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Bioactivity-Guided Fractionation for the Butyrylcholinesterase Inhibitory Activity of Furanocoumarins from *Angelica archangelica* L. Roots and Fruits

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ABSTRACT: Isolation and identification of the inhibitors of butyrylcholinesterase (BChE), obtained from the extracts of roots and fruits of *Angelica archangelica* L., are reported. Our results confirmed the weak inhibitory effect of *Angelica* roots on acetylcholinesterase activity. BChE inhibition was much more pronounced at a concentration of 100 μg/mL for hexane extracts and attained a higher rate than 50%. The TLC bioautography guided fractionation and spectroscopic analysis led to the isolation and identification of imperatorin from the fruit's hexane extract and of heraclenol-2′-*O*-angelate from the root's hexane extract. Both compounds showed significant BChE inhibition activity with $IC_{50} = 14.4 \pm 3.2\ \mu M$ and $IC_{50} = 7.5 \pm 1.8\ \mu M$, respectively. Only C8-substituted and C5-unsubstituted furanocoumarins were active, which could supply information about the initial structures of specific BChE inhibitors.

KEYWORDS: *Angelica archangelica* L., furanocoumarins, imperatorin, heraclenol-2′-*O*-angelate, cholinesterase inhibition, butyrylcholinesterase inhibition, Alzheimer's disease

■ INTRODUCTION

Angelica archangelica L., syn. *Angelica officinalis* HOFFM (*Apiaceae*) is native to Europe and Asia, especially to the Western Himalayas. *A. archangelica* is sometimes naturalized in eastern North America, in spite of the fact that a native American species *A. atropurpurea* has similar properties. Angelica is used to produce a spiced extract for confectionery and also is used in the manufacture of alcoholic bitters (vermouths) and herbal liqueurs such as Bénédictine, Becherovka, and Chartreuse. The fruit and root oil and the root extract are used as flavor enhancers.[1−3] Angelica root and fruit extracts are used in some countries as an appetite stimulant, an antispasmodic, and as medication for gastrointestinal symptoms such as bloating, poor digestion, eructation, and flatulence.[2,3] In Nordic countries, people have eaten the aerial parts and the roots for hundreds of years. Older phytotherapeutic books also describe its central nervous system-stimulant activity, similar to that of ginseng, and its properties as a nicotine antidote.[4,5] Some recent investigations have shown the anti-inflammatory, antioxidant, and antiproliferative as well as the calcium entry blocker activity of this plant.[6−9] The whole plant is rich in essential oil (0.35−1.3%), consisting of monoterpenes such as β-phellandren, α- phellandren, α-pinen, δ-3-caren, limonene, sabinen, myrcen,[2,10,11] and furanocoumarins; linear, xanthotoxin, bergapten, imperatorin, isoimperatorin, oxypeucedanin, phellopterin; simple, osthrutol, osthol, osthenol; and angular, angelicin, archangelicin, and 2′angeoloyl-3′-isovaleryl vaginate.[12−14] *Angelica* sp. extracts and coumarins were shown to inhibit the activity of acetylcholinesterase (AChE), but their potency was not very strong.[15−19] However, there is still no information about the effects of *Angelica* sp. and coumarins on butyrylcholinesterase activity (BChE).

Testing natural compounds and plant extracts which affect butyrylcholinesterase activity seems to be of importance in the treatment of Alzheimer's disease (AD), considering that BChE controls the level of extracellular ACh, which is decreased in the AD patient's brain.[20] Furthermore, BChE activity increases progressively as the severity of dementia advances. Clinical trials demonstrated that the treatment with AChE-selective or non-selective cholinesterase inhibitors (ChEI) improved cognitive function. However, this treatment suffered from drawbacks in the form of serious adverse reactions.[21] However, selective BChEI was reported to produce a significant increase in brain extra cellular AChE without triggering severe peripheral or central side effects.[20] So far, few BChE inhibitors with $IC_{50} < 15\ \mu M$, originating from natural sources, have been identified. They include steroidal alkaloids, isoquinoline alkaloids, piperidine alkaloids, and flavanones.[22−25] However, higher selectivity for BChE was demonstrated only for a few steroidal alkaloids.[22]

This article describes the cholinesterase inhibitory activity of *Angelica archangelica* root and fruit extracts and the bioguided isolation and identification of butyrylcholinesterase inhibitory furanocoumarins from the root and fruit hexane extracts.

■ MATERIALS AND METHODS

General Experimental Procedures. 1D and 2D NMR spectra (^{1}H, ^{13}C, DEPT-90, DEPT-135, COSY, HMQC, HMBC, and ROESY) were recorded on a Bruker DSX 400/100 MHz or a Varian 500/125 MHz

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spectrometer, using $CDCl_3$ as solvent and tetramethylsilane (TMS) as an internal standard. The HPLC-DAD-MS analysis was performed using a Dionex (Germany) UHPLC-3000 RS system equipped with a dual low-pressure gradient pump, an autosampler, a column compartment, a diode array detector, and an AmaZon SL ion trap mass spectrometer with an ESI interface (Bruker Daltonik GmbH, Germany). Enzymatic assays were carried out on a Shimadzu (Japan) 160A UV−vis spectrophotometer.

Chemicals. Osthol, xanthotoxin, and bergapten were purchased from ChromaDex (Santa Ana, CA). Isoimperatorin, isopimpinellin, and phellopterin isolated from *Angelica archangelica* were kindly provided by Professor K. Głowniak from the Department of Pharmacognosy with Medicinal Plant Laboratory, Medical University of Lublin (Poland). Acetylthiocholine iodide, *S*-butyrylthiocholine iodide, 1-naphthyl acetate, Fast Blue B salt, DTNB (5,5′-dithiobis [2-nitro-benzoic acid]), acetylcholinesterase from electric eel, butyrylcholinesterase from equine serum, galanthamine hydrobromide, and physostigmine hydrochloride were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Formic acid was obtained from Loba Feinchemie AG (Fischamend, Austria), and acetonitrile was obtained from Merck KGAa (Darmstadt, Germany). Water was of ultrapure quality (Millipore Corp., Molsheim, France). All substances used in the biological assays had purity >95%.

Plant Material. Roots and fruits of *Angelica archangelica* L. subsp. *archangelica* were collected in the second year of vegetation from plants cultivated in the Medical Plant Garden at the Department of Pharmacognosy, Medical University of Lublin (Poland). The plant was identified by Dr. W. Szypuła, Department of Botany and Pharmaceutical Biology, Warsaw Medical University. Additionally, the distinction between subsp. *Archangelica* and subsp. *litoralis* was made using microscopic analysis of the fruits, according to Blaschek et al.,[3] at the Department of Pharmacognosy and Molecular Basis of Phytotherapy, Warsaw Medical University, Poland. Voucher specimens (Nos. 07035 and 07036) are deposited at the Department of Pharmacognosy and Molecular Basis of Phytotherapy, Warsaw Medical University, Poland.

