# Acetylcholinesterase Inhibitors from Angelica polymorpha Stem 

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#### Abstract

Fourteen compounds were isolated from the stem of Angelica polymorpha. On the basis of spectral data, these compounds were identified as isoimperatorin (1), phellopterin (2), bergapten (3), xanthyletin (4), cnidilin (5), geijerine (6), (-)-3'-acetyl hamaudol (7), 7-demethylsuberosine (8), dehydrogeijerin (9), ( - )-hamaudol $(10),(+)$-visamminol (11), divaricatol (12), scopoletin (13), and decursidate (14), respectively. Among them, compounds $\mathbf{4 - 6}, \mathbf{8}, \mathbf{9}, 13$, and 14 were isolated for the first time from A. polymorpha. Dehydrogeijerin (6) and geijerin (9) were isolated for the first time from genus Angelica. All isolates tested for inhibitory activity against acetylcholinesterae. Compounds $\mathbf{1}$ to $\mathbf{1 3}$ showed acetylcholinesterase inhibitory activity with $\mathrm{IC}_{50}$ values ranging from 1.4 to $37.5 \mu \mathrm{M}$.


Keywords - Angelica polymorpha, Coumarins, Chromones, Acetylcholinesterase inhibitory effect

## Introduction

Angelica polymorpha is widely distributed in Korea, Japan and the Northeastern part of China. ${ }^{1}$ The roots of this plant have been used in China as a traditional medicine to treat the common cold, to act as an analgesic and to reduce inflammation. ${ }^{2}$ Up to now, over thirty compounds have been isolated from this plant, ${ }^{3-10}$ which mainly include coumarins and chromones.
To cholinergic hypothesis, one widely accepted explanation for onset Alzheimer's disease, ${ }^{11}$ suggests the use of acetylcholinesterase inhibitors as useful therapeutic agents for treating Alzheimer's disease. ${ }^{12-14}$ For this reason, many researchers have focused their study on finding acetylcholinesterase inhibitors from plant sources. As part of ongoing study to find acetylcholinesterase inhibitors from plants, we found that MeOH extract of $A$. polymorpha stem showed inhibitory activity against acetylcholinesterase.
The present study focuses on isolation of constituents of $A$. polymorpha stem and their acetylcholinesterase inhibitory activities.

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## Experimental

General experimental procedures - UV/Vis determinations were carried out using a V-530 spectrophotometer (JASCO, Tokyo, Japan). The optical rotations were measured using DIP 1000 digital polarimeter (JASCO, Tokyo, Japan). The MS spectrum was measured using an API 3200 LC/MS/MS system (AB Sciex, Concord, Canada). NMR spectra were recorded on an AVANCE 600 (Bruker, Rheinstetten, Germany). The chemical shifts were represented as parts per million (ppm) referenced to the residual solvent signal. Column chromatography was carried out using a Kieselgel 60, 63-200 $\mu \mathrm{m}$ and 40-63 $\mu \mathrm{m}$ (Merck, Darmstadt, Germany) and YMC gel ODS-A, $150 \mu \mathrm{~m}$ (YMC, Kyoto, Japan). TLC was performed on a glass backed Kieselgel $60 \mathrm{~F}_{254}$ and RP $\mathrm{F}_{254 \mathrm{~s}}$ plates. All other chemicals and reagents used were of analytical grade. Electric eel acetylcholinesterase, acetylthiocholine iodide, and 5-5'-thiobis-2-nitrobenzoic acid (DTNB) were purchased from Sigma (Sigma-Aldrich Co., St. Louis, USA).

Plant material - A stem of an A. polymorpha was collected from Mt. Samyung (Gangwon Province, Korea) in August, 2014. A voucher specimen (KNUH-S-1408-2) was deposited in the Herbarium of the College of Pharmacy, Kangwon National University, Korea.

