

Enzyme Inhibition and Phytochemical Profiling of *Ferula persica* Oleo-Gum-Resin Relevant to Alzheimer's Disease

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ABSTRACT

Numerous medicinal plants have proven valuable in managing Alzheimer's disease (AD). This research selected the oleo-gum-resin of *Ferula persica*, referred to as sagapenum, to examine its potential to inhibit enzymes linked to the development and advancement of AD. In addition, a previously unreported phytochemical profile was established. Inhibitory effects of dichloromethane, methanol, and aqueous extracts were evaluated in vitro against acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) employing the adapted Ellman's assay. The most effective extract underwent detailed phytochemical examination via multiple column chromatography approaches. The dichloromethane extract displayed marked selective inhibition of BuChE ($IC_{50} = 23.41 \mu\text{g/mL}$), relative to the standard drug donepezil ($IC_{50} = 1.97 \mu\text{g/mL}$). Analysis of this extract yielded the isolation and characterization of aurapten, farnesiferol A, umbelliprenin, farnesiferol C, farnesiferone A, karatavicinol, ferrocaulidin, and ligupersin A, all of which were screened for cholinesterase (ChE) inhibition. Farnesiferol A proved to be the strongest and most selective BuChE inhibitor ($IC_{50} = 31.46 \mu\text{g/mL}$). Furthermore, it demonstrated substantial inhibition of β -secretase 1 (BACE1) ($IC_{50} = 5.14 \mu\text{M}$), when benchmarked against the reference compound OM99-2 ($IC_{50} = 0.014 \mu\text{M}$), suggesting its potential as a multi-target agent for AD therapy. The pronounced selective BuChE inhibition by the dichloromethane extract of sagapenum, along with the activity of farnesiferol A—particularly its BACE1 suppression—could be instrumental in formulating novel dietary supplements for AD.

Keywords: Alzheimer's disease, *Ferula*, Oleo-gum-resin, Phytochemicals

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Introduction

Ferula persica Willd., native to Iran, is a member of the *Ferula* genus in the Apiaceae family. This family encompasses flowering, often aromatic plants that thrive mainly in temperate zones and are distributed extensively across the Mediterranean basin and Central Asia [1]. Iran hosts 112 genera, 316 species, and 75 endemic taxa from this family, among which the *Ferula* genus includes 30 species, 15 of them endemic. Locally, *Ferula* plants are known by names such as "Koma" or "Kema" [2]. *Ferula persica* occurs in northern and central Iran, Turkey, and Afghanistan, growing up to 1.5 meters tall [3] with robust, hollow, slightly fleshy stems and yellow flowers [4]. Compounds derived from *F. persica* predominantly feature sesquiterpene coumarins [5], sulfur-bearing molecules [6], and volatiles from essential oils [4]. These components underlie a range of pharmacological effects, including anti-inflammatory action [7], reversal of multi-drug resistance [8], depigmentation properties [9], as well as induction of apoptosis and cytotoxicity [10].

Sagapenum, called "Sakbinaj" in Persian, represents the oleo-gum-resin exuded from *F. persica* upon stem incision. It has a reddish or yellowish exterior with a white core [11] and has long been valued in traditional

medicine for purgative, anti-hysterical, and anti-diabetic actions [12], besides relieving joint stiffness and back pain. It has also been applied to manage epilepsy and paralysis, disorders marked by impaired sensory or motor function in affected body parts [11, 13]. Recent investigations into the antioxidant potential and composition of essential oils from *F. persica* oleo-gum-resin sourced from various Iranian locations, using diverse extraction techniques, revealed that microwave-assisted hydro-distillation and solvent-free microwave extraction delivered superior yields and bioactivity [14].