Preparation of Extracts. Accurately weighted 2 g of powdered plant material was extracted in a water bath at 60 °C under reflux with methanol (3 × 40 mL) or hexane (3 × 40 mL) for 30 min. The extracts were filtered and concentrated to dryness under reduced pressure at 60 °C. The dry extracts were stored at 4 °C and dissolved in an appropriate solvent shortly before the test.

HPLC with DAD and MS Detection. The extracts were dissolved in methanol to the concentration of 0.1 g of raw material per mL and were filtered through a Chromafil 0.45 μm filter (Macherey-Nagel, Germany) before injection (20 μL). Chromatographic separation was performed on a HypersilGOLD C-18, 10 × 2.1 mm, 1.9 μm column (TermoScientific, Bellefonte, PA). Column temperature was 25 °C. The mobile phase (A) was formic acid/water/acetonitrile (0.1:95:5, v/v/v), and the mobile phase (B) was formic acid/acetonitrile (0.1:100, v/v). A linear gradient solvent system was used for 0−28 min with 33−75% B. The flow rate was 0.2 mL/min. The column was equilibrated for 10 min between injections. UV spectra were recorded over a range of 200−400 nm, and chromatograms were acquired at 254 and 325 nm. The LC eluate was introduced directly into the ESI interface without splitting. Coumarins were analyzed in positive ion mode. The nebulizer pressure was 15 psi; dry gas flow was 8 L/min; dry temperature was 220 °C; and capillary voltage was 4.5 kV. The analysis was carried out using scans from m/z 100 to 2200. Coumarins were characterized by their UV−vis spectra, retention times, and mass spectra. The characteristics were compared with standards and/or those in the literature.[13,14]

Acetylcholinesterase and Butyrylcholinesterase Inhibition Colorimetric Assay. The assay was performed by the colorimetric Ellman method, using acetylthiocholine and butyrylthiocholine as substrates.[26]

Acetylcholinesterase or butyrylcholinesterase (40 μL of 0.45 U/mL in 50 mM TRIS-HCl buffer at pH 7.8) and extract solution at a concentration of 400−10 μg/mL or the compound solution at a concentration of 250−1 μM (100 μL) were added to 860 μL of 50 mM TRIS-HCl buffer (pH 7.8) and incubated at 4 °C for 30 min. The reaction was started by adding DTNB (20 μL, 3 mM in TRIS-HCl buffer pH 7.8) and acetylthiocholine or butyrylthiocholine (20 μL, 15 mM in TRIS-HCl buffer at pH 7.8). The reaction mixture was incubated for 20 min at 37 °C. The reaction was stopped by adding physostigmine (20 μL, 0.1 mM in methanol). The yellow product absorbance was measured at 412 nm.

The extracts and compounds were dissolved in methanol, the respective dilutions were made in 50 mM TRIS-HCl buffer at pH 7.8. The potential influence of methanol (1% in the assays) on enzyme activity was considered in controls. The purity of tested compounds was confirmed by TLC and HPLC methods. All substances used were of >95% purity.

Butyrylcholinesterase Inhibition Bioautographic Assay. The assay was performed by the bioautographic Marston method, using naphthyl acetate as a substrate.[27] The samples were dissolved in methanol to the concentration corresponding to 0.2 g of raw material per mL. Aliquots of 40 μL were spotted onto a TLC silica gel $60F_{254}$ plate (Merck) and developed with $CHCl_3$/EtOAc (95:5, v/v) as the eluent. The chromatogram was dried until complete solvent removal. The plate was sprayed with butyrylcholinesterase (6 U/mL in 50 mM TRIS-HCl buffer at pH 7.8 + BSA 1.5 mg/mL) and incubated at 37 °C for 20 min. Detection was performed by spraying the chromatogram with a mixture of naphthyl acetate solution (2.5 mg/mL in ethanol) and Fast Blue B salt solution (2.5 mg/mL in water). The presence of a white spot on a purple-colored plate indicated the presence of enzyme inhibitors.

BChE Inhibitor Isolation and Identification. Dried roots of *Angelica archangelica* (400 g) were extracted with hexane (3 × 800 mL) in a water bath at 70 °C for 5 h each time. The collected filtrates were evaporated at 60 °C, giving a residue of 9.7 g. The hexane-soluble residue was subjected to silica gel column chromatography (24 × 4 cm; 0.063−0.100 mm; Merck) and eluted with $CHCl_3$ to obtain 100 fractions of 20 mL, which were pooled into 11 main fractions (A−K) on the basis of their TLC profile and inhibitory activity. Fraction J (1.5 g) was rechromatographed on a silica gel column (45 × 2 cm; 0.063−0.100 mm; Merck) with a hexane/EtOAc gradient (90:10 → 75:25) of 4 steps, 750 mL each. One hundred seventy-five fractions were collected and pooled into 7 main fractions (J1−J7) on the basis of their TLC profile and inhibitory activity. Using HPLC analysis, the most active fraction J6 was found to consist of at least 3 main compounds. These compounds were separated on a silica gel RP-18 column (45 × 2 cm; 0.040−0.06 mm), using MeOH−MeCN−H_2O−THF (30:15:60:5, v/v/v/v) as an eluent. 150 fractions of 10 mL were collected and pooled into 8 main fractions to yield compound 1 (50 mg) from fraction 4, compound 2 (20 mg) from fraction 6, and compound 3 (24 mg) from fraction 8. The separation and isolation are illustrated in Figure 1A.

The structures of all isolated compounds were determined by mass spectroscopy and 1D and 2D NMR experiments.