Extraction and isolation - The air dried stem of $A$. polymorpha was cut into small pieces and extracted with hot $\mathrm{MeOH}(3.0 \mathrm{~kg}, 20 \mathrm{~L} \times 2)$ for 4 hrs. All extracts were
combined and concentrated in vacuo at $40^{\circ} \mathrm{C}$. The MeOH extract ( 367 g ) was suspended in water and the successively partitioned with $n$-hexane, $\mathrm{CHCl}_{3}$, and $n$ - BuOH , leaving a residual water soluble fraction. Each soluble fraction was evaporated in vacuo to yield residues of $n$-hexane fraction (fr.) ( 23.5 g ), $\mathrm{CHCl}_{3}$ fr. ( 16.6 g ), and $n$ - BuOH fr. ( 24.5 g ). Among the solvent fractions, $n$-hexane fr. and $\mathrm{CHCl}_{3}$ fr. showed inhibition rates of $58.8 \%$ and $57.0 \%$ against acetylcholinesterase at $100 \mu \mathrm{~g} / \mathrm{ml}$, respectively. To isolate active compounds from these two fractions, various column chromatography were performed. The $n$-hexane soluble fraction ( 20 g ) was applied to silica gel column chromatography ( $63-200 \mu \mathrm{~m}, 10 \times 50 \mathrm{~cm}, 600 \mathrm{~g}$ ) using isocratic elution with benzene: EtOAc ( $5: 1,100 \mathrm{~mL}$ each), in order to divide the fraction into seven fractions (Fr. 1 - Fr. 7). Fr. $2(5.1 \mathrm{~g})$ was applied to further chromatography ( $63-200 \mu \mathrm{~m}, 5 \times 50 \mathrm{~cm}, 180 \mathrm{~g}$ ) to yield four sub-fractions (Fr. 2-1 - Fr. 2-4). Fr. 2-2 ( 0.9 g ) was applied to further chromatography on a flash column (Redisep ${ }^{\circledR}$ ODS, 43 g ) and silica gel ( $100 \mathrm{~g}, 63-200 \mu \mathrm{~m}, 3 \times 50 \mathrm{~cm}$ ) using isocratic elution with $\mathrm{MeOH}: \mathrm{H}_{2} \mathrm{O}(75: 25$; flow rate : 20 $\mathrm{mL} / \mathrm{min}$ ) and benzene : EtOAc ( $99: 1,50 \mathrm{~mL}$ each), to give compound $\mathbf{1}(65.4 \mathrm{mg})$. Fr. 2-3 ( 1.6 g ) was applied to further chromatography on a flash column (Redisep ${ }^{\circledR}$ ODS, 43 g ) using isocratic elution with MeOH : $\mathrm{H}_{2} \mathrm{O}(80$ : 20; flow rate : $30 \mathrm{~mL} / \mathrm{min}$ ) to yield three sub-fractions ( Fr . 2-3-1 - Fr. 2-3-3). Fr. 2-3-1 ( 0.7 g ) was applied to further chromatography on a silica gel ( $40-63 \mu \mathrm{~m}, 2.5 \times 30 \mathrm{~cm}$, 30 g ) and ODS (YMC gel, $150 \mu \mathrm{~m}, 3 \times 50 \mathrm{~cm}, 70 \mathrm{~g}$ ) using isocratic elution with $n$-hexane: EtOAc $(4: 1,30 \mathrm{~mL}$ each) and $\mathrm{MeOH}: \mathrm{H}_{2} \mathrm{O}(70: 30,20 \mathrm{~mL}$ each $)$ to give compound $3(7.0 \mathrm{mg})$ and $\mathbf{4}(10.0 \mathrm{mg})$. Fr. 2-3-2 ( 0.5 g ) was applied to further chromatography on a silica gel ( $40-63 \mu \mathrm{~m}, 2.5 \times 50 \mathrm{~cm}, 30 \mathrm{~g}$ ) using isocratic elution with $n$-hexane : EtOAc ( $5: 1,20 \mathrm{~mL}$ each) to give compound $2(66.1 \mathrm{mg})$ and compound $5(7.0 \mathrm{mg})$, respectively. Fr. 2-4 ( 2.8 g ) was applied to further chromatography on a flash column (Redisep ${ }^{\circledR}$ ODS, 43g) using isocratic elution with $\mathrm{MeOH}: \mathrm{H}_{2} \mathrm{O}(70: 30$; flow rate : $20 \mathrm{~mL} / \mathrm{min}$ ) to give compound $9(7.7 \mathrm{mg})$. Fr. $3(4.5 \mathrm{~g})$ was applied to further chromatography on a flash column (Redisep ${ }^{\text {® }}$ ODS, 130 g ) using isocratic elution with $\mathrm{MeOH}: \mathrm{H}_{2} \mathrm{O}$ ( $70: 30$; flow rate : $30 \mathrm{~mL} / \mathrm{min}$ ) to yield seven subfractions (Fr. 2-3-1 - Fr. 2-3-7). Fr. 2-3-3 (0.3 g) was applied to further chromatography on a silica gel (40-63 $\mu \mathrm{m}, 2.5 \times 30 \mathrm{~cm}, 30 \mathrm{~g})$ isocratic elution with benzene : EtOAc (19:1, 20 mL each) to give compound 6 ( 70.0 $\mathrm{mg})$. Fr. 2-3-4 $(0.4 \mathrm{~g})$ was re-chromatographed on a silica gel ( $40-63 \mu \mathrm{~m}, 2.5 \times 30 \mathrm{~cm}, 30 \mathrm{~g}$ ) isocratic elution with benzene : EtOAc (19: 1, 20 mL each) to give compound
$8(29.6 \mathrm{mg})$. Fr. 2-3-5 ( 0.7 g ) was re-chromatographed on a silica gel ( $40-63 \mu \mathrm{~m}, 2.5 \times 30 \mathrm{~cm}, 30 \mathrm{~g}$ ) isocratic elution with benzene : EtOAc (19:1, 20 mL each) to give compound $7(118.1 \mathrm{mg})$. Fr. $6(1.5 \mathrm{~g})$ was applied to further chromatography on a silica gel ( $63-200 \mu \mathrm{~m}, 10 \times 50 \mathrm{~cm}$, 300 g ) using isocratic elution with $n$-hexane : EtOAc ( 2 : $1,100 \mathrm{~mL}$ each) to yield four sub-fractions (Fr.6-1 - Fr. 6-4). Fr. 6-3 ( 0.9 g ) was re-chromatographed on a flash column (Redisep ${ }^{\text {® }}$ ODS, 43 g ) using isocratic elution with $\mathrm{MeOH}: \mathrm{H}_{2} \mathrm{O}(70: 30$; flow rate : $20 \mathrm{~mL} / \mathrm{min}$ ) to give compound $10(348.6 \mathrm{mg})$. The $\mathrm{CHCl}_{3}$ soluble fraction $(16 \mathrm{~g})$ was applied to silica gel medium pressure liquid chromatography (63-200 $\mu \mathrm{m}$, Büchi, $5 \times 40 \mathrm{~cm}, 400 \mathrm{~g}$ ) using isocratic elution with benzene : EtOAc (2:1, 100 mL each), in order to divide the fraction into seven fractions (CFr. $1-$ CFr. 7). CFr. 3 ( 0.9 g ) was applied to further chromatography on a flash column (Redisep ${ }^{\text {® }}$ ODS, 43 g ) using isocratic elution with $\mathrm{MeOH}: \mathrm{H}_{2} \mathrm{O}(60$ : 30; flow rate : $20 \mathrm{~mL} / \mathrm{min}$ ) to yield five sub-fractions (CFr. 3-1 - CFr. 3-5). CFr. 3-4 ( 0.5 g ) was re-chromatographed on a silica gel ( $40-63 \mu \mathrm{~m}, 2.5 \times 50 \mathrm{~cm}, 100 \mathrm{~g}$ ) isocratic elution with $\mathrm{CHCl}_{3}: \mathrm{MeOH}(49: 1,50 \mathrm{~mL}$ each $)$ to give compounds $\mathbf{1 1}(3.2 \mathrm{mg})$ and $12(4.4 \mathrm{mg})$. CFr. 4 $(1.1 \mathrm{~g})$ was applied to further chromatography on a flash column (Redisep ${ }^{\circledR}$ ODS, 130 g ) using gradient elution with $\mathrm{MeOH}: \mathrm{H}_{2} \mathrm{O}(40: 30$; flow rate $: 30 \mathrm{~mL} / \mathrm{min}) \rightarrow$ $\mathrm{MeOH}: \mathrm{H}_{2} \mathrm{O}(50: 50$; flow rate $: 30 \mathrm{~mL} / \mathrm{min})$ to yield five sub-fractions (CFr. 4-1 - CFr. 4-5). CFr. 4-2 (0.3 g) and CFr. 4-3 $(0.4 \mathrm{~g})$ were applied to chromatography on a silica gel ( $40-63 \mu \mathrm{~m}, 2.5 \times 30 \mathrm{~cm}, 40 \mathrm{~g}$ ) isocratic elution with benzene : $\operatorname{EtOAc}(3: 1,20 \mathrm{~mL}$ each $)$ to give compounds $\mathbf{1 3}(32.6 \mathrm{mg})$ and $\mathbf{1 4}(112.2 \mathrm{mg})$.