Alzheimer's disease (AD) is a progressive neurodegenerative illness manifesting as memory deterioration and cognitive dysfunction. It involves cholinergic dysfunction, particularly through the breakdown of acetylcholine (ACh)—a vital neurotransmitter in the central nervous system, neuromuscular junctions, and visceral motor ganglia—by acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Accordingly, cholinesterase (ChE) inhibition has become a cornerstone therapeutic approach for alleviating AD symptoms [15].

In addition to the cholinergic pathway, cerebral accumulation of amyloid- β ($A\beta$) peptides constitutes a key pathological feature of AD, with β -secretase 1 (BACE1) serving as a pivotal enzyme in $A\beta$ formation. Many medicinal plants have revealed significant AD-relevant bioactivities [16-22], with promising outcomes in clinical studies [23], positioning them as prime sources for AD drug development. Traditional Persian medicine has historically employed diverse botanicals to bolster memory, motivating their exploration in modern studies [24-27]. Oleo-gum-resins from species like *Ferula gummosa* (galbanum) [28] and *Boswellia serrata* [29] have similarly been advocated for cognitive support. The present investigation centers on sagapenum (oleo-gum-resin from *Ferula persica*) to explore its phytochemical constituents and inhibitory potential against AD-associated enzymes (ChEs and BACE1).

Materials and Methods

Ethical considerations

Approval for this investigation was granted by the Research Ethics Committee at the Institute of Pharmaceutical Sciences, Tehran University of Medical Sciences ([IR.TUMS.TIPS.REC.1400.217]).

Chemicals

The following reagents were sourced from Sigma-Aldrich (United States): acetylcholinesterase (AChE, E.C. 3.1.1.7, Type V-S, freeze-dried powder from electric eel, 1000 units), butyrylcholinesterase (BChE, E.C. 3.1.1.8, equine serum origin), 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (ATCI), and butyrylthiocholine iodide (BTCl). Salts were acquired from Merck (Germany). The human BACE1 ELISA kit (Invitrogen) came from Thermo Fisher Scientific Inc. (United States) (ex-PanVera Corporation, Madison, WI). OM99-2 was supplied by Bachem (Switzerland). All solvents originated from Dr. Mojallali Industrial Chemical Complex Co. (Iran) and underwent distillation before use.

Plant material

Sagapenum oleo-gum-resin was purchased from a traditional pharmaceutical bazaar in Tehran, Iran. It was verified taxonomically and a reference specimen was stored in the herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences, assigned voucher code PMP-1824.

Extraction

Dichloromethane fraction: To enable easy pulverization, 300 g of sagapenum was frozen. It was then exhaustively extracted with dichloromethane (3×1 L) across three successive days. The pooled solutions were reduced under vacuum, and the product was further dried in a vacuum oven to deliver the dichloromethane fraction.

Methanol and aqueous fractions: The defatted residue (48.8 g) was subjected to maceration in 80% methanol (3×250 mL) over 72 hours, yielding the methanol fraction after vacuum concentration. The leftover solid was subsequently treated with distilled water (3×250 mL) for 72 hours to generate the aqueous fraction.

Compound isolation

A portion of the dichloromethane fraction (250 g) was applied to a silica gel column (30–75 mesh, Merck, dimensions 20×8 cm) and separated using a stepwise gradient of petroleum ether to ethyl acetate (10:0 progressing to 0:10), resulting in ten main fractions (D1–D10) evaluated by TLC with UV detection (254 and 366