*Heraclenol-2′-O-angelate (**1**).* Pale yellowish amorphous powder; $[\alpha]^{25}_{D}$ −14.1 (c 1.0, MeOH); UV (MeOH) λ_{max} 299, 247, 224 nm; IR (KBr) ν_{max} 3469, 2976, 1714, 1587, 1402, 1332, 1232, 1154, 1098, 830, 757 cm^{-1}; ESI-MS m/z 409.1 $[M + Na]^+$; ^1H NMR ($CDCl_3$): δ 6.33 (1H, d, J = 9.6 Hz, H-3), 7.74 (1H, d, J = 9.6 Hz, H-4), 7.35 (1H, s, H-5), 7.64 (1H, d, J = 2 Hz, H-11), 6.79 (1H, d, J = 2 Hz, H-12), 4.89 (1H, dd, J = 3.2, 11.2 Hz, H-13a), 4.67 (1H, dd, J = 6.8, 11.2 Hz, H-13b), 5.33 (1H, dd, J = 3.2, 6.8 Hz, H-14), 6.08 (1H, qd, J = 7.2, 1.2, 1.2 Hz, H-18), 1.97 (3H, dd, J = 7.2, 1.6 Hz, H-19), 1.87 (3H, d, J = 1.6 Hz, H-20), 1.38 (3H, s, H-21), 1.33 (3H, s, H-22). ^{13}C NMR ($CDCl_3$): 160.10 (C-2), 114.69 (C-3), 144.24 (C-4), 113.54 (C-5), 125.91 (C-6), 147.77 (C-7), 131.36 (C-8), 143.11 (C-9), 116.43 (C-10), 146.65 (C-11), 106.74 (C-12), 72.47 (C-13), 76.68 (C-14), 71.80 (C-15), 167.30 (C-16), 127. 46 (C-17), 138.97 (C-18), 15.83 (C-19), 20.49 (C-20), 26.37 (C-21), 26.38 (C-22).

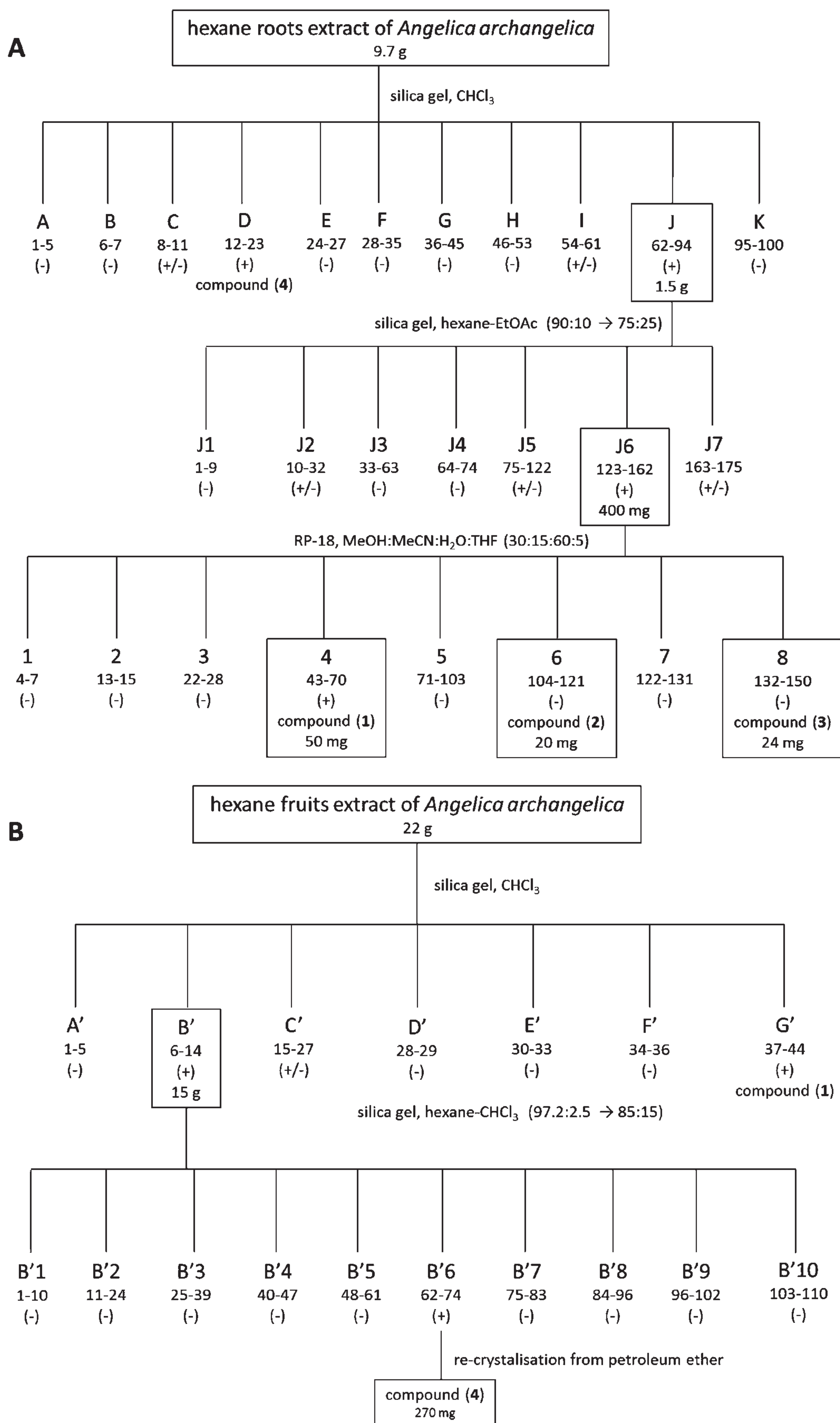

Figure 1. Schemes of root (A) and fruit (B) hexane extracts of *Angelica archangelica* fractionation and BChE inhibitor isolation. (+), presence of BChE inhibitor; (+ /−), presence of weak BChE inhibitor; (−), no BChE inhibitor in the bioautographic test.

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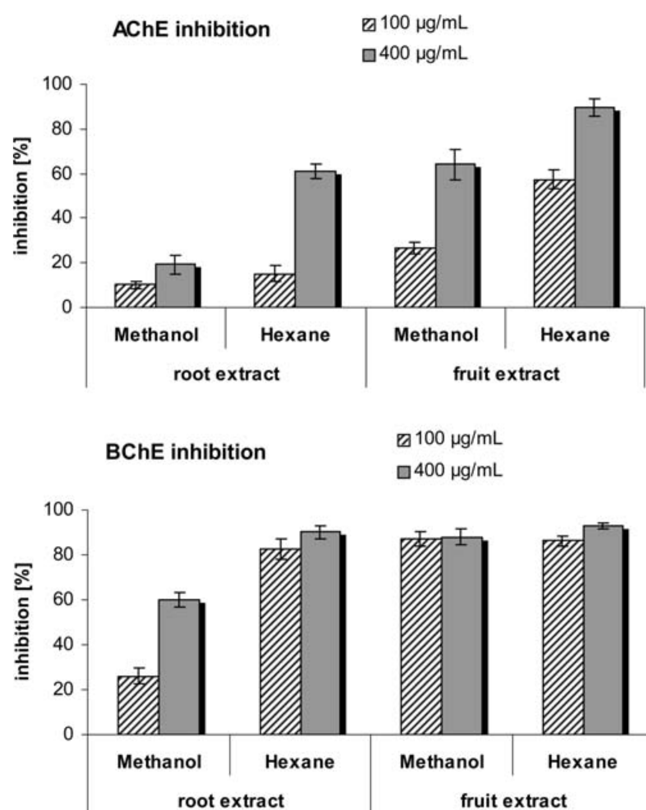

Figure 2. AChE and BChE inhibition by root and fruit methanolic and hexane extracts of *Angelica archangelica* at concentrations of 100 μg/mL and 400 μg/mL. Data represent the mean ± SD of at least three independent experiments, each performed in duplicated samples.

