio).¹⁸ The solution was subsequently subjected to incubation at 80 °C for 30 min and the absorbance was read at 600 nm using a Unico 4802 UV-visible double beam spectrophotometer (Dayton, NJ, USA). The analyses were run in triplicate and compared to that of trolox as the reference.

Data processing for antioxidant activity assays

The scavenging effect on DPPH, DMPD and nitric oxide radicals, and the metal-chelation capacity of the extracts was calculated using Eq. (2) and the results were expressed as inhibition level (*I* / %):

$$I = 100 \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \quad (2)$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test sample), and A_{sample} is the absorbance of the extracts. The analyses were run in triplicate and the results are expressed as average values \pm SEM.

For the FRAP and PRAP assays, the analyses were also achieved in triplicate and increased absorbance of the reaction meant increased reducing power in both assays.

Statistical analysis of data

Data obtained from *in vitro* enzyme inhibition and antioxidant experiments were expressed as the mean standard error (SEM). Statistical differences between the reference and the sample groups were evaluated by one way ANOVA. Dunnett multiple comparison tests were used as *post hoc* tests. $p < 0.05$ was considered to be significant ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$ and $****p < 0.0001$).

RESULTS

Yield as mass percentages of the extracts are given in Table I. Variations from no to moderate effects were observed with the umbelliferous extracts in the ChE and TYR inhibitory assays performed at 100 $\mu\text{g mL}^{-1}$ (Fig. 1). The inhibitory activity of the extracts varied between 7.26 \pm 1.86 (CS) and 32.52 \pm 3.27 % (HP) against AChE, none to 46.16 \pm 1.42 % (HP) against BChE, and 2.56 \pm 0.96 and 16.73 \pm 2.80 % against TYR.

Although the extracts screened possessed either no or low to modest antioxidant activity at 100 $\mu\text{g mL}^{-1}$, the HP extract exerted the highest scavenging activity toward DPPH (33.99 \pm 2.41 %) and DMPD (10.12 \pm 1.13 %) radicals (Fig. 2).

On the other hand, seven of the extracts showed no radical scavenging activity slightly over 50 % as follows: PAF (54.91 \pm 1.98 %) > LA (54.34 \pm 1.13 %) > AMM (53.88 \pm 0.22 %) > AS (53.48 \pm 0.93 %) > TA (52.88 \pm 0.48 %) > PC (51.09 \pm 0.16 %) > PAN (50.05 \pm 0.49 %), Fig. 2. The FRAP and PRAP values of

the extracts were revealed to change from a low to moderate level as compared to the references, while their metal-chelating capacity was diminutive (Table II).

TABLE I. Yields, total phenol and flavonoid contents of the extracts

Species	Extract yield mass %	Total phenol content ^a ± SEM ^b	Total flavonoid content ^c ± SEM
<i>Apium graveolens</i> (AG)	36.92	15.28±0.99	20.73±0.45
<i>Angelica sylvestris</i> var. <i>sylvestris</i> (ASS)	12.57	43.86±1.33	10.58±0.75
<i>Artemisia squamata</i> (AS)	28.21	106.43±5.30	34.91±0.45
<i>Astrantia maxima</i> subsp. <i>maxima</i> (AMM)	14.98	67.30±3.31	34.06±2.54
<i>Coriandrum sativum</i> (CS)	4.73	10.24±0.17	— ^d
<i>Foeniculum vulgare</i> (FV)	14.17	12.70±0.33	2.22±0.90
<i>Heracleum platytaenium</i> (HP)	16.31	5.55±1.16	—
<i>Ligusticum alatum</i> (LA)	14.67	82.06±11.27	10.79±0.45
<i>Petroselinum crispum</i> (PC)	27.89	22.54±6.63	33.75±0.60
<i>Pimpinella affinis</i> (PAF)	20.84	61.67±9.28	22.43±3.74
<i>Pimpinella anisum</i> (PAN)	11.36	34.61±3.48	13.12±0.15
<i>Smyrniolum olusatrum</i> (SO)	12.03	40.35±5.63	18.19±1.94
<i>Tordylium apulum</i> (TA)	19.57	56.05 ± 1.66	44.11 ± 2.39

^aData expressed in mg equivalent of gallic acid to 1 g of extract; ^bstandard error mean SEM ($n = 3$); ^cdata expressed in mg equivalent of quercetin to 1 g of extract; ^d not able to calculate due to very low absorbance