Compound $1-{ }^{1} \mathrm{H}-\mathrm{NMR}\left(600 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta: 8.15$ ( $1 \mathrm{H}, \mathrm{d}, J=9.8 \mathrm{~Hz}, \mathrm{H}-4$ ), $7.59\left(1 \mathrm{H}, \mathrm{d}, J=2.3 \mathrm{~Hz}, \mathrm{H}-2^{\prime}\right)$, 7.14 ( $1 \mathrm{H}, \mathrm{s}, \mathrm{H}-8$ ), 6.95 ( $1 \mathrm{H}, \mathrm{d}, J=2.3 \mathrm{~Hz}, \mathrm{H}-3$ '), 6.26 ( $1 \mathrm{H}, \mathrm{d}, J=9.8 \mathrm{~Hz}, \mathrm{H}-3$ ), 5.54 ( $1 \mathrm{H}, \mathrm{t}, J=6.9 \mathrm{~Hz}, \mathrm{H}-2 \mathrm{C}$ ), $4.92(2 \mathrm{H}, \mathrm{d}, J=6.9 \mathrm{~Hz}, \mathrm{H}-1$ "), 1.80 and 1.70 (each $3 \mathrm{H}, \mathrm{s}$, $\left.\left(\mathrm{CH}_{3}\right)_{2}\right] ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(150 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta: 161.29(\mathrm{C}-2)$, 158.13 (C-7), 152.66 (C-9), 148.97 (C-5), 144.89 (C-2), 139.83 (C-3"), 139.58 (C-4), 119.10 (C-2"), 114.18 (C-6), 112.54 (C-10), 107.50 (C-3), 105.06 (C-3'), 94.20 (C-8), $69.74(\mathrm{C}-1 "), 25.82\left(\mathrm{CH}_{3}\right), 18.22\left(\mathrm{CH}_{3}\right)$; ESI-MS $m / z, 293$ $[\mathrm{M}+\mathrm{Na}]^{+}$

Compound $2-{ }^{1} \mathrm{H}-\mathrm{NMR}\left(600 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta: 8.09$ ( $1 \mathrm{H}, \mathrm{d}, J=9.8 \mathrm{~Hz}, \mathrm{H}-4$ ), $7.60\left(1 \mathrm{H}, \mathrm{d}, J=2.2 \mathrm{~Hz}, \mathrm{H}-2^{\prime}\right)$, $6.98\left(1 \mathrm{H}, \mathrm{d}, J=2.2 \mathrm{~Hz}, \mathrm{H}-3^{\prime}\right), 6.24(1 \mathrm{H}, \mathrm{d}, J=9.8 \mathrm{~Hz}, \mathrm{H}-$ 3), 5.58 ( $1 \mathrm{H}, \mathrm{t}, J=7.2 \mathrm{~Hz}, \mathrm{H}-2$ "), 4.83 ( $2 \mathrm{H}, \mathrm{d}, J=7.2 \mathrm{~Hz}$, $\mathrm{H}-1$ "), $4.16\left(3 \mathrm{H}, \mathrm{s},-\mathrm{OCH}_{3}\right), 1.72$ and 1.68 [each $3 \mathrm{H}, \mathrm{s}$, $\left.\left(\mathrm{CH}_{3}\right)_{2}\right] ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(150 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta: 160.53(\mathrm{C}-2)$, 150.79 (C-7), 145.08 (C-2'), 144.38 (C-5), 144.32 (C-9),
139.59 (C-3"), 139.42 (C-4), 126.82 (C-8), 119.88 (C-2"), 114.49 (C-6), 112.69 (C-3), 107.49 (C-10), 105.10 (C-3'), $70.35(\mathrm{C}-1 \mathrm{l}), 60.74\left(\mathrm{OCH}_{3}\right), 25.80\left(\mathrm{CH}_{3}\right), 18.06\left(\mathrm{CH}_{3}\right)$; ESI-MS $m / z, 323\left[\mathrm{M}^{+} \mathrm{H}\right]^{+}$
Compound $3-{ }^{1} \mathrm{H}-\mathrm{NMR}\left(600 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta: 8.15$ $(1 \mathrm{H}, \mathrm{d}, J=9.8 \mathrm{~Hz}, \mathrm{H}-4), 7.60\left(1 \mathrm{H}, \mathrm{d}, J=2.5 \mathrm{~Hz}, \mathrm{H}-2^{\prime}\right)$, $7.13(1 \mathrm{H}, \mathrm{s}, \mathrm{H}-8), 7.02\left(1 \mathrm{H}, \mathrm{d}, J=2.5 \mathrm{~Hz}, \mathrm{H}-3^{\prime}\right), 6.27$ $(1 \mathrm{H}, \mathrm{d}, J=9.8 \mathrm{~Hz}, \mathrm{H}-3), 4.27\left(3 \mathrm{H}, \mathrm{s}, \mathrm{OCH}_{3}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}$ $\left(150 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta: 161.23(\mathrm{C}-2), 158.39$ (C-7), 152.72 (C-9), 149.59 (C-5), 144.79 (C-2'), 139.26 (C-4), 112.69 (C-6), 112.56 (C-3), 106.42 (C-10), 105.04 (C-3'), 93.85 (C-8), $60.10\left(\mathrm{OCH}_{3}\right)$; ESI-MS $m / z, 217[\mathrm{M}+\mathrm{H}]^{+}$