Byakangelicin-2′-O-angelate (**2**). White amorphous powder; $[\alpha]^{25}_{D}$ −15.4 (c 1.0, MeOH); UV (MeOH) λ_{max} 310, 260, 248 nm; IR (KBr) ν_{max} 3440, 2976, 1737, 1590, 1483, 1350, 1159, 1070, 817, 746 cm^{-1}; ESI-MS m/z 439.2 $[M + Na]^+$. ^{1}H NMR ($CDCl_3$): δ 6.24 (1H, d, J = 9 Hz, H-3), 8.08 (1H, d, J = 9 Hz, H-4), 7.56 (1H, d, J=2 Hz, H-11), 6.97 (1H, d, J=2 Hz, H-12), 4.70 (1H, dd, J = 3.2, 11.2 Hz, H-13a), 4.52 (1H, m, H-13b), 5.30 (1H, dd, J = 3.2, 6.8 Hz, H-14), 6.08 (1H, qd, J = 7.2, 1.2, 1.2 Hz, H-18), 1.97 (3H, dd, J = 7.3, 1.4 Hz, H-19), 1.89 (3H, bt, J = 1.3 Hz, H-20), 1.38 (3H, s, H-21), 1.33 (3H, s, H-22), 4.17 (3H, s, OCH_3). ^{13}C NMR ($CDCl_3$): 160.11 (C-2), 112.71 (C-3), 139.33 (C-4), 144.57 (C-5), 114.42 (C-6), 149.96 (C-7), 127.54 (C-8), 143.66 (C-9), 107.37 (C-10), 145.06 (C-11), 105.14 (C-12), 72.83 (C-13), 76.69 (C-14), 71.83 (C-15), 167.35 (C-16), 126.61 (C-17), 138.91 (C-18), 15.85 (C-19), 20.54 (C-20), 26.54 (C-21), 26.54 (C-22), 60.65 (O-CH_3).

Byakangelicin-2′-O-isovalerate (**3**). White amorphous powder; $[\alpha]^{25}_{D}$ +75,5 (c 0.5, MeOH); UV (MeOH) λ_{max} 312, 267, 249 nm; IR (KBr) ν_{max} 3352, 2962, 1736, 1483, 1350, 1071, 746 cm^{-1}; ESI-MS m/z 441.2 $[M + Na]^+$. ^{1}H NMR ($CDCl_3$): δ 6.26 (1H, d, J = 9.6 Hz, H-3), 8.10 (1H, d, J = 9 Hz, H-4), 7.60 (1H, s, H-11), 6.99 (1H, s, H-12), 4.70 (1H, dd, J = 2, 11 Hz, H-13a), 4.52 (1H, m, H-13b), 5.22 (1H, bd, H-14), 2.34 (1H, m, H-17), 2.13 (1H, m, H-18), 0.97 (3H, bd, H-19), 0.97 (3H, bd, H-20), 1.32 (3H, s, H-21), 1.36 (3H, s, H-22), 4.17 (3H, s, OCH_3). ^{13}C NMR ($CDCl_3$): 160.59 (C-2), 113.39 (C-3), 139.84 (C-4), 144.21 (C-5), 114.97 (C-6), 150.47 (C-7), 127.07 (C-8), 145.12 (C-9), 107.96 (C-10), 145.62 (C-11), 105.71 (C-12), 73.36 (C-13), 77.14 (C-14), 72.23 (C-15), 173.35 (C-16), 43.89 (C-17), 26.06 (C-18), 22.89 (C-19), 22.89 (C-20), 26.81 (C-21), 27.04 (C-22), 61.32 (O−CH_3).

Dried fruits of *Angelica archangelica* (200 g) were extracted with hexane (3 × 700 mL) in a water bath at 70 °C for 5 h each time. The collected filtrates were evaporated at 60 °C giving a residue of 22 g. The hexane-soluble residue was subjected to silica gel column chromatography (24 × 4 cm; 0.063−0.100 mm; Merck) and eluted with $CHCl_3$ to obtain 50 fractions of 20 mL, which were pooled into 6 main fractions (A′−G′) on the basis of their TLC profile and inhibitory activity. Fraction B′ (15 g) was rechromatographed on a silica gel column (50 × 5 cm; 0.063−0.100 mm; Merck) with a gradient of hexane/$CHCl_3$ (97.5:2.5 → 85:15) of 5 steps, 1000 mL each. One hundred ten fractions were collected and pooled into 10 main fractions (B′1−B′10) on the basis of their TLC profile and inhibitory activity. Compound **4** (270 mg) was isolated from fraction B′6 by recrystallization from petroleum ether. Compound **4** was identified as imperatorin by spectroscopic analysis.[12] The separation and isolation are illustrated in Figure 1B.

Statistics. The results were expressed as the mean ± SD of three independent experiments performed in duplicate. Statistical analysis was performed by Student's t test, and $p < 0.05$ IC_{50} values were obtained from dose−effect curves by linear regression.