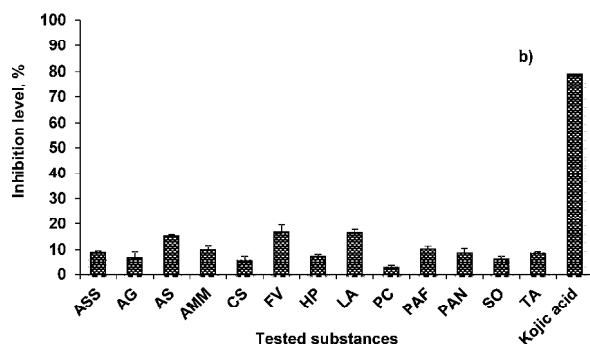
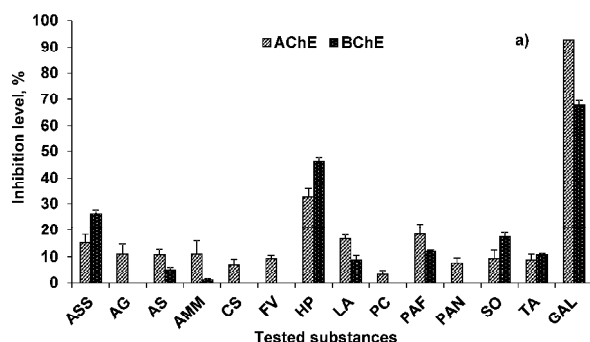


Fig. 1. a) Acetylcholinesterase (AChE) butyrylcholinesterase (BChE) and b) tyrosinase (TYR) inhibitory activity (inhibition level ± SEM) of the Apiaceae extracts and references (GAL: galanthamine) and kojic acid at 100 $\mu\text{g mL}^{-1}$.

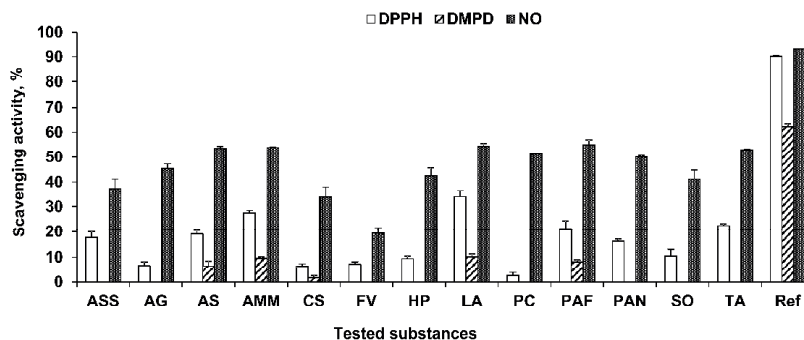


Fig. 2. DPPH (2,2-diphenyl-1-picrylhydrazyl), DMPD (*N,N*-dimethyl-*p*-phenylendiamine), and NO (nitric oxide) radical scavenging activity (scavenging activity \pm SEM) of the Apiaceae extracts and references (quercetin for DPPH and DMPD radicals and gallic acid for NO radical) at 100 μ g mL⁻¹.

TABLE II. Ferric- (FRAP) and phosphomolibdenum-reducing antioxidant power (PRAP) activities and metal-chelating capacity of the extracts at 1000 μ g mL⁻¹; $p < 0.0001$

Extract	FRAP (absorbance \pm SEM ^a at 700 nm ^b)	PRAP (absorbance \pm SEM at 600 nm ^b)	Metal-chelating capacity \pm SEM, %
AG	0.231 \pm 0.007	0.202 \pm 0.003	13.39 \pm 2.37
AS	0.332 \pm 0.005	0.191 \pm 0.003	5.70 \pm 0.99
ASS	0.314 \pm 0.004	0.203 \pm 0.005	9.63 \pm 1.61
AMM	0.445 \pm 0.006	0.188 \pm 0.012	6.33 \pm 2.33
CS	0.195 \pm 0.002	0.188 \pm 0.001	18.94 \pm 1.51
FV	0.241 \pm 0.003	0.211 \pm 0.027	12.33 \pm 1.61
HP	0.219 \pm 0.001	0.180 \pm 0.033	8.38 \pm 1.09
LA	0.541 \pm 0.003	0.193 \pm 0.006	4.64 \pm 0.42
PC	0.175 \pm 0.001	0.246 \pm 0.017	23.92 \pm 1.92
PAF	0.399 \pm 0.012	0.200 \pm 0.001	14.23 \pm 0.65
PAN	0.319 \pm 0.006	0.172 \pm 0.003	7.38 \pm 0.04
SO	0.242 \pm 0.014	0.222 \pm 0.002	25.49 \pm 1.12
TA	0.343 \pm 0.006	0.216 \pm 0.012	15.96 \pm 3.01
Quercetin ^c	1.491 \pm 0.041		
Trolox ^d		1.871 \pm 0.012	
EDTA ^e			75.08 \pm 1.16