Compound $4-{ }^{1} \mathrm{H}-\mathrm{NMR}\left(600 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta: 7.57$ ( $1 \mathrm{H}, \mathrm{d}, J=9.4 \mathrm{~Hz}, \mathrm{H}-4$ ), 7.04 ( $1 \mathrm{H}, \mathrm{s}, \mathrm{H}-5$ ), $6.71(1 \mathrm{H}, \mathrm{s}, \mathrm{H}-$ 8), $6.33\left(1 \mathrm{H}, \mathrm{d}, J=9.9 \mathrm{~Hz}, \mathrm{H}-3^{\prime}\right), 6.21(1 \mathrm{H}, \mathrm{d}, J=9.4 \mathrm{~Hz}$, $\mathrm{H}-3), 5.68$ ( $1 \mathrm{H}, J=9.9 \mathrm{~Hz}, \mathrm{H}-4$ '), 1.46 [ $\left.6 \mathrm{H}, \mathrm{s},\left(\mathrm{CH}_{3}\right)_{2}\right]$; ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(150 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta: 161.19$ (C-2), 156.84 (C-7), 155.44 (C-9), 144.35 (C-4), 131.23 (C-3'), 124.77 (C-5), 120.78 (C-4'), 118.52 (C-6), 113.02 (C-3), 112.78 (C-10), $104.39(\mathrm{C}-8), 77.73(\mathrm{C}-2), 28.34\left(\mathrm{CH}_{3} \times 2\right)$; ESIMS $m / z, 229[\mathrm{M}+\mathrm{H}]^{+}$

Compound $5-{ }^{1} \mathrm{H}-\mathrm{NMR}\left(600 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta: 8.12$ $(1 \mathrm{H}, \mathrm{d}, J=9.8 \mathrm{~Hz}, \mathrm{H}-4), 7.63\left(1 \mathrm{H}, \mathrm{d}, J=2.2 \mathrm{~Hz}, \mathrm{H}-2^{\prime}\right)$, $6.95(1 \mathrm{H}, \mathrm{d}, J=2.2 \mathrm{~Hz}, \mathrm{H}-3 '), 6.29(1 \mathrm{H}, \mathrm{d}, J=9.8 \mathrm{~Hz}, \mathrm{H}-$ 3), 5.53 ( $1 \mathrm{H}, \mathrm{t}, J=7.1 \mathrm{~Hz}, \mathrm{H}-2$ "), 4.79 ( $2 \mathrm{H}, \mathrm{d}, J=7.1 \mathrm{~Hz}$, $\mathrm{H}-1$ "), $4.12\left(3 \mathrm{H}, \mathrm{s}, \mathrm{OCH}_{3}\right), 1.79$ and 1.66 [each $3 \mathrm{H}, \mathrm{s}$, $\left.\left(\mathrm{CH}_{3}\right)_{2}\right] ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(150 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta: 160.55(\mathrm{C}-2)$, 149.69 (C-7), 145.21 (C-2'), 143.58 (C-5), 143.44 (C-9), 139.85 (C-3"), 139.75 (C-4), 128.61 (C-8), 119.22 (C-2"), 116.54 (C-6), 112.90 (C-3), 108.96 (C-10), 105.09 (C-3'), $70.55(\mathrm{C}-1 \mathrm{l}), 61.69\left(\mathrm{OCH}_{3}\right), 25.82\left(\mathrm{CH}_{3}\right), 18.16\left(\mathrm{CH}_{3}\right)$; ESI-MS $m / z, 323[\mathrm{M}+\mathrm{Na}]^{+}$

