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

ILKAY EDROGAN ORHAN[1], FATMA TOSUN[1], KRYSTYNA SKALICKA-WOŹNIAK[2,*]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey and [2]Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, 1 Chodzki Str, 20-093 Lublin, Poland (kskalicka@pharmacognosy.org)

Abstract: Neurobiological activity of the methanol extracts of thirteen Umbelliferae (Apiaceae) plants was tested against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and tyrosinase (TYR) using high-throughput screening technique. Although the extracts displayed none to low profile of inhibition against 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, antioxidant potential of the extracts was searched through radical scavenging, metal-chelating capacity, and reducing power experiments and exerted modest levels of activity varying according to the method. The extracts had better ability to scavenge nitric oxide radical (19.47 ± 2.09 % to 54.91 ± 1.98 %). Since these species are known to be rich in coumarins, our quantitative high-performance liquid chroatography (HPLC) analysis indicated 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

RUNNING TITLE: NEUROBIOLOGICAL ACTIVITY OF APIACEAE PLANTS

INTRODUCTION

Alzheimer's disease (AD), the most common form of dementia, is a neurodegenerative disorder with a progressive nature affecting the elderly population particularly over the age of 60. Cholinesterase (ChE) enzyme family, consisting of two sister enzymes as acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8), catalyzes hydrolysis of acetylcholine (ACh), which has been proved to be in lower amount in the brains of AD patients than usual. Consequently, ChE inhibitors have been most frequently prescribed drug class for the modern treatment of AD such as tacrine, rivastigmine, donepezil, and galanthamine.[1] After AD, Parkinson's disease (PD) is another common neurodegenerative disorder worldwide with clear motor symptoms instigated by degeneration of nigrostriatal dopaminergic neurons often accompanied with cognitive conditions. It has been 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 amount and, therefore, inhibition of TYR may also help to 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 to mention that age-associated disorders with neurodegenerative character such as AD and PD are usually linked to oxidative damage and, thus, neuroprotective effect is correlated with prevention of oxidative stress involved in the over production of reactive oxygen species along with metal dysregulation.[4] Based on all the relevant data reported up to date, it has become rational to imply a multi-target approach for the treatment of AD and PD.

Regarding our ongoing research on finding new inhibitors of ChE and TYR from herbal sources since the year of 2000, we have found some promising results with coumarin-rich plants from Umbelliferae (Apiaceae) such as *Angelica officinalis* against ChEs,[5] and taking this findings into account, we have now aimed to investigate neurobiological effect of the methanol extracts prepared from thirteen randomly selected umbelliferous edible plants including *Apium graveolens* L. (AG), *Angelica sylvestris* L. var. *sylvestris* (ASS), *Artedia squamata* 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), *Smyrnium olusatrum* L. (SO), and *Tordylium apulum* L. (TA)

through their ChE and TYR inhibitory activity using ELISA (enzyme-linked immunosorbent assay) microtiter assays. Relevantly, antioxidant potential of the extracts was evaluated using six in vitro high-throughput assays based on radical scavenging, metal-chelating, and reducing power mechanisms. Coumarin analysis was carried out on the extracts of HP and ASS using an high-performance liquid chromatography (HPLC) technique.

EXPERIMENTAL

Plant materials

The samples of the umbelliferous plants studied were collected throughout Turkey. The plants were identified by Prof. Dr. Mecit Vural from Department of Biology, Faculty of Arts and Sciences, Gazi University (Ankara, Turkey) and the voucher specimens are kept in the Herbarium of Faculty of Pharmacy, Gazi University (Ankara, Turkey). The collection sites and herbarium numbers of the plants are listed in Table I.

Table I. Collection sites and herbarium numbers of the plant species.