Table 1. Inhibition of Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) by Tested Extracts, Compounds, and Reference Inhibitors[a]

extract/compound	AChE IC_{50} (μM)	BChE IC_{50} (μM)
roots hexane extract	315 ± 20 (μg/mL)	16 ± 5 (μg/mL)
fruits hexane extract	73 ± 7 (μg/mL)	9 ± 2 (μg/mL)
xanthotoxin	>250	>250
isopimpinelin	>250	>250
bergapten	>250	>250
heraclenol-2′-*O*-angelate	NA[b]	7.5 ± 1.8
byakangelicin-2′-*O*-angelate	NA[b]	NA[b]
byakangelicin-2′-*O*-isovalerate	NA[b]	NA[b]
imperatorin	156 ± 15	14.4 ± 3.2
phellopterin	>250	>250
osthol	>250	>250
isoimperatorin	>250	>250
physostigmine	0.21 ± 0.1	1.73 ± 0.4
galanthamine	0.37 ± 1.1	8.3 ± 2.6

[a] IC_{50} values means ± standard deviation were obtained from dose−effect curves by linear regression performed in at least three independent experiments (each in duplicated samples). [b] NA, not active at a concentration of 1000 μM

RESULTS AND DISCUSSION

Our results revealed a weak effect of Angelica root extracts and also some effect of the fruit extract, especially the hexane one, on AChE activity.[28] Hexane extracts from both roots and fruits were proven to achieve BChE inhibition at a rate of over 50% at a concentration of 100 μg/mL. The BChE inhibition by all extracts was dose-dependent at a concentration range of 400−10 μg/mL; at concentrations of 100 and 400 μg/mL, both hexane extracts and methanolic fruit extracts showed close to 95% of enzyme inhibition (Figure 2). Interestingly, Angelica extracts showed a selective inhibition of BChE (Figure 2). The IC_{50} values are presented in Table 1.

As compared with standards and/or literature data[13,14] of UV−vis spectra and mass spectra, the HPLC-DAD/MS profiles of both hexane extracts of fruits and roots showed the presence of oxypeucedanin hydrate, xanthotoxin, isopimpinelin, bergapten, osthenol, oxypeucedanin, imperatorin, phellopterin, osthol,

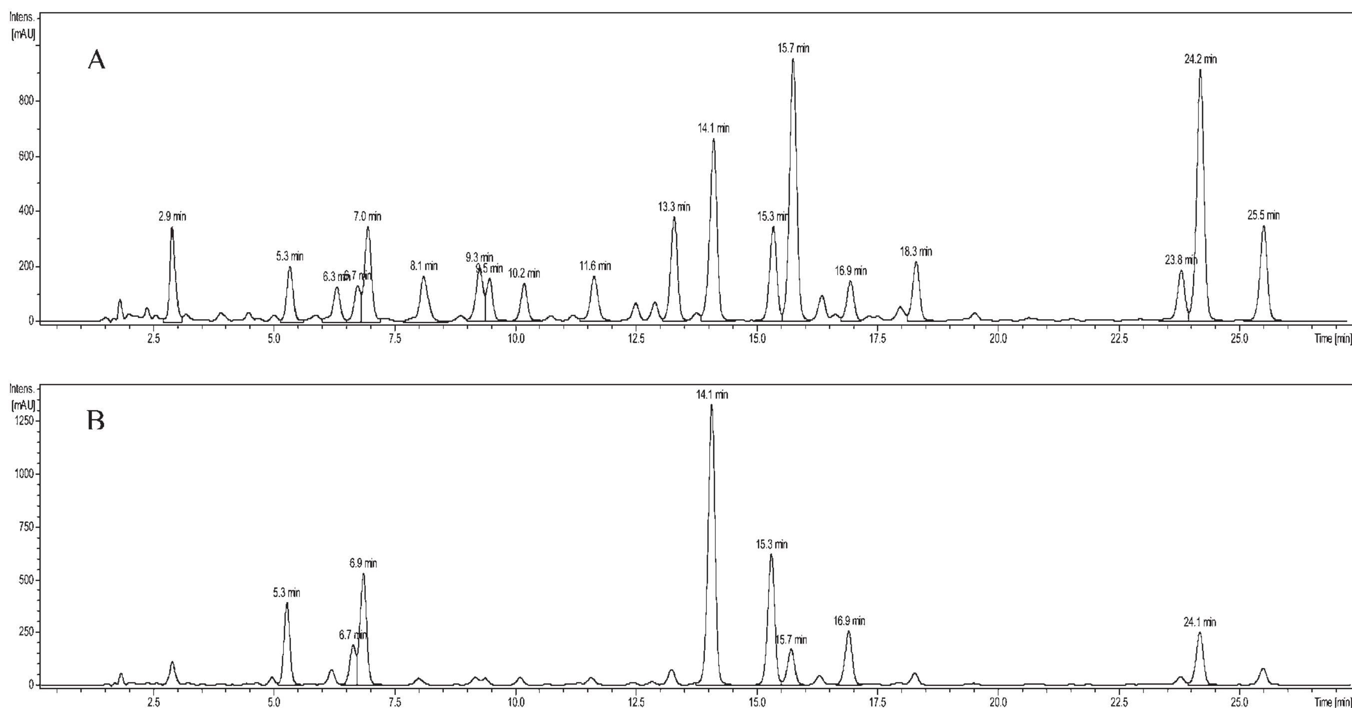

Figure 3. HPLC chromatograms at 325 nm of hexane extracts from roots (A) and fruits (B) of *Angelica archangelica*. Retention time t_R (min): 2.9, oxypeucedanin hydrate; 5.3, xanthotoxin; 6.7, isopimpinelin; 7.0, bergapten; 8.1, osthenol; 9.3, oxypeucedanin; 10.2, heraclenol-2′-*O*-angelate; 11.6, byakangelicin-2′-*O*-angelate; 13.3, byakangelicin-2′-*O*-isovalerate; 14.1, imperatorin; 15.3, phellopterin; 15.7, osthol; 16.9, isoimperatorin; 24.2, archangelicin; 25.5, 2′-angeloyl-3′-isovaleryl vaginate.

Table 2. Characterization by HPLC/DAD/MS of Coumarins Identified in Root and Fruit Hexane Extracts of *Angelica archangelica*