^aStandard error mean ($n = 3$); ^bhigher absorbance indicated the greater antioxidant activity; ^creference for FRAP assay; ^dreference for PRAP assay; ^eethylenediaminetetraacetic acid, reference for metal-chelating capacity assay

Spectrophotometric determination of total phenol and flavonoid amounts in the umbelliferous extracts indicated that the richest extract in terms of total phenol was LA (82.06 \pm 11.27 mg g⁻¹ extract), while the PC extract had the highest amount of total flavonoids (33.75 \pm 0.60 mg g⁻¹ extract, Table I).

Since the most active extract against the ChE enzymes belonged to HP, this extract was subjected to HPLC-DAD analysis and the following coumarins were quantified: xanthotoxin (2.97 \pm 0.019 mg 100 g⁻¹), angelicin (1.74 \pm 0.033 mg 100

g^{-1}), isopimpinellin ($0.31 \pm 0.003 \text{ mg } 100 \text{ g}^{-1}$), bergapten ($2.51 \pm 0.045 \text{ mg } 100 \text{ g}^{-1}$), and pimpinellin ($7.73 \pm 0.159 \text{ mg } 100 \text{ g}^{-1}$). In the HP extract, the simple coumarin, osthol, was also identified, but its quantitative analysis was not realized because the concentration of osthol was out of the range. In the ASS extract that had the second highest BChE inhibition after HP, angelicin ($0.31 \pm 0.015 \text{ mg } 100 \text{ g}^{-1}$) and imperatorin ($2.36 \pm 0.033 \text{ mg } 100 \text{ g}^{-1}$) were determined using HPLC-DAD in the same manner (the chromatogram are presented in Fig. S-1 of the Supplementary material).

DISCUSSION

A number of coumarin derivatives along with coumarin-rich plants were reported to possess notable inhibitory potential against AChE and BChE, which prompted the realization of the current study. For instance, a significant anti-AChE activity was determined for the root methanol extract of *Angelica gigas* (Umbelliferae), which led to the isolation of twelve coumarin derivatives and, among them, decursin was identified as the most promising one due to its marked AChE inhibitory as well as its *in vivo* memory-enhancing effect.¹⁹ Later, two other coumarin derivatives (nodakenin and decursinol) isolated from *A. gigas* were also revealed to have AChE inhibitory activity.²⁰ Additionally, the dichloromethane, ethanol, and aqueous extracts of *A. graveolens* were previously demonstrated to have low inhibition toward AChE and BChE at $200 \mu\text{g mL}^{-1}$, in accordance with the present data on this species.

A limitation of this study was that the ChE or TYR inhibitory effects of the coumarin standards identified in the HP and ASS extracts could not be confirmed due to scarcity in their amounts. Nevertheless, in an earlier study,⁵ three coumarin compounds, imperatorin ($83.98 \pm 0.99 \%$), xanthotoxin ($88.04 \pm 0.83 \%$) and bergapten ($86.69 \pm 2.56 \%$), identified in *Angelica officinalis*, were shown to have strong BChE inhibition, which was also supported by molecular docking experiments. Since presence of xanthotoxin and bergapten in HP and imperatorin in ASS was confirmed by the HPLC analysis, these coumarins could be considered most likely to contribute to the moderate ChE inhibitory effect exhibited by HP as well as ASS. Furthermore, isopimpinellin, earlier obtained from *Angelica acutiloba* with AChE inhibitory effect, might be suggested to contribute to some extent to the relevant activity of HP.²¹ Previously, the methanol and petroleum ether extracts of HP were reported to exert $49.86 \pm 1.56 \%$ and $49.28 \pm 1.28 \%$ against AChE and $65.51 \pm 1.63 \%$ and $56.59 \pm 1.62 \%$ against BChE, respectively, at $200 \mu\text{g mL}^{-1}$. The extract yielded 8 furocoumarins, elucidated as psoralen, bergapten, xanthotoxin, pimpinellin, isopimpinellin, sphondin, byakangelicin, and heracle-nol.²² In the same study,²² pimpinellin was determined to cause $78.57 \pm 2.86 \%$ inhibitions of AChE and $82.17 \pm 1.66 \%$ BChE inhibitions. As it was quantified as the major coumarin in the HP extract in the present study, pimpinellin might be