Plant species	Collection site	Herbarium numbers
Apium graveolens (AG)	Izmir-Kusadasi	GUE 2092
Angelica sylvestris var. *sylvestris* (ASS)	Giresun-Sebinkarahisar	GUE 1972
Artedia squamata (AS)	Karabuk-Safranbolu	GUE 2015
Astrantia maxima subsp. *maxima* (AMM)	Trabzon-Zigana Pass	GUE 1990
Coriandrum sativum (CS)	Ankara-Beypazari	GUE 1896
Foeniculum vulgare (FS)	Zonguldak-Kozlu	GUE 1894
Heracleum platytaenium (HP)	Trabzon-Hamsikoy	GUE 1933
Ligusticum alatum (LA)	Giresun-Sebinkarahisar	GUE 1968
Petroselinum crispum (PC)	Ankara-Golbasi	GUE 1912
Pimpinella affinis (PAF)	Trabzon-Altindere	GUE 1966
Pimpinella anisum (PAN)	Izmir-Cesme	GUE 1895
Smyrnium olusatrum (SO)	Istanbul-Baltalimani	GUE 1886
Tordylium apulum (TA)	Istanbul-Sariyer	GUE 1884

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 until dryness. The extracts were kept in freezer until the experiments were performed. Yield percentages (w/w) of the extracts are given in Table II.

Table II. Yields, total phenol and flavonoid contents of the extracts.

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

[a] Data expressed in mg equivalent of gallic acid to 1 g of extract

[b] Standard error mean S. E. M. (n=3)

[c] Data expressed in mg equivalent of quercetin to 1 g of extract

[d] Not able to calculate due to very low absorbance

Phytochemical content of the extracts

Determination of total phenol and flavonoid contents in the extracts

Total phenol content of the extracts was determined in accordance with Folin-Ciocalteau's reagent (Sigma, St. Louis, MO, USA).[6] In brief, a number of dilutions of gallic acid (50-500 μg 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-Ciocalteau's reagent and 600 μL of sodium carbonate in test tubes. The tubes were then vortexed and incubated at 40°C for 30 min. Afterwards, absorption was measured at 760 nm at a Unico 4802 UV-visible double beam spectrophotometer (Dayton, NJ, USA). Total flavonoid content of the extracts was established by aluminum chloride colorimetric method.[7] To sum up, a number of dilutions of quercetin (50-500 μg 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 as well as distilled water. Following incubation for 30 min at room temperature,

absorbance of the reaction mixtures was measured at wavelength of 415 nm with a Unico 4802 UV-visible double beam spectrophotometer (Dayton, NJ, USA). The total phenol and flavonoid contents of the extracts were 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 Shimadzu system (Shimadzu, Japan) equipped with an automatic degasser (DGU-20A 3R), a quaternary pump (LC-20AD), an autosampler (SIL-20A HT) and diod-array detector (DAD) (SPD-M20A). Chromatographic separation was carried out on a Zorbax Eclipse XDB C18 (Agilent) (250 mm × 4.6 mm, 5 μm) at 20 ºC. The flow rate of mobile phase was maintained at 1 mL/min and the injection volume was 10 μL. The LC pumps, autosampler, column oven, and DAD were monitored and controlled by use of LabSolutions 5.51 software (Shimadzu). The gradient of methanol (A) and water (B) was used as follows: 0 min – 20 % A in B, 10 min – 50 % A in B, 15 min – 60 % A in B, 40 min – 60 % A in B, 42 min – 100 % A, 44 min – 100 % A (cleaning of the column), 48-60 – 20 % A in B (stabilization of the column).