Comound $6-{ }^{1} \mathrm{H}-\mathrm{NMR}\left(600 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta: 7.73$ $(1 \mathrm{H}, \mathrm{s}, \mathrm{H}-5), 7.66(1 \mathrm{H}, \mathrm{d}, J=9.5 \mathrm{~Hz}, \mathrm{H}-4), 6.84(1 \mathrm{H}, \mathrm{s}$, $\mathrm{H}-8), 6.60\left(1 \mathrm{H}, \mathrm{br} \mathrm{s}, \mathrm{H}-2^{\prime}\right), 6.29(1 \mathrm{H}, \mathrm{d}, J=9.5 \mathrm{~Hz}, \mathrm{H}-3)$, $3.94\left(3 \mathrm{H}, \mathrm{s}, \mathrm{OCH}_{3}\right), 2.23$ and 1.98 [each $\left.3 \mathrm{H}, \mathrm{s},\left(\mathrm{CH}_{3}\right)_{2}\right]$; ${ }^{13} \mathrm{C}-\mathrm{NMR}\left(150 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta: 190.51$ (C-1'), 160.77 (C-7), 160.499 (C-2), 157.35 (C-9), 156.70 (C-3'), 143.33 (C-4), 130.36 (C-5), 128.28 (C-6), 124.85 (C-2'), 113.96 $(\mathrm{C}-3), 112.20(\mathrm{C}-10), 99.65(\mathrm{C}-8), 56.29\left(\mathrm{OCH}_{3}\right), 28.12$ $\left(\mathrm{CH}_{3}\right), 21.45\left(\mathrm{CH}_{3}\right) ;$ ESI-MS m/z, $259[\mathrm{M}+\mathrm{H}]^{+}$
Compound $7-[\alpha]_{\mathrm{D}}{ }^{15}-100.7^{\circ}$ (c, 0.1 in MeOH ); ${ }^{1} \mathrm{H}-$ NMR ( $600 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta: 6.33(1 \mathrm{H}, \mathrm{s}, \mathrm{H}-8), 5.99(1 \mathrm{H}$, s, H-3), 5.11 ( $\left.1 \mathrm{H}, \mathrm{dd}, J=5.2,5.5 \mathrm{~Hz}, \mathrm{H}-3^{\prime}\right), 2.98$ ( 1 H , dd, $\left.J=5.2,17.5 \mathrm{~Hz}, \mathrm{H}-\mathrm{H}^{\prime} \mathrm{a}\right), 2.77$ ( $1 \mathrm{H}, \mathrm{dd}, J=5.2,17.5 \mathrm{~Hz}, \mathrm{H}-$ $\left.4^{\prime} \mathrm{b}\right), 2.33\left(3 \mathrm{H}, \mathrm{s}, 2-\mathrm{CH}_{3}\right), 2.07\left(3 \mathrm{H}, \mathrm{s}, 3^{\prime}-\mathrm{COCH}_{3}\right), 1.36$ and 1.33 [each $3 \mathrm{H}, \mathrm{s}, 2^{\prime}-\left(\mathrm{CH}_{3}\right) \times 2$ ]; ${ }^{13} \mathrm{C}-\mathrm{NMR}(150 \mathrm{MHz}$, $\left.\mathrm{CDCl}_{3}\right) \delta: 182.53(\mathrm{C}-4), 170.30\left(3^{\prime}-\mathrm{COCH}_{3}\right), 166.79(\mathrm{C}-$ 2), 159.52 (C-5), 158.72 (C-7), 156.20 (C-9), 108.37 (C3), 104.42 (C-10), 102.38 (C-6), 94.79 (C-8), 69.74 (C-
$\left.3^{\prime}\right), 24.67\left(2^{\prime}-\mathrm{CH}_{3}\right), 23.01\left(2^{\prime}-\mathrm{CH}_{3}\right), 22.60\left(\mathrm{C}-4^{\prime}\right), 21.06$ $\left(3 '-\mathrm{COCH}_{3}\right), 20.55\left(2-\mathrm{CH}_{3}\right)$; ESI-MS m/z, $319[\mathrm{M}+\mathrm{H}]^{+}$
Compound 8 - ${ }^{1} \mathrm{H}-\mathrm{NMR}\left(600 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta: 7.67$ $(1 \mathrm{H}, \mathrm{d}, J=9.4 \mathrm{~Hz}, \mathrm{H}-4), 7.20(1 \mathrm{H}, \mathrm{s}, \mathrm{H}-5), 7.06(1 \mathrm{H}, \mathrm{s}$, $\mathrm{H}-8), 6.23(1 \mathrm{H}, \mathrm{d}, J=9.4 \mathrm{~Hz}, \mathrm{H}-3), 5.32(1 \mathrm{H}, \mathrm{t}, J=7.3$ $\mathrm{Hz}, \mathrm{H}-2^{\prime}$ ), 3.37 ( $2 \mathrm{H}, \mathrm{d}, J=7.3 \mathrm{~Hz}, \mathrm{H}-\mathrm{l}^{\prime}$ ), 1.78 and 1.73 [each $\left.3 \mathrm{H}, \mathrm{s},\left(\mathrm{CH}_{3}\right)_{2}\right] ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(150 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta:$ 162.75 (C-2), 158.72 (C-7), 154.04 (C-9), 144.58 (C-4), 134.68 (C-3'), 128.22 (C-5), 126.15 (C-6), 121.12 (C-2'), 112.18 (C-10), 111.98 (C-3), 103.12 (C-8), 28.24 (C-1'), $25.82\left(\mathrm{CH}_{3}\right), 17.87\left(\mathrm{CH}_{3}\right) ;$ ESI-MS $m / z, 231[\mathrm{M}+\mathrm{H}]^{+}$

Compound $9-{ }^{1} \mathrm{H}-\mathrm{NMR}\left(600 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta: 7.86$ ( $1 \mathrm{H}, \mathrm{s}, \mathrm{H}-5$ ), 7.70 ( $1 \mathrm{H}, \mathrm{d}, J=9.4 \mathrm{~Hz}, \mathrm{H}-4$ ), 6.89 ( $1 \mathrm{H}, \mathrm{s}$, $\mathrm{H}-5), 6.33(1 \mathrm{H}, \mathrm{d}, J=9.4 \mathrm{~Hz}, \mathrm{H}-3), 4.00\left(3 \mathrm{H}, \mathrm{s}, \mathrm{OCH}_{3}\right)$, $2.89\left(2 \mathrm{H}, \mathrm{d}, J=6.8 \mathrm{~Hz}, \mathrm{H}-2^{\prime}\right), 2.24\left(1 \mathrm{H}, \mathrm{m}, \mathrm{H}-3^{\prime}\right), 0.96$ $\left[6 \mathrm{H}, \mathrm{d}, J=6.7 \mathrm{~Hz},\left(\mathrm{CH}_{3}\right)_{2}\right] ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(150 \mathrm{MHz}, \mathrm{CDCl}_{3}\right)$ $\delta: 200.58$ (C-1'), 161.26 (C-7), 160.32 (C-2), 157.77 (C9), 143.33 (C-4), 130.55 (C-5), 126.23 (C-6), 114.17 (C3), $112.30(\mathrm{C}-10), 99.72(\mathrm{C}-8), 56.21\left(\mathrm{OCH}_{3}\right), 52.71(\mathrm{C}-$ $\left.2^{\prime}\right), 24.99\left(\mathrm{C}-3^{\prime}\right), 22.71\left[\left(\mathrm{CH}_{3}\right)_{2}\right]$; ESI-MS $m / z, 261[\mathrm{M}+\mathrm{H}]^{+}$

Compound $\mathbf{1 0}-[\alpha]_{\mathrm{D}}{ }^{15}-75.3^{\circ}(\mathrm{c}, 0.1$ in MeOH$)$; ${ }^{1} \mathrm{H}-$ NMR ( $600 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta: 12.96(1 \mathrm{H}, \mathrm{s}, 5-\mathrm{OH}), 6.29$ ( $1 \mathrm{H}, \mathrm{s}, \mathrm{H}-8$ ), $5.97(1 \mathrm{H}, \mathrm{s}, \mathrm{H}-3), 3.86\left(1 \mathrm{H}, \mathrm{br} \mathrm{s}, \mathrm{H}-3^{\prime}\right), 2.93$ ( $1 \mathrm{H}, \mathrm{dd}, J=4.9,17.1 \mathrm{~Hz}, \mathrm{H}-4$ '), 2.75 ( 1 H , dd, $J=4.9$, $\left.17.1 \mathrm{~Hz}, \mathrm{H}-4^{\prime}\right), 2.32\left(3 \mathrm{H}, \mathrm{s}, 2-\mathrm{CH}_{3}\right), 1.39,1.34$ [each 3 H , s, gem- $\left.\left(\mathrm{CH}_{3}\right)_{2}\right] ;{ }^{13} \mathrm{C}$-NMR ( $150 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta: 182.50$ (C-4), 166.81 (C-2), 159.66 (C-5), 158.98 (C-7), 156.18 (C-9), 108.29 (C-3), 104.31 (C-10), 102.93 (C-6), 94.79 (C-8), 78.48 (C-2'), 68.69 (C-3'), 25.35 (C-4'), 24.83 $\left(\mathrm{gem}-\mathrm{CH}_{3}\right), 22.09\left(\mathrm{gem}-\mathrm{CH}_{3}\right), 20.54\left(2-\mathrm{CH}_{3}\right) ;$ ESI-MS m/ $z, 299[\mathrm{M}+\mathrm{H}]^{+}$