nm). Promising fractions D4 (6.4 g), D5 (13.18 g), D7 (20.0 g), and D9 (14.67 g) were advanced to purification on higher-resolution silica gel (230–400 mesh, 70 × 5 cm columns). Both D4 and D7 were resolved with petroleum ether–ethyl acetate (7:3), producing 12 and 24 subfractions each. Gradient elution of D5 (petroleum ether–ethyl acetate 8:2 to 7:3) gave 19 subfractions. Fraction D9 (14.67 g) was processed with chloroform–ethyl acetate (10:0 to 7:3) to yield 35 subfractions. Targeted subfractions—D4-2 (140 mg), D4-4 (70 mg), D5-14 (100 mg), D5-18 (80 mg), D7-23 (123 mg), and D9-17 (112 mg)—were refined via reverse-phase silica gel chromatography (20 × 1 cm), isolating compounds 1 (25 mg), 2 (29 mg), 3 (9 mg), 4 (7 mg), 5 (12 mg), and 6 (15 mg) in that order. Subfraction D9-15 (450 mg) was fractionated on normal-phase silica gel (30–75 mesh, Merck, 50 × 5 cm) to obtain compounds 7 (19 mg) and 8 (5 mg).

Identification of the purified compounds was achieved through ¹H- and ¹³C-NMR analysis in CDCl₃.

In vitro evaluation of cholinesterase inhibition

Potential to block acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) was tested using an updated version of Ellman's spectrophotometric assay, quantifying the colored product 5-thio-2-nitrobenzoic acid released during enzyme-mediated breakdown of thiocholine-based substrates [18].

Assessment of BACE1 inhibition

Inhibition of BACE1 was measured according to a published method [18]. Samples were initially dissolved in DMSO and then adjusted to working concentrations in assay buffer (final DMSO level: 6% v/v). In opaque black 96-well plates, 10 μL substrate was combined with 10 μL of each test concentration. Enzyme (10 μL BACE1) was introduced to launch the reaction, followed by dark incubation at 25 °C for 90 minutes. Quenching was performed with 10 μL of 2.5 M sodium acetate. Resulting fluorescence was recorded at 544 nm (excitation) and 590 nm (emission) using a BMG LABTECH Polarstar reader (Germany).

Results and Discussion

Successive extraction of sagapenum produced dichloromethane (251.20 g), methanol (4.85 g), and aqueous (36.86 g) fractions using the respective solvents. These fractions were then tested for their ability to inhibit cholinesterases, with the findings presented in **Table 1**.

Table 1. Cholinesterase inhibitory activity of different extracts of sagapenum^a

Extract/Control	AChE Inhibition IC ₅₀ (μg/mL)	BuChE Inhibition IC ₅₀ (μg/mL)
Dichloromethane extract	> 500	23.41 ± 0.58
Methanol extract	> 500	> 500
Aqueous extract	> 500	> 500
Donepezil (positive control)	0.03 ± 0.002	1.97 ± 0.38

^a mean ± SD

No fraction showed inhibitory effects against AChE. However, the dichloromethane fraction proved to be the most effective and selective toward BuChE, leading to its choice for in-depth phytochemical exploration that resulted in the isolation of compounds 1–8 (**Figure 1**). Structural assignment of compounds 1–8 was accomplished through analysis of their ¹H- and ¹³C-NMR spectra. For instance, the ¹H-NMR data for the most active isolate (compound 2) revealed five coumarin-related aromatic protons at 7.65, 7.37, 6.84, 6.82, and 6.26 ppm (corresponding to H4, H5, H6, H8, and H3). The downfield doublet for H4, coupled to H3 with J = 9.5 Hz, was particularly diagnostic. Terminal olefinic protons (H12') appeared in the 4.70–4.80 ppm region, while the geminal protons at C11' displayed doublets of doublets between 4.05–4.30 ppm. The hydroxyl-bearing methine proton H3' resonated at 3.28 ppm. Upfield signals between 1.32–2.37 ppm accounted for the aliphatic protons at positions 1', 2', 5', 6', 7', and 9'. The three methyl groups gave rise to sharp singlets at 1.07 (H13'), 0.84 (H14'), and 1.01 ppm (H15').