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, isoimperatorin (ChromaDex, USA), xanthotoxin, bergapten, imperatorin (Sigma Aldrich), byacangelicol, heraclenin, byakangelicin, and phellopterin (PhytoLab, GmbH & Co. KG, Germany). Quantitative determination was performed at 254 and 320 nm. Quantitative analysis of pimpinellin was done when pimpinellin was calculated to angelicin (the lack of standard of pimpinellin). In order to confirmed identification of coumarin derivatives a HPLC-coupled with and 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 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′-Dithio-bis(2-nitrobenzoic)acid (DTNB; Sigma, St. Louis, MO, USA) was used for the measurement of the anticholinesterase activity. All reagents and conditions were same as described in our 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 by 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-thio-2-nitrobenzoate anion as a result of 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), the 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 1.14.1.8.1; 30 U, mushroom tyrosinase, Sigma) by the samples was determined using the modified dopachrome method with L-3,4-dihydroxyphenylalanine (L-DOPA) as substrate.[12] The assays were conducted in 96-well microplate using ELISA microplate reader (VersaMax Molecular Devices, USA) to measure 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 in each well. Results were compared with control (DMSO). Baicalein (Sigma, St. Louis, MO, USA) was used as the reference.

Data processing for enzyme inhibition assays

The measurements and calculations were evaluated by using Softmax PRO 4.3.2.LS software. Percentage of inhibition of AChE/BChE was determined by comparison of rates of reaction of test samples relative to blank sample (ethanol in phosphate buffer pH=8). Extent of

the enzymatic reaction was calculated based on the Eq. (1).

$$E = (C - T)/C \times 100 \quad (1)$$

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

Data are expressed as average inhibition ± standard error mean (S.E.M.) 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 µL) and reference dissolved in ethanol (75 %) were mixed with 2700 µL of DPPH solution (1.5×10^{-4} mol L^{-1}). Remaining DPPH amount 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 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 up with 50 µL of the extract dilutions and 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 done in triplicate.

Nitric oxide (NO) radical scavenging activity

The scavenging activity of the extracts 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 left to incubation 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 %

naphthylethylenediamine dihydrochloride). The absorbance of the occurred 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 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 inhibition of ferrozine-Fe^{2+} complex formation was calculated.

Ferric-reducing antioxidant power assay (FRAP)

FRAP of the samples was tested using the assay of Oyaizu.[17] Different concentrations of the extracts were mixed with 2500 µL of phosphate buffer (pH 6.6) and 2500 µL of potassium ferricyanide. Later, the mixture was incubated at 50°C for 20 min and, then, trichloroacetic acid (10 %) was added. After the mixture was shaken vigorously, this solution was mixed with distilled water and ferric chloride (0.1 %). After 30 min of incubation, 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 reference.

Phosphomolibdenum-reducing antioxidant power (PRAP) assay

In order to perform PRAP assays on the extracts, each dilution was mixed 10 % phosphomolybdic acid solution in ethanol (w/v).[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). Analyses were run in triplicate and compared to that of quercetin as the reference.

Data processing for antioxidant activity assays

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

$$I\% = [A_{blank} - A_{sample}/A_{blank}] \times 100$$

(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. Analyses were run in triplicate and the results were expressed as average values with S.E.M. (Standard error of the mean).

For 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 ANOVA (one way). Dunnett's multiple comparison tests were used as post hoc tests. $p < 0.05$ was considered to be significant [*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$].

RESULTS

As displayed in Fig. 1, variations from none to moderate effect were observed with the umbelliferous extracts in ChE and TYR inhibitory assays performed at 100 µg mL^{-1}. Inhibitory activity of the extracts varied between 7.26 ± 1.86 (CS) and 32.52 ± 3.27 % (HP) against AChE, none to 46.16 ± 1.42 % (HP) against BChE, and 2.56 ±0.96 and 16.73 ± 2.80 % against TYR.