Compound $11-[\alpha]_{\mathrm{D}}{ }^{15}+112.6^{\circ}(\mathrm{c}, 0.1$ in MeOH$)$; ${ }^{1} \mathrm{H}-$ NMR ( $600 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta: 12.93(1 \mathrm{H}, \mathrm{s}, 5-\mathrm{OH}), 6.30$ $(1 \mathrm{H}, \mathrm{s}, \mathrm{H}-8), 6.01(1 \mathrm{H}, \mathrm{s}, \mathrm{H}-3), 4.76(1 \mathrm{H}, \mathrm{dd}, J=9.4,8.2$ $\left.\mathrm{Hz}, \mathrm{H}-2^{\prime}\right), 3.17$ ( $\left.1 \mathrm{H}, \mathrm{dd}, J=9.4,15.7 \mathrm{~Hz}, \mathrm{H}-3^{\prime}\right), 3.09(1 \mathrm{H}$, $\left.\mathrm{dd}, J=8.2,15.7 \mathrm{~Hz}, \mathrm{H}-3^{\prime}\right), 2.33\left(3 \mathrm{H}, \mathrm{s}, 2-\mathrm{CH}_{3}\right), 1.34,1.23$ [each 3H, s, gem- $\left(\mathrm{CH}_{3}\right)_{2}$ ]; ${ }^{13} \mathrm{C}$-NMR ( $\left.150 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta$ : 182.64 (C-4), 166.54 (C-7), 165.80 (C-2), 158.34 (C-5), 156.68 (C-9), 108.86 (C-3), 108.64 (C-6), 105.49 (C-10), 91.73 (C-8), 88.85 (C-2'), 71.91 (C-1"), 26.78 (C-3"), 25.88 (C-2"), 23.88 (C-2-CH33), 20.42 (C-3'); ESI-MS $m / z$, $277[\mathrm{M}+\mathrm{H}]^{+}$

Compound $12-{ }^{1} \mathrm{H}-\mathrm{NMR}\left(600 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta: 12.90$ $(1 \mathrm{H}, \mathrm{s}, 5-\mathrm{OH}), 6.34(1 \mathrm{H}, \mathrm{s}, \mathrm{H}-8), 6.32(1 \mathrm{H}, \mathrm{s}, \mathrm{H}-3), 5.11$ ( $1 \mathrm{H}, \mathrm{dd}, J=5.1,4.6 \mathrm{~Hz}, \mathrm{H}-3$ '), 4.55 ( 2 H , br s, $2-\mathrm{CH}_{2}$ ), $2.99\left(1 \mathrm{H}, \mathrm{dd}, J=5.1,17.7 \mathrm{~Hz}, \mathrm{H}-4^{\prime} \beta\right), 2.78(1 \mathrm{H}$, dd, $J=4.6,17.7 \mathrm{~Hz}, \mathrm{H}^{\prime} 4^{\prime} \alpha$ ), 2.07 ( $3 \mathrm{H}, \mathrm{s}, 2^{\prime \prime}-\mathrm{CH}_{3} \mathrm{COO}$ ), 1.37 , 1.34 [each 3 H , s, gem- $\left(\mathrm{CH}_{3}\right)_{2}$ ]; ${ }^{13} \mathrm{C}-\mathrm{NMR}(150 \mathrm{MHz}$, $\left.\mathrm{CDCl}_{3}\right) \delta: 182.56(\mathrm{C}-4), 170.35\left(\mathrm{COCH}_{3}\right), 167.98(\mathrm{C}-2)$, 159.62 (C-5), 159.06 (C-9), 155.87 (C-7), 106.38 (C-3),
104.86 (C-10), 94.96 (C-8), 69.68 (C-3'), 61.42 (C-2$\left.\mathrm{CH}_{2} \mathrm{OH}\right), 24.67\left(\mathrm{C}-3 '-\mathrm{CH}_{3}\right), 23.04\left(\mathrm{C}-3 '-\mathrm{CH}_{3}\right), 22.60(\mathrm{C}-$ $\left.4^{\prime}\right), 21.06\left(\mathrm{COCH}_{3}\right)$; ESI-MS m/z, $335[\mathrm{M}+\mathrm{H}]^{+}$

Compound $13-{ }^{1} \mathrm{H}-\mathrm{NMR}\left(600 \mathrm{MHz}\right.$, acetone- $\mathrm{d}_{6}$ ) $\delta$ : $7.83(1 \mathrm{H}, \mathrm{d}, J=9.7 \mathrm{~Hz}, \mathrm{H}-4), 7.18(1 \mathrm{H}, \mathrm{s}, \mathrm{H}-5), 6.78(1 \mathrm{H}$, $\mathrm{s}, \mathrm{H}-8), 6.16(1 \mathrm{H}, \mathrm{d}, J=9.7 \mathrm{~Hz}, \mathrm{H}-3), 3.89(3 \mathrm{H}, \mathrm{s}$, $\left.\mathrm{OCH}_{3}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}\left(150 \mathrm{MHz}\right.$, acetone-d $\left.\mathrm{d}_{6}\right) \delta: 160.44(\mathrm{C}-$ 2), 150.93 (C-9), 150.23 (C-7), 145.06 (C-6), 143.77 (C4), 112.39 (C-3), 111.19 (C-10), 109.07 (C-5), 102.81 (C8), $55.82\left(\mathrm{OCH}_{3}\right)$; ESI-MS m/z, $193[\mathrm{M}+\mathrm{H}]^{+}$