In the ¹³C-NMR spectrum of compound 2, the ester carbonyl (C2) was located at 162.1 ppm. Coumarin skeleton carbons C3 through C10 produced signals at 113.2, 143.2, 128.6, 113.0, 161.0, 101.6, 155.7, and 112.5 ppm in the aromatic range. Olefinic carbons C8' and C12' were assigned to peaks at 146.5 and 111.3 ppm. Within the aliphatic portion, oxygen-substituted carbons C3' and C11' were the most downfield at 79.0 and 68.1 ppm. Other

aliphatic carbons resonated at 32.3 (1'), 27.6 (2'), 39.0 (4'), 46.6 (5'), 23.0 (6'), 34.8 (7'), 56.5 (9'), 37.7 (10'), and 68.1 ppm (11'). The methyl carbons appeared at 28.4 (13'), 22.1 (14'), and 15.7 ppm (15').

Compound 1

Aurapten (C₁₉H₂₂O₃); ¹H-NMR (CDCl₃, 500 MHz): δH (ppm): 7.65 (1H, d, *J*=9.4 Hz, H4), 7.38 (1H, d, *J*=8.3 Hz, H5), 6.86 (1H, dd, *J*=8.3, 2.3 Hz, H6), 6.83 (1H, d, *J*=2.3 Hz, H8), 6.26 (1H, d, *J*=9.4 Hz, H3), 5.48 (1H, t, *J*=6.6 Hz, H2'), 5.11-5.09 (1H, m, H6'), 4.62 (2H, d, *J*=6.6 Hz, H1'), 2.16-2.11 (4H, m, H4', H5'), 1.78 (3H, s, H10'), 1.68 (3H, s, H9'), 1.62 (3H, s, H8'). ¹³C-NMR (CDCl₃, 125 MHz): δC (ppm): 162.3 (C2), 160.9 (C7), 155.6 (C9), 143.4 (C4), 142.1 (C3'), 131.7 (C7'), 128.8 (C5), 123.7 (C6'), 118.4 (C2'), 113.4 (C6), 112.8 (C3), 112.1 (C10), 101.4 (C8), 65.5 (C1'), 39.5 (C4'), 26.0 (C5'), 25.5 (C9'), 17.7 (C10'), 16.9 (C8') [30, 31].

Compound 2

Farnesiferol A (C₂₄H₃₀O₄) ¹H-NMR (CDCl₃, 500 MHz) : δH (ppm): 7.65 (1H, d, *J* = 9.5 Hz, H4), 7.37 (1H, d, *J* = 8.0 Hz, H5), 6.85-6.82 (2H, m, H6, H8), 6.26 (1H, d, *J* = 9.5 Hz, H3), 4.85-4.84 (1H, m, H12'a), 4.75-4.74 (1H, m, H12'b), 4.30 (1H, dd, *J* = 9.6, 5.5 Hz, H11'a), 4.05 (1H, dd, *J* = 9.6, 5.6 Hz, H11'b), 3.30-3.27 (1H, m, H3'), 2.38-2.35 (1H, m, H7'a), 2.23 (1H, t, *J* = 5.5 Hz, H9'), 2.11-2.03 (1H, m, H7'b), 1.74-1.69 (1H, m, H2'a), 1.68-1.65 (1H, m, H1'a), 1.61-1.56 (2H, m, H6'), 1.49-1.46 (1H, m, 2'b), 1.44-1.43 (1H, m, 1'b), 1.33 (1H, dd, *J* = 12.6, 2.6 Hz, H5'), 1.07 (3H, s, H13'), 1.01 (3H, s, H15'), 0.84 (3H, s, H14'). ¹³C-NMR (CDCl₃, 125 MHz): δC (ppm): 162.1 (C2), 161.0 (C7), 155.7 (C9), 146.5 (C8'), 143.2 (C4), 128.6 (C5), 113.2 (C3), 113.0 (C6), 112.5 (C10), 111.3 (C12'), 101.6 (C8), 79.0 (C3'), 68.1 (C11'), 56.5 (C9'), 46.6 (C5'), 39.0 (C4'), 37.7 (C10'), 34.8 (C7'), 32.3 (C1'), 28.4 (C13'), 27.6 (C2'), 23.0 (C6'), 22.1 (C14'), 15.7 (C15') [32].