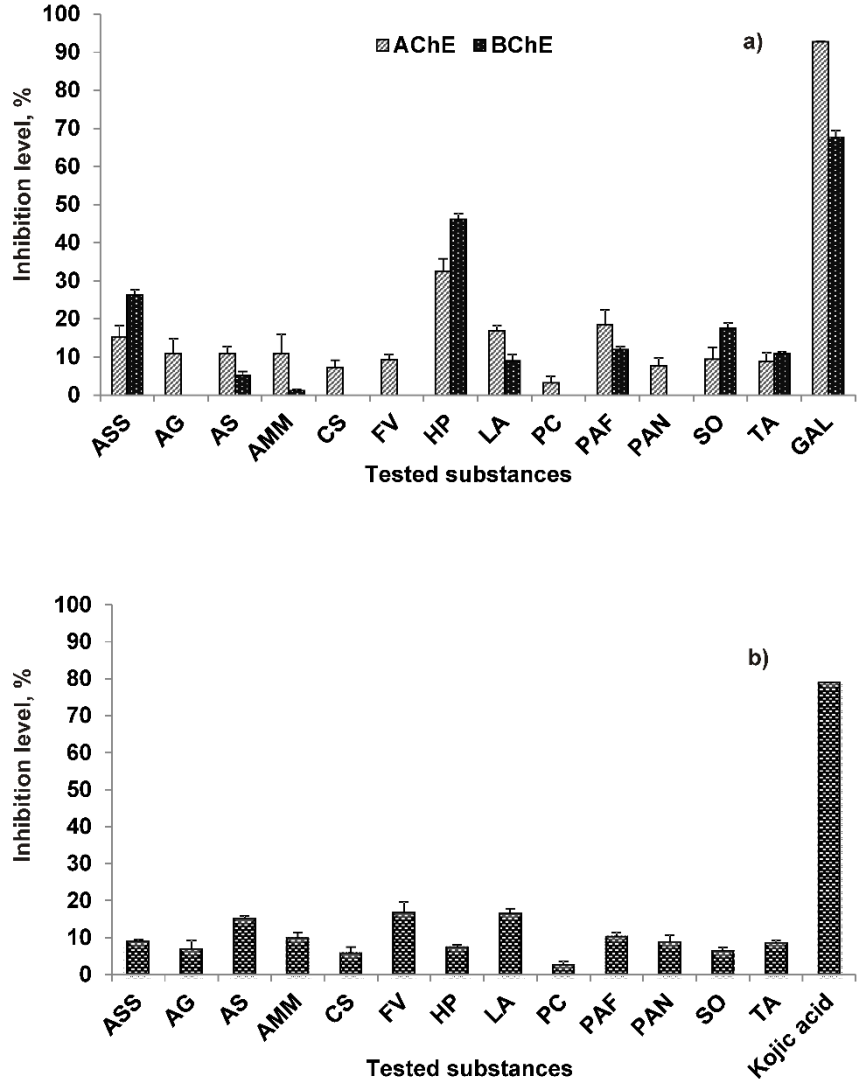

Fig. 1. (a) Acetylcholinesterase (AchE) and (butyrylcholinesterase) BChE (b) tyrosinase (TYR) inhibitory activity (% ± S.E.M.) of the Apiaceae extracts and references (GAL: Galanthamine) and kojic acid at 100 µg mL^{-1}

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

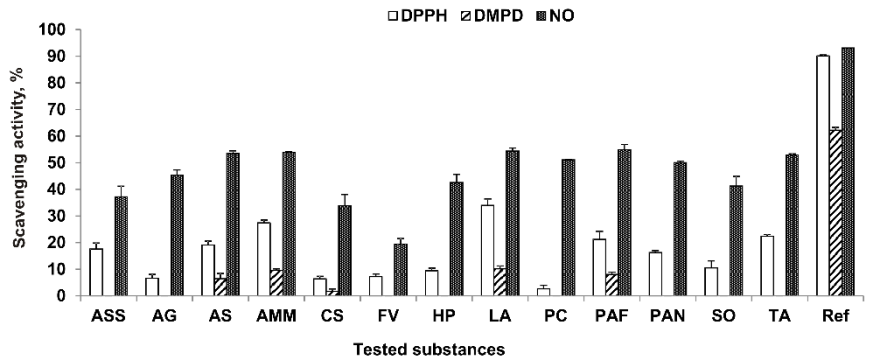

Fig. 2. DPPH (2,2-diphenyl-1-picrylhydrazyl), DMPD (N,N-dimethyl-*p*-phenylendiamine), and NO (nitric oxide) radical scavenging activity (% ± S.E.M.) of the Apiaceae extracts and references (quercetin for DPPH and DMPD radicals and gallic acid for NO radical) at 100 μg mL^{-1}