Compound $14-[\alpha]_{\mathrm{D}}{ }^{11}-255.1^{\circ}$ (c, 0.1 in MeOH ); ${ }^{1} \mathrm{H}-$ NMR ( 600 MHz , acetone- $\mathrm{d}_{6}$ ) $\delta: 7.61(1 \mathrm{H}, \mathrm{d}, J=15.9 \mathrm{~Hz}$, H-7), $7.30(1 \mathrm{H}, \mathrm{d}, J=1.9 \mathrm{~Hz}, \mathrm{H}-2), 7.29(2 \mathrm{H}, \mathrm{d}, J=8.4$ Hz, H-2', H-6'), 7.11 (1H, dd, $J=1.9,8.2 \mathrm{~Hz}, \mathrm{H}-6), 6.87$ $(1 \mathrm{H}, \mathrm{d}, J=8.2 \mathrm{~Hz}, \mathrm{H}-5), 6.83(2 \mathrm{H}, \mathrm{d}, J=8.4 \mathrm{~Hz}, \mathrm{H}-3 ', \mathrm{H}-$ $\left.5^{\prime}\right), 6.38(1 \mathrm{H}, \mathrm{d}, J=15.9 \mathrm{~Hz}, \mathrm{H}-8), 4.92\left(1 \mathrm{H}, \mathrm{m}, \mathrm{H}-7{ }^{\prime}\right)$, $4.22\left(2 \mathrm{H}, \mathrm{m}, \mathrm{H}-8\right.$ '), $3.90\left(3 \mathrm{H}, \mathrm{s}, \mathrm{OCH}_{3}\right) ;{ }^{13} \mathrm{C}-\mathrm{NMR}(150$ MHz , acetone- $\mathrm{d}_{6}$ ) $\delta: 166.72$ (C-9), 156.91 (C-4'), 149.23 (C-4), 147.90 (C-3), 145.08 (C-7), 132.57 (C-1'), 127.54 (C-2', C-6'), 126.56 (C-1), 123.11 (C-6), 115.27 (C-5), 115.02 (C-3', C-5'), 114.86 (C-8), 110.45 (C-2), 71.20 (C7'), 69.14 (C-8'), $55.49\left(\mathrm{OCH}_{3}\right)$; ESI-MS m/z, $331[\mathrm{M}+\mathrm{H}]^{+}$

Determination of acetylcholinesterase inhibitory activity - The acetylcholinesterase inhibition assay was
measured according to the method of Ellman et. al. ${ }^{15}$ with a slight modification. Tested compounds were dissolved in DMSO. The reaction mixture had a final volume of 1 mL , contained a sodium phosphate buffer $(100 \mathrm{mM}, \mathrm{pH}$ 8.0 ), up to $10 \mu \mathrm{~L}$ of the tested sample solution, and $20 \mu \mathrm{~L}$ of acetylcholinesterase ( $5 \mathrm{U} / \mathrm{mL}$ ), which were mixed and incubated for 10 min at $37^{\circ} \mathrm{C}$. The reactions were started with the addition of $40 \mu \mathrm{~L}$ of 10 mM dithionitrobenzoic acid (DTNB) and $10 \mu \mathrm{~L}$ of 75 mM acetylthiocholine iodide (ATCI) as a substrate. The hydrolysis was monitored by following the formation of the yellow 5-thio-2-nitrobenzoate anion at 412 nm for 6 min using a spectrophotometer.

A control reaction was carried out using water instead of compounds.

Inhibition activity $(\%)=\left[1-\left(\Delta \mathrm{A}_{\text {sample }} / \Delta \mathrm{A}_{\text {control }}\right)\right] \times 100$
$\Delta \mathrm{A}_{\text {sample }}$ is the absorbance of the tested compounds and $\Delta \mathrm{A}_{\text {control }}$ is the absorbance of the control.

Measurements were performed twice, and the concentration of each test sample giving $50 \%$ activity inhibition $\left(\mathrm{IC}_{50}\right)$ was estimated from the least-squares regression line of the logarithmic concentration plotted against the remaining activity. Galanthamine hydrobromide was used as a reference compound.


1. $\mathrm{R}_{1}=\mathrm{O}$
2. $\mathrm{R}_{1}=\mathrm{OCH}_{3}$

3. $\mathrm{R}_{1}=\mathrm{OCH}_{3}$
$\mathrm{R}_{2}=\mathrm{H}$
4. $\mathrm{R}_{1}=\mathrm{O}$
$\mathrm{R}_{2}=\mathrm{OCH}_{3}$

5. 



9.


11
13. $\mathrm{R}_{1}=\mathrm{OCH}_{3} \quad \mathrm{R}_{2}=\mathrm{OH}$

7. $\mathrm{R}_{1}=\mathrm{OH}$
10. $\mathrm{R}_{1}=\mathrm{OH}$
12. $\mathrm{R}_{1}=\mathrm{OH}$

$\mathrm{R}_{2}=\mathrm{OH}$





14

Fig. 1. Structures of 1-14.