Compound 3

Umbelliprenin (C₂₄H₃₀O₃) ¹H-NMR (CDCl₃, 500 MHz): δH (ppm): 7.64 (1H, d, *J* = 9.50 Hz, H4), 7.37 (1H, d, *J* = 8.1 Hz, H5), 6.86 (1H, dd, *J* = 8.1, 2.5 Hz, H6), 6.86 (1H, d, *J* = 2.5 Hz, H8), 6.25 (1H, d, *J* = 9.5 Hz, H3), 5.48 (1H, t, *J* = 7.5 Hz, H2'), 5.11-5.09 (2H, m, H6', 10'), 4.61 (2H, d, *J* = 7.5 Hz, H1'), 2.15-1.95 (8H, m, H 4', 5', 8', 9'), 1.77 (3H, s, H12'), 1.68 (3H, s, H13'), 1.61-1.60 (6H, m, H 14', 15') [33].

Compound 4

Farnesiferol C (C₂₄H₃₀O₄) ¹H-NMR (CDCl₃, 500 MHz): δH (ppm): 7.65 (1H, d, *J* = 9.5 Hz, H4), 7.38 (1H, d, *J* = 8.4 Hz, H5), 6.86 (1H, dd, *J* = 8.4, 2.2 Hz, H6), 6.83 (1H, d, *J* = 2.2 Hz, H8), 6.25 (1H, d, *J* = 9.5 Hz, H3), 5.46 (1H, t, *J* = 6.5 Hz, H9'), 4.61 (2H, d, *J* = 6.5 Hz, H11'), 3.72 (1H, d, *J* = 5.4 Hz, H3'), 2.10-2.01 (2H, m, H7'), 1.92-1.87 (1H, m, 1'a), 1.78 (3H, s, H12'), 1.70-1.66 (2H, m, 1'b, 2'a), 1.49-1.42 (3H, m, 2'b, 6'), 1.35 (3H, s, H13'), 1.20-1.17 (1H, m, H5'), 1.05 (3H, s, H15'), 1.02 (3H, s, H14'). ¹³C-NMR (CDCl₃, 125 MHz): δC (ppm): 161.9 (C7), 161.1 (C2), 155.8 (C9), 143.3 (C4), 142.5 (C8'), 128.7 (C5), 118.5 (C9'), 113.2 (C3), 112.9 (C6), 112.3 (C10), 101.6 (C8), 86.6 (C10'), 86.1 (C3'), 65.3 (C11'), 55.3 (C5'), 45.2 (C4'), 39.6 (C2'), 38.9 (C1'), 26.1 (C14'), 25.8 (C6'), 25.7 (C7), 23.4 (15'), 18.9 (C13'), 16.7 (C12') [34].