On the other hand, seven of the extracts showed NO radical scavenging activity slightly over 50 % as follows; PAF (54.91 ± 1.98 %) > LA (54.34 ± 1.13 %) > AMM (53.88 ± 0.22 %) > AS (53.48 ± 0.93 %) > TA (52.88 ± 0.48 %) > PC (51.09 ± 0.16 %) > PAN (50.05 ± 0.49 %) (Fig. 2). FRAP and PRAP values of the extracts were revealed to change from low to moderate level as compared to the references, while their metal-chelating capacity was diminutive (Table III).

Table III. Ferric- (FRAP) and phosphomolibdenum-reducing antioxidant power (PRAP) activities and metal-chelating capacity of the extracts at 1000 μg mL^{-1}

	FRAP (Absorbance at 700 nm[a] ± ± S.E.M.[b])	PRAP (Absorbance at 600 nm[a] ± S.E.M.)	Metal-chelating capacity (%±S.E.M.)
AG	0.231 ± 0.007****	0.202 ± 0.003****	13.39 ± 2.37****
AS	0.332 ± 0.005****	0.191 ± 0.003****	5.70 ± 0.99****
ASS	0.314 ± 0.004****	0.203 ± 0.005****	9.63 ± 1.61****
AMM	0.445 ± 0.006****	0.188 ± 0.012****	6.33 ± 2.33****
CS	0.195 ± 0.002****	0.188 ± 0.001****	18.94 ± 1.51****
FV	0.241 ± 0.003****	0.211 ± 0.027****	12.33 ± 1.61****
HP	0.219 ± 0.001****	0.180 ± 0.033****	8.38 ± 1.09****
LA	0.541 ± 0.003****	0.193 ± 0.006****	4.64 ± 0.42****
PC	0.175 ± 0.001****	0.246 ± 0.017****	23.92 ± 1.92****
PAF	0.399 ± 0.012****	0.200 ± 0.001****	14.23 ± 0.65****
PAN	0.319 ± 0.006****	0.172 ± 0.003****	7.38 ± 0.04****
SO	0.242 ± 014****	0.222 ± 0.002****	25.49 ± 1.12****
TA	0.343 ± 0.006****	0.216 ± 0.012****	15.96 ± 3.01****

Quercetin[c]	1.491 ± 0.041		
Trolox[d]		1.871 ± 0.012	
EDTA[e]			75.08 ± 1.16

[a] Higher absorbance indicated the greater antioxidant activity.

[b] Standard error mean (n=3)

[c] Reference for FRAP assay

[d] Reference for PRAP assay

[e] EDTA (ethylenediaminetetraacetic acid) - reference for metal-chelating capacity assay

[$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$, $^{****}p < 0.0001$]

Spectrophotometric determination of total phenol and flavonoid amounts in the umbelliferous extracts indicated that the richest extract in terms of total phenol belonged to LA (82.06 ± 11.27 mg g^{-1} extract), while the PC extract had the highest amount of total flavonoids (33.75 ± 0.60 mg g^{-1} extract) (Table II).

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 ± 0.019 mg 100 g^{-1}), angelicin (1.74 ± 0.033 mg 100 g^{-1}), isopimpinellin (0.31 ± 0.003 mg 100 g^{-1}), bergapten (2.51 ± 0.045 mg 100 g^{-1}), and pimpinellin (7.73 ± 0.159 mg 100 g^{-1}) (Fig. 3). In HP extract also simple coumarin, osthol, was identified, however, its quantitative analysis was not done because concentration of osthol was out of the range. In the ASS extract that had the second highest BChE inhibition after HP, angelicin (0.31 ± 0.015 mg 100 g^{-1}) and imperatorin (2.36 ± 0.033 mg 100 g^{-1}) were amounted using HPLC-DAD in the same manner (Fig. 3).