compound	retention time t_R (min)	UV λ_{max} (nm)	(+) ESI-MS *m*/*z*	MW
(**1**) oxypeucedanin hydrate	2.9	249, 260, 309	305.1 $[M + H]^+$	304
(**2**) xanthotoxin	5.3	248, 301	217.0 $[M + H]^+$	216
(**3**) unknown	6.3	249, 259, 311	319.1 $[M + H]^+$	318
(**4**) isopimpinelin	6.7	273, 312	247.0 $[M + H]^+$	246
(**5**) bergapten	7	249, 267, 310	217.0 $[M + H]^+$	216
(**6**) osthenol	8.1	325	231.1 $[M + H]^+$	230
(**7**) oxypeucedanin	9.3	249, 262, 309	287.1 $[M + H]^+$	286
(**8**) unknown	9.5	322	715.2 $[2M + Na]^+$, 369.1 $[M + Na]^+$	346
(**9**) heraclenol-2′-*O*-angelate	10.2	248, 301	795.1 $[2M + Na]^+$, 409.1 $[M + Na]^+$	386
(**10**) byakangelicin-2′-*O*-angelate	11.6	247, 262, 310	855.3 $[2M + Na]^+$, 439.2 $[M + Na]^+$	416
(**11**) byakangelicin-2′-*O*-isovalerate	13.3	249, 267, 309	859.3 $[2M + Na]^+$, 441.2 $[M + Na]^+$	418
(**12**) imperatorin	14.1	247, 301	271.1 $[M + H]^+$	270
(**13**) phellopterin	15.3	269, 310	301.1 $[M + H]^+$	300
(**14**) osthol	15.7	322	245.1 $[M + H]^+$	244
(**15**) isoimperatorin	16.9	250, 262, 310	271.1 $[M + H]^+$	270
(**16**) unknown	18.3	321	795.1 $[2M + Na]^+$, 409.1 $[M + Na]^+$	386
(**17**) unknown	23.8	321	875.1 $[2M + Na]^+$, 449.1 $[M + Na]^+$	426
(**18**) archangelicin	24.2	321	875.3 $[2M + Na]^+$, 449.2 $[M + Na]^+$	426
(**19**) 2′-angeloyl-3′-isovaleryl vaginate	25.5	321	879.4 $[2M + Na]^+$, 451.2 $[M + Na]^+$	428

isoimperatorin, archangelicin, and 2′-angeloyl-3′-isovaleryl vaginate (Figure 3 and Table 2). Since there was no information about the inhibition of BChE by this group of compounds, we performed a bioautographic assay to identify the compounds responsible for the observed effect. As a result, two BChE inhibitors were found, one in the fruit extract and the other in the root extract, both of which were present in high concentrations (Figure 4). Next, we isolated and identified by NMR spectroscopy imperatorin as a dominating active compound from fruits. The bioguided fractionation of the root hexane extract using silica gel with $CHCl_3$ and with hexane gradient in EtOAc (90:10 → 75:25) afforded a purified fraction containing a BChE inhibitor. The HPLC-DAD analysis

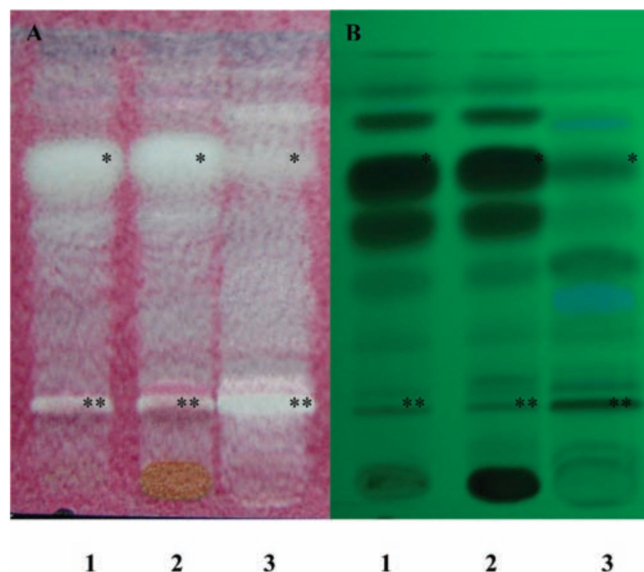

Figure 4. TLC of *Angelica archangelica* extracts (A) showing butyrylocholinesterase inhibitory activity; white spots indicate inhibition. (B) TLC plate in UV-254 nm. The samples were dissolved in methanol to the concentration corresponding to 0.2 g of raw material per mL. Aliquots of 40 μL were spotted onto a TLC silica gel $60F_{254}$ plate and developed with $CHCl_3$/EtOAc (95:5, v/v) eluent. **1**, fruits hexane extract; **2**, fruit methanolic extract; **3**, roots hexane extract. The white area on plate A corresponds to the enzyme inhibitors; *, imperatorin; **, heraclenol-2′-*O*-angelate.

showed the presence of at least three furanocoumarins. Further separation on a silica gel RP-18 column using MeOH−MeCN−H_2O−THF (30:15:60:5) resulted in the isolation of three compounds. Their structures were confirmed as heraclenol-2′-*O*-angelate, byakangelicin-2′-*O*-angelate, and byakangelicin-2′-*O*-isovalerate on the basis of their molecular mass and ^1H and ^{13}C NMR spectra (Figure 5). All compounds were isolated previously from *Angelica archangelica*.[32]

Among the identified (xanthotoxin, isopimpinelin, bergapten, phellopterin, osthol, and isoimperatorin) and isolated (imperatorin, heraclenol-2′-*O*-angelate, byakangelicin-2′-*O*-angelate, and byakangelicin-2′-*O*-isovalerate) compounds, only imperatorin was found to inhibit both enzyme activities with IC_{50} = 14.4 μM for BChE and IC_{50} = 156 μM for AChE (Table 1). The obtained results explain the inhibitory effect of Angelica fruits extract, rich in imperatorin, on both enzymes. While the AChE inhibitory activity of imperatorin was described in previous studies,[17,18] this is the first report on BChE inhibitory activity of this compound. Imperatorin was shown to also display other CNS activities such as anticonvulsant, γ-aminobutyric acid degradation inhibitory properties, and $GABA_A$ modulating properties.[29−31] Heraclenol-2′-*O*-angelate showed significant BChE inhibitory activity with IC_{50} = 7.5 μM, whereas byakangelicin-2′-*O*-angelate and byakangelicin-2′-*O*-isovalerate were inactive in both cholinesterase activity assays (Table 1). Moreover, heraclenol-2′-*O*-angelate revealed selective inhibitory activity against BChE. Imperatorin and heraclenol-2′-*O*-angelate appear to have weaker activity in comparison with that of well known cholinesterase inhibitors, physostigmine (IC_{50} = 1.73 μM) and comparable to those of galanthamine (IC_{50} = 8.3 μM), but both furanocoumarins, especially heraclenol-2′-*O*-angelate, showed stronger selectivity toward BChE than standard compounds (Table 1). The BChE