Compound 5

Farnesiferone A (C₂₄H₂₈O₄); ¹H-NMR (CDCl₃, 500 MHz): δH (ppm): 7.63 (1H, d, *J* = 9.6 Hz, H4), 7.36 (1H, d, *J* = 8.4 Hz, H5), 6.80 (1H, d, *J* = 8.4 Hz, H6), 6.79 (1H, d, *J* = 1.7 Hz, H8), 6.26 (1H, d, *J* = 9.6 Hz, H3), 4.90 (1H, s, H12'a), 4.82 (1H, s, H12'b), 4.26 (1H, dd, *J* = 8.2, 4.4 Hz, H11'a), 4.04 (1H, dd, *J* = 8.2, 4.4 Hz, H11'b), 2.78 (1H, dtd, *J* = 15.7, 14.8, 5.8 Hz, H2'a), 2.41-2.38 (1H, m, H2'b), 2.37-2.36 (1H, m, H9'), 2.36-2.35 (1H, m, H7'a), 2.06-2.16 (1H, m, H7'b), 1.94 (1H, dtd, *J* = 15.7, 14.9, 5.8 Hz, H1'a), 1.80 (1H, dd, *J* = 12.6, 3.3 Hz, H5') 1.73-1.67 (1H, m, H1'b), 1.61-1.53 (2H, m, H6'), 1.21 (3H, s, H15'), 1.15 (3H, s, H14'), 1.06 (3H, s, H13'). ¹³C-NMR (CDCl₃, 125 MHz): δC (ppm): 215.7 (C3'), 161.7 (C2), 161.1 (C7), 156.5 (C9), 146.0 (C8'), 143.3 (C4), 128.7 (C5), 113.2 (C3), 113.0 (C6), 112.6 (C10), 112.2 (C12'), 101.6 (C8), 68.1 (C11'), 56.0 (C9'), 47.9 (C4'), 47.7 (C5'), 37.4 (C10'), 35.5 (C1'), 35.0 (C7'), 32.1 (C2'), 25.8 (C13'), 23.8 (C6'), 22.3 (C15'), 21.1 (C14') [33].

Compound 6

Karatavicinol (C₂₄H₃₂O₅); ¹H-NMR (CDCl₃, 500 MHz): δH (ppm): 7.65 (1H, d, *J* = 9.5 Hz, H₄), 7.36 (1H, d, *J* = 8.6 Hz, H₅), 6.85 (1H, dd, *J* = 8.6, 2.2 Hz, H₆), 6.8 (1H, d, *J* = 2.2 Hz, H₈), 6.24 (1H, d, *J* = 9.5 Hz, H₃), 5.45 (1H, t, *J* = 6.0 Hz, H₂'), 5.18 (1H, t, *J* = 5.9 Hz, H₆'), 4.61 (2H, d, *J* = 6.0 Hz, H₁'), 3.36-3.34 (1H, m, H₁₀'), 2.35-2.03 (8H, m, H₉', H₈', H₄', H₅'), 1.73 (3H, s, H₁₂'), 1.62 (3H, s, H₁₃'), 1.19 (3H, s, H₁₅'), 1.15 (3H, s, H₁₄'). ¹³C-NMR (CDCl₃, 125 MHz): δC (ppm): 162.1 (C₂), 161.4 (C₇), 155.9 (C₉), 143.5 (C₄), 142.0 (C₃'), 135.5 (C₇'), 128.6 (C₅), 124.2 (C₆'), 118.7 (C₂'), 113.3 (C₆), 112.8 (C₃), 112.4 (C₁₀'), 101.4 (C₈), 78.1 (C₁₀'), 72.8 (C₁₁'), 65.5 (C₁'), 39.4 (C₈'), 36.6 (C₄'), 29.6 (C₅'), 26.4 (C₉'), 25.9 (C₁₄'), 23.3 (C₁₂'), 16.8 (C₁₃'), 15.8 (C₁₅') [35].

Compound 7

Ferocaulidin (C₂₄H₂₈O₅); ¹H-NMR (CDCl₃, 500 MHz): δH (ppm): 7.65 (1H, d, *J* = 9.4 Hz, H₄), 7.39 (1H, d, *J* = 8.5 Hz, H₅), 6.85-6.83 (2H, m, H₈, H₆), 6.27 (1H, d, *J* = 9.4 Hz, H₃), 5.90 (1H, s, H₇'), 4.28 (1H, dd, *J* = 9.8, 2.8 Hz, H₁₁'a), 4.19-4.16 (1H, m, H₁₁'b), 3.39-3.37 (1H, s, H₃'), 2.80-2.77 (1H, s, H₉'), 2.64 (1H, s, H₅'), 2.01-1.99 (2H, m, 1'a, 2'a), 1.96 (3H, s, H₁₂'), 1.70-1.65 (2H, m, 1'b, 2'b), 1.24 (3H, s, H₁₄'), 1.20 (3H, s, H₁₃'), 1.04 (3H, s, H₁₅'). ¹³C-NMR (CDCl₃, 125 MHz): δC (ppm): 200.0 (C₆'), 161.2 (C₇), 160.9 (C₂), 155.8 (C₉), 155.4 (C₈'), 143.2 (C₄), 129.7 (C₇'), 128.9 (C₅), 113.4 (C₃), 112.9 (C₆), 112.8 (C₁₀'), 101.3 (C₈), 76.2 (C₃'), 65.7 (C₁₁'), 57.3 (C₅'), 54.9 (C₉'), 41.8 (C₁₀'), 36.5 (C₄'), 31.7 (C₁'), 27.9 (C₁₄'), 24.4 (C₂'), 21.8 (C₁₂'), 21.7 (C₁₃'), 16.3 (C₁₅') [36].