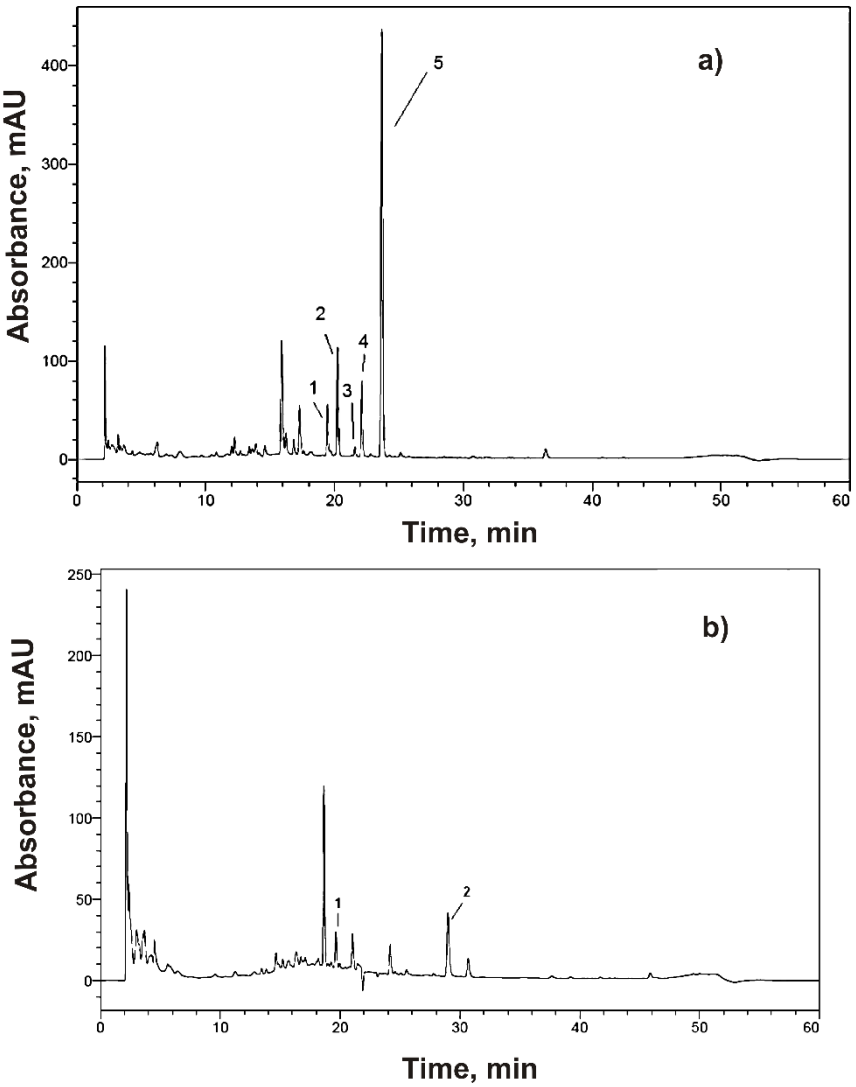

Fig. 3. HPLC chromatograms of a) the HP extract (*Heracleum platytaenium* Boiss.), and b) the ASS extract (*Angelica sylvestris* L. var. *sylvestris)*

DISCUSSION

A number of coumarin derivatives along with coumarin-rich plants have been reported to own notable inhibitory potential against AChE and BChE, which prompted us to perform the current study. For instance; a significant anti-AChE activity was determined with 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 μg mL^{-1}, in accordance with our data on this species.