## Results and Discussion

Compounds 1-5, 7, 8, and 10-14 were identified as isoimperatorin, ${ }^{16}$ phellopterin, ${ }^{16}$ bergapten, ${ }^{16}$ xanthyletin,,${ }^{17,18}$ cnidilin, ${ }^{19,20}(-)$-3'-acetylhamaudol, ${ }^{21} 7$-demethylsuberosine, ${ }^{22}$ $(-)$-hamudol, ${ }^{16}(+)$-visamminol, ${ }^{23}$ divaricatol, ${ }^{24}$ scopoletin, ${ }^{25}$ and decursidate, ${ }^{26,27}$ respectively, by comparing their physico-chemical and spectral data with those of literature values. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ spectrum of $\mathbf{6}$ exhibited two doublets at $\delta 7.66$ and 6.29 (each $1 \mathrm{H}, J=9.4 \mathrm{~Hz}$ ), and two singlet signals at $\delta 7.73$ and 6.84 (each 1 H ). Furthermore, ${ }^{1} \mathrm{H}-$ NMR spectrum of 6 exhibited two methyl signals at $\delta$ 2.23 and 1.98 (each $3 \mathrm{H}, \mathrm{s}$ ), and a broad singlet at $\delta 6.60$, and a methoxyl signal at $\delta 3.94$. These data showed $\mathbf{6}$ has a 6,7-disubstitued coumarin skeleton. ${ }^{28}{ }^{13} \mathrm{C}$-NMR spectrum of 6 exhibited characteristic signals assignable to a senecioyl moiety at $\delta 190.51156 .70,124.85,28.12$ and 21.45. HMBC spectrum showed a correlation between the methoxyl group at $\delta_{\mathrm{H}} 3.94$ and $\mathrm{C}-7$ at $\delta_{\mathrm{C}} 160.77$. This result showed that a methoxyl group attached at C-7 and a senecioyl group attached at C-6. On the basis of these data and on those previously reported in the literature, ${ }^{29,30,31}$ 6 was identified as dehydorgeijerin. ${ }^{1} \mathrm{H}$ - and ${ }^{13} \mathrm{C}-\mathrm{NMR}$ spectra of 9 is similar to those of 6 with the exception those of senecioly moiety. ${ }^{1} \mathrm{H}-\mathrm{NMR}$ spectrum of 9 exhibited a doublet at $2.89(2 \mathrm{H}, J=6.8 \mathrm{~Hz})$, a multiplet $(1 \mathrm{H})$ at $\delta 2.24$, and a doublet at $0.96(6 \mathrm{H}, J=6.7 \mathrm{~Hz})$, which showed that 9 has an isovaleryl moiety in the skeleton instead of senecioyl moiety. ${ }^{13} \mathrm{C}$-NMR spectrum of 9 exhibited signals at $\delta 200.58,52.71,24.99$, and 22.71 , which confirmed presence of an isovaleryl moiety in the skeleton of 9 . Comparing these data with those of literature, ${ }^{29,32} 9$ was identified as geijerin. Dehydrogeijerin (6) and geijerine (9) were isolated for the first time from genus Angelica. All isolates can divide five types of skeleton, simple coumarins $(\mathbf{6}, \mathbf{8}, \mathbf{9}, \mathbf{1 3})$, furanocoumarins $(\mathbf{1}, \mathbf{2}, \mathbf{3}, \mathbf{5})$, pyranocoumrins (4), chromones $(\mathbf{7}, \mathbf{1 0}, \mathbf{1 1}, \mathbf{1 2})$ and a ferulate (14). To determine the inhibitory activity of each compound, all isolates were tested for their inhibitory activity against acetylcholinesterase (Table 1). Acetylcholinesterase inhibition activities have been reported previously for tested compounds, isoimperatorin (1), xanthyletin (4), 7-demethylsuberosin (8), and scopoletin (13). ${ }^{33-35}$ As shown in Table 1, bergapten (3) exhibited a potent acetylcholinesterase inhibition activity with an $\mathrm{IC}_{50}$ value of $1.4 \mu \mathrm{M}$. Phellopterin (2) and cnidin (5) showed moderate acetylcholinesterase inhibition activity with $\mathrm{IC}_{50}$ values of 4.0 and $6.3 \mu \mathrm{M}$, respectively. Xanthyletin (4), dehydrogeijerin (6), demethylsuberosin (8), geijerin (9), (+)-visamminol (11) and divaricatol (12) showed mild acetylcholinesterase

Table 1. Acetylcholinesterase inhibitory activity of compounds 1-14

| Tested compounds | $\mathrm{IC}_{50}{ }^{1)}(\mu \mathrm{g} / \mathrm{ml})$ | $\mathrm{IC}_{50}(\mu \mathrm{M})$ |
| :---: | :---: | :---: |
| $\mathbf{1}$ | 7.7 | 28.5 |
| $\mathbf{2}$ | 1.3 | 4.0 |
| $\mathbf{3}$ | 0.3 | 1.4 |
| $\mathbf{4}$ | 2.0 | 8.8 |
| $\mathbf{5}$ | 1.9 | 6.3 |
| $\mathbf{6}$ | 2.5 | 9.7 |
| $\mathbf{7}$ | 4.0 | 12.6 |
| $\mathbf{8}$ | 2.0 | 8.7 |
| $\mathbf{9}$ | 2.7 | 10.4 |
| $\mathbf{1 0}$ | 6.9 | 23.2 |
| $\mathbf{1 1}$ | 2.4 | 8.7 |
| $\mathbf{1 2}$ | 4.3 | 12.9 |
| $\mathbf{1 3}$ | 7.2 | 37.5 |
| $\mathbf{1 4}$ | $>100$ | - |
| Galanthamine-hydrobromide $\left.{ }^{2}\right)$ | 0.3 | 0.8 |

${ }^{1)}$ The inhibitory activity dose that reduced $50 \%$ of aceylcholineseterase activity and expressed as mean of two different experiments.
${ }^{2)}$ A positive control.
inhibition activity with an $\mathrm{IC}_{50}$ values of $8.9,9.7,8.7$, $10.4,8.7$, and $12.9 \mu \mathrm{M}$, respectively; whereas isoimperatorin (1), (-)-hamaudol (10) and scopeoletin (13) showed weak acetylcholinesterase inhibition activity with $\mathrm{IC}_{50}$ values of $28.5,23.2$, and $37.5 \mu \mathrm{M}$, respectively, in the present study. Kang et al. ${ }^{35}$ studied structure-activity relationship of some coumarins and suggested that pyrone moiety in the coumarin skeleton may plays an important role on the acetylcholinesterase inhibitory activity. We isolated one pyranocoumarin, four furanocoumarins, and four simple coumarins, which gave us information to discuss structure-activity relationships between furanocoumarins and simple coumarins. From this data, methoxyl moiety at the C-5 position of furanocourmarin was considered required to induce exhibition of acetylcholinesterase inhibitory activity. In the inhibitory activity of chromone derivatives against acetylcholinesterase, furan moiety of chromone skeleton may play an important role in the acetylcholinesterase inhibition, though the number of tested compounds was very small.

In conclusion, this data shows that $A$. polymorpha extracts have potent acetylcholinesterase inhibitory activity, suggesting that it may be a useful therapeutic agent for Alzheimer's disease.

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