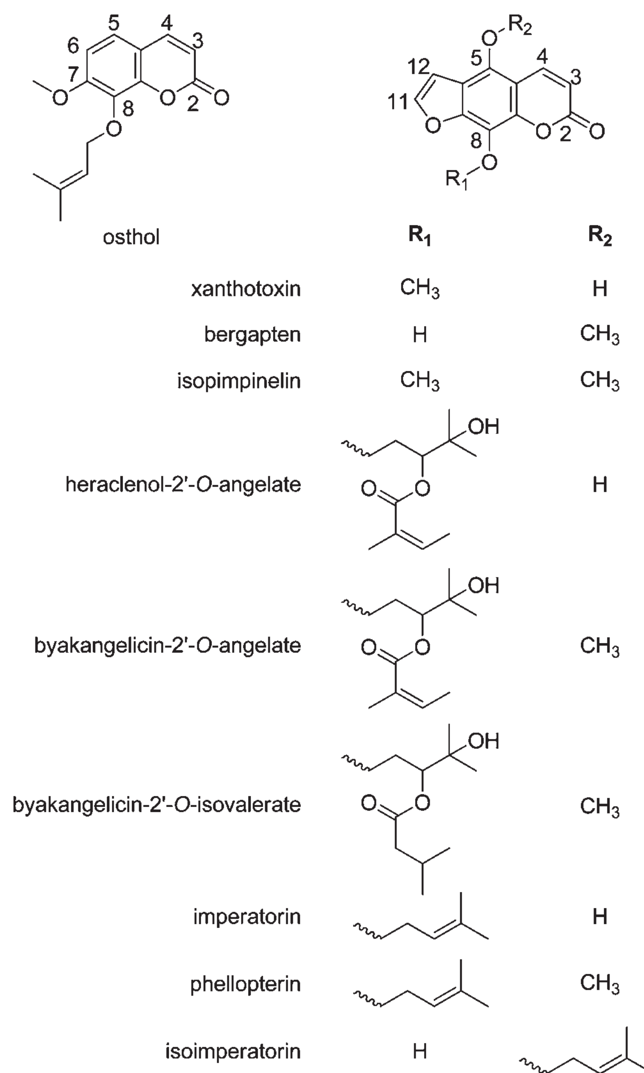

Figure 5. Structures of tested compounds.

inhibitory activity strongly depends on coumarin structure. Only C-8 substituted furanocoumarins were active. Osthol, a simple coumarin as well as a fraction from roots which contained angular furanocoumarin archangelicin (not shown), exhibited no such activity. However, the methoxy group in C-8, as in xanthotoxin, was not sufficient to produce the inhibitory activity. Isoimperatorin, which differs structurally from imperatorin only in the substitution site, and phellopterin, which differs from imperatorin by possessing an additional methoxy group in the C-5 position, were both inactive against BChE. Other C-5 furanocoumarins (bergapten and isopimpinelin) were also found to be inactive. Interestingly, byakangelicin-2′-*O*-angelate and byakangelicin-2′-*O*-isovalerate, the C-5 and C-8 substituted furanocoumarin, showed no activity, which suggests that the C-5 free structure was also indispensable. Moreover, the most active heraclenol-2′-*O*-angelate was an ester similar to acetylcholine. However, all tested furanocoumarins showed weak or no inhibitory activity against AChE. Our results support the findings of Kang et al.[15] that not furanocoumarins but only pyranocoumarins, especially those with free hydroxyl group at C-3′ such as decursinol, are able to inhibit the activity of this enzyme (IC_{50}= 28 μM).

Our observations may be explained by the fact that AChE has a characteristic narrow gorge leading to its active site, while BChE's gorge is wider.[33,34] That is probably why AChE can hydrolyze only small acyl esters in contrast to BChE, which can accommodate larger acyl groups and hence larger substrates. Accordingly, we supposed that only furanocoumarins with a longer and more branched side chain would be more selective toward BChE because they are not able to pass through the narrow gorge to AChE's active site. So far, few BChE inhibitors with $IC_{50} < 15\ \mu M$, originating from natural sources, have been discovered. They include steroidal alkaloids, isoquinoline alkaloids, piperidine alkaloids, and flavanones.[22−25] However, higher selectivity for BChE was demonstrated only for a few steroidal alkaloids.[22]

In conclusion, four compounds, namely, heraclenol-2′-*O*-angelate (**1**), byakangelicin-2′-*O*-angelate (**2**), byakangelicin-2′-*O*-isovalerate (**3**), and imperatorin (**4**), were isolated from *Angelica* roots and fruits. Imperatorin and heraclenol-2′-*O*-angelate exhibited significant BChE inhibitory activity with $IC_{50} = 14.4 \pm 3.2\ \mu M$ and $IC_{50} = 7.5 \pm 1.8\ \mu M$, respectively. Our results demonstrate that Angelica extracts may act as a CNS stimulant causing the inhibition of acetylcholine degradation. Imperatorin and more potent heraclenol-2′-*O*-angelate seem to be interesting initial structures for the synthesis of specific butyrylcholineterase inhibitors.

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REFERENCES

(1) Bhat, Z. A.; Kumar, D.; Shah, M. Y. *Angelica archangelica* Linn. is an angel on earth for the treatment of diseases. *Int. J. Nutr. Pharmacol. Neurol. Dis.* **2011**, *1*, 36–50.

(2) Wichtl, M. *Herbal Drugs and Phytopharmaceuticals*, 3rd ed.; Medpharm Scientific Publishers: Stuttgart, Germany, 2004; pp 38−41.

(3) Blaschek, W.; Hänsel, R.; Keller, K.; Reichling, J.; Rimpler, H.; Schneider, G. *Hagers Handbuch der Pharmazeutischen Praxis, (Drogen A-K)*; Springer-Verlag, Berlin, 1998; pp 95−110 (in German).

(4) Muszyński, J. *Vademecum Fitoterapii*, 3rd ed.; WPLiS: Warszawa, Poland, 1959; pp 55−56 (in Polish).

(5) Fournier, P. *Le Livre des Plantes Médicinales et Vénéneuses de France*; Paul Lechevalier Ēditeur, Paris, France, 1947; pp 93−97 (in French).

(6) Roos, G.; Waiblinger, J.; Zschocke, S.; Liu, J. H; Klaiber, I.; Kraus, W.; Bauer, R. Isolation, identification and screening for COX-1 and 5-LO-inhibition of coumarins from *Angelica archangelica*. *Pharmaceut. Pharmacol. Lett.* **1997**, *7*, 157–160.

(7) Schempp, H.; Weiser, D.; Kelber, O.; Elstner, E. F. Radical scavenging and anti-inflammatory properties of STW 5 (Iberogast®) and its components. *Phytomedicine* **2006**, *13*, 36–44.

(8) Härmälä, P.; Vuorel,a, H.; Hiltunem, R.; Nyiredy, S.; Sticher, O.; Törnquist, K.; Kaltia, S. Strategy for the isolation and identification of coumarins calcium antagonistic properties from the roots of *Angelica archangelica*. *Phytochem. Anal.* **1992**, *3*, 42–48.

(9) Sigurdsson, S.; Ögmundsdottir, H. M.; Gudbjarnason, S. Antiproliferative effect of *Angelica archangelica* fruits. *Z. Naturforsch., C: J. Biosci.* **2004**, *59*, 523–527.