A limitation to our study was that we could not confirm the ChE or TYR inhibitory effects of the coumarin standards identified in HP and ASS extracts due to scarcity in their amounts. Nevertheless, in our earlier study, three coumarin compounds, imperatorin (83.98 ± 0.99 %), xanthotoxin (88.04 ± 0.83 %), and bergapten (86.69 ± 2.56 %) identified in *Angelica officinalis*, were shown to have strong BChE inhibition, which was also supported by molecular docking experiments.[5] Since presence of xanthotoxin and bergapten in HP and imperatorin in ASS was found by our HPLC analysis, these coumarins can be considered most likely to donate to moderate ChE inhibitory effect of HP as well as ASS. Besides, isopimpinellin, earlier obtained from Angelica acutiloba with AChE inhibitory effect, might be suggested to contribute to relevant activity of HP to some extent.[21] Previously, the methanol and petroleum ether extracts of HP were reported to exert 49.86 ± 1.56 % and 49.28 ± 1.28 % against AChE and 65.51 ± 1.63 % and 56.59 ± 1.62 % against BChE, respectively at 200 μg mL^{-1}, respectively, which yielded 8 furocoumarins elucidated as psoralen, bergapten, xanthotoxin, pimpinellin, isopimpinellin, sphondin, byakangelicin, and heraclenol.[22] In the same study, pimpinellin was determined to cause 78.57 ± 2.86 % of AChE inhibition and 82.17 ± 1.66 of BChE inhibition. As it was quantified as the major coumarin in the HP extract in our present study, pimpinellin seems to be the major contributor to ChE inhibitory effect of this plant. Additionally, the remarkable anticholinesterase effect of xanthotoxin and bergapten were also confirmed in that study by Dincel et al.,[22] which supported our former data on the same compounds.[5] Moreover, the weak DPPH radical scavenging activity of HP was stated in the aforementioned study, which is again consistent with our data. CS, used for memory-enhancing purpose in Iranian folk medicine, was previously deduced to have a very low AChE inhibitory along with DPPH scavenging effect, which supports our present finding on CS.[23] The root ethanol extract of FV was formerly found to display neither AChE nor BChE inhibitory effect pertinent to our current results on the fruit methanol extract of FV.[24] Consistent with our data, the methanol extracts of both PC (root) and PAN (fructus) used for memory impairment in Danish folk medicine were ineffective in AChE inhibition assay.[25] On the other hand, there has been no report on ChE inhibitory effect of SO and TA up to date.

TYR inhibitory activity of umbelliferous plants has been searched, which again led to 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'-epicleomiscosin from *Rhododendron collettianum* showed a potent TYR-inhibiting effect.[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 had only 29.6 % of 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 our data on these species, which might be resulted from phytochemical differences.

CONCLUSION

Taken together, the methanol extracts of thirteen umbelliferous plant species have been screened for their ChE and TYR inhibitory along with 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 seems to be the active substances. As far as we know, this study is the first on ChE and TYR inhibition and antioxidant activities of *Angelica sylvestris* var. *sylvestris* (ASS), *Artedia squamata* (AS), *Astrantia maxima* subsp. *maxima* (AMM), *Ligusticum alatum* (LA), *Smyrnium olusatrum* (SO), and *Tordylium apulum* (TA).