speculated to be the major contributor to the ChE inhibitory effect of this plant. Additionally, the remarkable anticholinesterase effect of xanthotoxin and bergapten was also confirmed in the study by Dincel *et al.*,²² that supported former data on the same compounds.⁵ Moreover, the weak DPPH radical scavenging activity of HP was found in the aforementioned study, which is again consistent with the present data. CS, used for memory-enhancing purpose in Iranian folk medicine, was previously deduced to have a very low AChE inhibitory and DPPH scavenging effect, which supports the present finding on CS.²³ The root ethanol extract of FV was formerly found to display neither AChE nor BChE inhibitory effects pertinent to the current results on the fruit methanol extract of FV.²⁴ Consistent with the present data, the methanol extracts of both PC (root) and PAN (fruit) used for memory impairment in Danish folk medicine were ineffective in the AChE inhibition assay.²⁵ On the other hand, there has hitherto been no report on the ChE inhibitory effect of SO and TA.

The TYR inhibitory activity of umbelliferous plants was researched, which again led to the isolation of some coumarins as the active constituents. For instance; 9-hydroxy-4-methoxypsoralen from *Angelica dahurica*, aloesin from *Aloe vera*, esculetin from *Euphorbia lathyris*, and 8'-*epi*-cleomiscosin A from *Rhododendron collettianum* showed potent TYR-inhibiting effects.²⁶ Among the plant species tested herein, CS was reported to possess 47.76±2.50 and 49.2 % of inhibition of TYR in two early studies, while FV showed only 29.6 % TYR inhibition.^{27,28} Adhikari *et al.* also described TYR inhibitory effects 41.7±2.2 % for CS and 22.4±6.0 % for FV, and 45.8±16.9 % for PC at 50 µg mL⁻¹,²⁹ in contrary to the present data on these species, which might be the result of phytochemical differences.

CONCLUSION

In summary, the methanol extracts of thirteen umbelliferous plant species were screened for their ChE and TYR inhibitory effects and their antioxidant activity. The findings obtained in this study revealed that HP shows some notable inhibition against AChE and BChE and the coumarins found in this plant seem to be the active substances. To the best of our knowledge, this study is the first on ChE and TYR inhibition and the antioxidant activities of *Angelica sylvestris* var. *sylvestris* (ASS), *Artemisia squamata* (AS), *Astrantia maxima* subsp. *maxima* (AMM), *Ligusticum alatum* (LA), *Smyrniololus atrum* (SO) and *Tordylium apulum* (TA).

SUPPLEMENTARY MATERIAL

Collection sites and herbarium numbers of the plant species as well as HPLC chromatograms of the HP extract and the ASS extract are available electronically from <http://www.shd.org.rs/JSCS/>, or from the corresponding author on request.

ИЗВОД

АНТИХОЛИНЕСТЕРАЗНИ, АНТИТИРОЗИНАЗНИ И АНТИОКСИДАТИВНИ ПОТЕНЦИЈАЛ БИЉАКА ФАМИЛИЈЕ UMBELLIFERAE И ХРОМАТОГРАФСКИ ПРОФИЛ САСТОЈАКА БИЉАКА *Heracleum platytaenium* Boiss. И *Angelica sylvestris* L. var. *sylvestris*ILKAY EDROGAN ORHAN¹, FATMA TOSUN¹ и KRYSTYNA SKALICKA-WOŹNIAK²¹Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey и ²Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, Lublin, Poland

Неуробиолошка активност метанолних екстраката тринаест биљака фамилије Umbelliferae (Апицеае) је тестирана спрам ацетилхолинестеразе (АChЕ), бутирилхолинестеразе (ВChЕ) и тирозиназе (TYR). Екстракти нису испољили или су имали слаб инхибиторни ефекат на ензиме, а највећу антихолинестеразну активност је имао екстракт биљке *Heracleum platytaenium* (32,52±3,27 % спрам АChЕ и 46,16±1,42 % спрам ВChЕ) у концентрацији 100 µg mL⁻¹. Пошто су неуродегенеративне промене повезане са оксидативним оштећењима, антиоксидативни потенцијал екстраката је утврђиван у тесту хватања слободних радикала, одређивањем хелатног капацитета и мерењем редукујућег потенцијала. Активност екстраката није била велика, а највећа се показала у хватању слободних радикала азот-оксида (од 19,47±2,09 до 54,91±1,98 %). Ове биљне врсте садрже доста кумарина, а квантитативном HPLC анализом је утврђено присуство ксантотоксина, ангелицина, изопимпинелина, бергаптена и пимпинелина у *Heracleum platytaenium*, као и ангелицина и императорина у *Angelica sylvestris* var. *sylvestris*.

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