(10) Wedge, D. E.; Klun, J. A.; Tabanca, B. D.; Ozek, T.; Baser, K. H. C.; Liu, Z.; Zhang, S.; Cantrell, C. L.; Zhang, J. Bioactivity-Guided Fractionation and GC/MS fingerprinting of *Angelica sinensis* and *Angelica archangelica* root components for antifungal and mosquito deterrent activity. *J. Agric. Food Chem.* **2009**, *57*, 464–470.

(11) Nivinskiene, O.; Butkiene, R.; Mockute, D. The seed (fruit) essential oils of *Angelica archangelica* L. growing wild in Lithuania. *J. Essent. Oil Res.* **2007**, *19*, 477–481.

(12) Harkar, S.; Razdan, T. K.; Waight, E. S. Steroids, chromone and coumarins from *Angelica officinalis*. *Phytochemistry* **1984**, *23*, 419–426.

(13) Eava, M.; Rauha, J.-P.; Vuorela, P.; Vuorela, H. Computer-assisted, high-performance liquid chromatography with mass spectrometric detection for the analysis of coumarins in *Peucedanum palustre* and *Angelica archangelica*. *Phytochem. Anal.* **2004**, *15*, 167–174.

(14) Härmälä, P.; Kaltia, S.; Vuorela, H.; Hiltunem, R. A furanocoumarin from *Angelica archangelica*. *Planta Med.* **1992**, *58*, 287–288.

(15) Kang, S. Y.; Lee, K. Y.; Sung, S. H.; Park, M. J.; Kim, Y. C. Coumarins isolated from *Angelica gigas* inhibit acetylcholinesterase: structure-activity relationships. *J. Nat. Prod.* **2001**, *64*, 683–685.

(16) Choudhary, M. I.; Khalid, A.; Sultani, S. Z.; Rahman, A. A new coumarin from *Murraya paniculata*. *Planta Med.* **2002**, *68*, 81–83.

(17) Kim, D. K.; Lim, J. P.; Yang, J. H.; Eom, D. O.; Eun, J. S.; Leem, K. H. Acetylcholinesterase inhibitors from the roots of *Angelica dahurica*. *Arch. Pharm. Res.* **2002**, *25*, 856–859.

(18) Urbain, A.; Marston, A.; Hostettmann, K. Coumarins from *Peucedanum ostruthium* as inhibitors of acetylcholinesterase. *Pharm. Biol.* **2005**, *48*, 647–650.

(19) Orhan, I.; Tosun, F.; Şener, B. Coumarin, anthroquinone and stilbene derivatives with anticholinesterase activity. *Z. Naturforsch., C: J. Biosci.* **2008**, *65*, 366–370.

(20) Giacobini, E. Selective inhibitors of butyrylocholinesterase: a valid alternative for therapy of Alzheimer's disease? *Drugs Aging* **2001**, *18*, 891–898.

(21) Lane, R. M.; Potkin, S. G.; Enz, A. Targeting acetylcholinesterase and butyrylcholinesterase in dementia. Int. *J. Neuropsychopharmacol.* **2006**, *9*, 101–124.

(22) Choudhary, M. I.; Devkota, K. P.; Nawaz, S. A.; Ranjit, R.; Rahman, A. Cholinesterase inhibitory pregnane-type steroidal alkaloids from *Sarcococca hookeriana*. *Steroids* **2005**, *70*, 295–303.

(23) Cho, K.-M.; Yoo, I.-D.; Kim, W.-G. 8-hydroxydihydrochelerythrine and 8-hydroxydihydrosanguinarine with a potent acetylcholinesterase inhibitory activity from *Chelidonium majus* L. *Biol. Pharm. Bull.* **2006**, *29*, 2317–2320.

(24) Ferheen, S.; Ahmed, E.; Afza, N.; Malik, A.; Shah, M. R.; Nawaz, S. A.; Choudhary, M. I. Haloxylines A and B, antifungal and cholinesterase inhibiting piperidine alkaloids from *Haloxylon sarcornicum*. *Chem. Pharm. Bull.* **2005**, *53*, 570–572.

(25) Ahmad, I.; Anis, I.; Malik, A.; Nawaz, S. A.; Choudhary, M. I. Cholinesterase inhibitory constituents from *Onosma hispida*. *Chem. Pharm. Bull.* **2003**, *51*, 412–414.

(26) Ellman, G. L.; Courtney, K. D.; Andres., V.; Featherstone, R. M. A new and rapid colometric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **1961**, *7*, 88–95.

(27) Marston, A.; Kissling, J.; Hostettmann, K. A rapid TLC bioautographic method for the detection of acetylcholinesterase and butyrylcholinesterase inhibitors in plants. *Pythochem. Anal.* **2002**, *13*, 51–54.

(28) Sigurdsson, S.; Gudbjarnason, S. Z. Inhibition of acetylcholinesterase by extracts and constituents from *Angelica archangelica* and *Geranium sylvaticum*. *Z. Naturforsch., C: J. Biosci.* **2007**, *62*, 689–893.

(29) Luszczki, J. J.; Głowniak, K.; Czuczwar, S. J. Imperatorin enhances the protective activity of convential antieleptic drugs against maximal electroshock-induced seizures in mice. *Eur. J. Pharmacol.* **2007**, *574*, 133–139.

(30) Choi, S. Y.; Ahn, E. M.; Song, E. M.; Kim, D. W.; Kang, J. H.; Kwon, O. S.; Kang, T. C.; Baek, N. I. In vitro GABA-transaminase inhibitory compounds from the roots of *Angelica dahurica*. *Phytother. Res.* **2005**, *19*, 839–845.

(31) Zaugg, J.; Eickmeier, E.; Rueda, D. C.; Hering., S.; Hamburger, M. HPLC-based activity profiling of *Angelica pubescens* roots for new positive $GABA_A$ receptor modulators in *Xenopus* oocytes. *Fitoterapia* **2011**, *82 434440.*

(32) Sun, H.; Jakupovic, J. Further heraclenol derivatives from *Angelica archangelica*. *Pharmazie* **1986**, *41*, 888–889.

(33) Soreq, H.; Seidman, S. Acetylcholinesterase: New roles for an old actor. *Nat. Rev. Neurosci.* **2001**, *2* (4), 294–302.

(34) Darvesh, S.; Hopkins, D. A.; Geula, C. Neurobiology of butyrylcholinesterase. *Nat. Rev. Neurosci.* **2003**, *4* (2), 131–138.