REFERENCES

1. 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, *CNS Neurol. Disord.: Drug Targets* **13** (2014) 391
2. D. Devos, C. Moreau, K. Dujardin, I. Cabantchik, L. Defebvre, R. Bordet, *Clin. Ther.* **35** (2013) 1640
3. T. Hasegawa, A. Treis, N. Patenge, F. C. Fiesel, W. Springer, P. J. Kahle, *J. Neurochem.* **105** (2008) 1700
4. V. Kumar, K. D. Gill, *Neurotoxicology* **41** (2014) 154
5. F. S. Senol, K. Skalicka-Woźniak, M. T. H. Khan, I. E. Orhan, B. Sener, K. Głowniak, *Phytochem. Lett*. **4** (2011) 462
6. V. L. Singleton, J. A. Rossi, *Am. J. Enol. Viticult*. **16** (1965) 144
7. R. Woisky, A. Salatino, *J. Apicol. Res*. **37** (1998) 99
8. K. Skalicka-Woźniak, T. Mroczek, E. Kozioł, J. *Sep. Sci.* **38** (2015) 179
9. M. Liu, X. Shi, W. Yang, S. Liu, N. Wang, R. Shi, S. Qiao, Q. Wang, Y. Wang, *Biomed. Chromatogr*. **25** (2011) 783
10. G. L. Ellman, K. D. Courtney, V. Andres, R. M. Featherstone, *Biochem. Pharmacol*. **7** (1961) 88
11. I. E. Orhan, F. S. Senol, A. R. Gulpinar, M. Kartal, N. Sekeroglu, M. Deveci, Y. Kan, B. Sener, *Food. Chem. Toxicol*. **47** (2009) 1304

12. T. Masuda, D. Yamashita, Y. Takeda, S. Yonemori, *Biosci. Biotechnol. Biochem.* **69** (2005) 197
13. M. S. Blois, *Nature* **181** (1958) 1199
14. K. Schlesier, M. Harvat, V. Bohm, R. Bitsch, R. *Free. Radic. Res*. **36** (2002) 177
15. I. Marcocci, J. J. Marguire, M. T. Droy-Lefaiz, L. Packer, *Biochem. Biophys. Res. Commun.* **201** (1994) 748
16. M. T. Chua, Y. T. Tung, S. T. Chang, *Bioresour. Technol*. **99** (2008) 1918
17. M. Oyaizu, *Jap. J. Nutr*. **44** (1986) 307
18. G. Falcioni, D. Fedeli, L. Tiano, I. Calzuola, L. Mancinelli, V. Marsili, *J. Food. Sci.* **67** (2002) 2918
19. S. Y. Kang, K. Y. Lee, M. J. Park, Y. C. Kim, G. T. Markelonis, T. H. Oh, Y. C. Kim, *Neurobiol. Learn Mem*. **79** (2003) 11
20. D. H. Kim, D. Y. Kim, Y. C. Kim, J. W. Jung, S. Lee, B. H. Yoon, J. H. Cheong, Y. S. Kim, S. S. Kang K. H. Ko, J. H. Ryu, *Life Sci*. **80** (2007) 1944
21. M. Miyazawa, T. Tsukamoto, J. Anzai, Y. Ishikawa, *J. Agric. Food Chem.* **52** (2004) 4401
22. D. Dincel, S. D. Hatipoglu, A. C. Goren, G. Topcu, *Turk. J. Chem*. **37** (2013) 675
23. H. R. Adhami, H. Farsam, L. Krenn, *Phytother. Res*. **25** (2011) 1148
24. S. Khattak, Saeed-Ur-Rehman; H. U. Shah, T. Khan, M. Ahmad, *Nat. Prod. Res.* 19 (2005) 567
25. A. Adsersen, B. Gauguin, L. Gudiksen, A. K. Jäger, *J. Ethnopharmacol*. **104** (2006) 418
26. T. S. Chang, *Int. J. Mol. Sci*. **10** (2009) 2440
27. F. Khanom, H. Kayahara, K. Tadasa, *Biosci. Biotechnol. Biochem*. **64** (2000) 1967
28. P. K. Mukherjee, S. Badami, A. M. Wahile, S. Rajan, B. Suresh, *J. Nat. Rem.* **½** (2001) 125
29. A. Adhikari, H. P. Devkota, A. Takano, K. Masuda, T. Nakane, P. Basnet, N. Skalko-Basnet, *Int. J. Cosmetic. Sci.* **30** (2008) 353.