Compound 8

Ligupersin A (C₂₄H₂₈O₈), appearing as a white powdery solid without crystalline form; ¹H-NMR (CDCl₃, 500 MHz): δ_H (ppm): 7.65 (1H, d, **J** = 9.5 Hz, H₄), 7.40 (1H, d, **J** = 8.4 Hz, H₅), 6.85 (1H, dd, **J** = 8.4, 2.4 Hz, H₆), 6.83 (1H, s, H₈), 6.28 (1H, d, **J** = 9.5 Hz, H₃), 5.92 (1H, s, H₇'), 4.27 (1H, dd, **J** = 9.8, 3.2 Hz, H₁₁'a), 4.18-4.15 (1H, m, H₁₁'b), 3.25 (1H, dd, **J** = 10.8, 4.8 Hz, H₃'), 2.65-2.70 (1H, m, H₉'), 2.20 (1H, s, H₅'), 1.95 (3H, s, H₁₅'), 1.76-1.67 (2H, m, H₂'), 1.66-1.56 (2H, m, H₁'), 1.30 (3H, s, H₁₃'), 1.20 (3H, s, H₁₄'), 1.04 (3H, s, H₁₂') [36].

Every purified compound (1-8) was tested for its potential to block cholinesterases, with details outlined in **Table 2**. None proved effective against AChE. Compound 8 displayed modest inhibition, reaching 26.38% at a concentration of 40 µg/mL. Regarding BuChE blockade, compound 2 ranked as the superior selective blocker across the series (IC₅₀ = 31.46 µg/mL).

The performance of compound 2 (identified as farnesiferol A) was additionally probed for BACE1 blockade employing a fluorescence resonance energy transfer (FRET) assay, adhering to the kit guidelines. Results indicated robust potency (IC₅₀ = 5.14 ± 0.50 µM) versus the benchmark OM99-2 (IC₅₀ = 0.014 ± 0.003 µM). OM99-2 functions as a peptidomimetic agent with eight residues that strongly binds to human brain memapsin 2. Alzheimer's disease (AD) unfolds as a gradual neurodegenerative ailment driven by diverse pathological processes contributing to its initiation and escalation. Cholinesterase inhibitors (ChEIs) continue to serve as valuable options for symptom relief in AD [37]. Growing focus has shifted to the extra-cholinergic roles of AChEIs, especially in modulating brain amyloid-β (Aβ) plaque formation. AChE bound to these plaques amplifies the harmful effects of AChE-Aβ aggregates and modifies their characteristics [38, 39]. The value of targeted BuChE suppression has emerged, owing to BuChE's backup function in acetylcholine breakdown during later disease phases [40]. Thus, specific BuChE blockers are regarded as effective approaches for managing severe AD cases [41]. Aβ buildup stands as a primary AD indicator, where BACE1 crucially facilitates the shift of amyloid precursor protein (APP) into toxic Aβ peptides [42].

Efforts to create safer therapeutics with fewer adverse reactions have spotlighted natural compounds, valued for their wealth of potent phytochemicals [43].

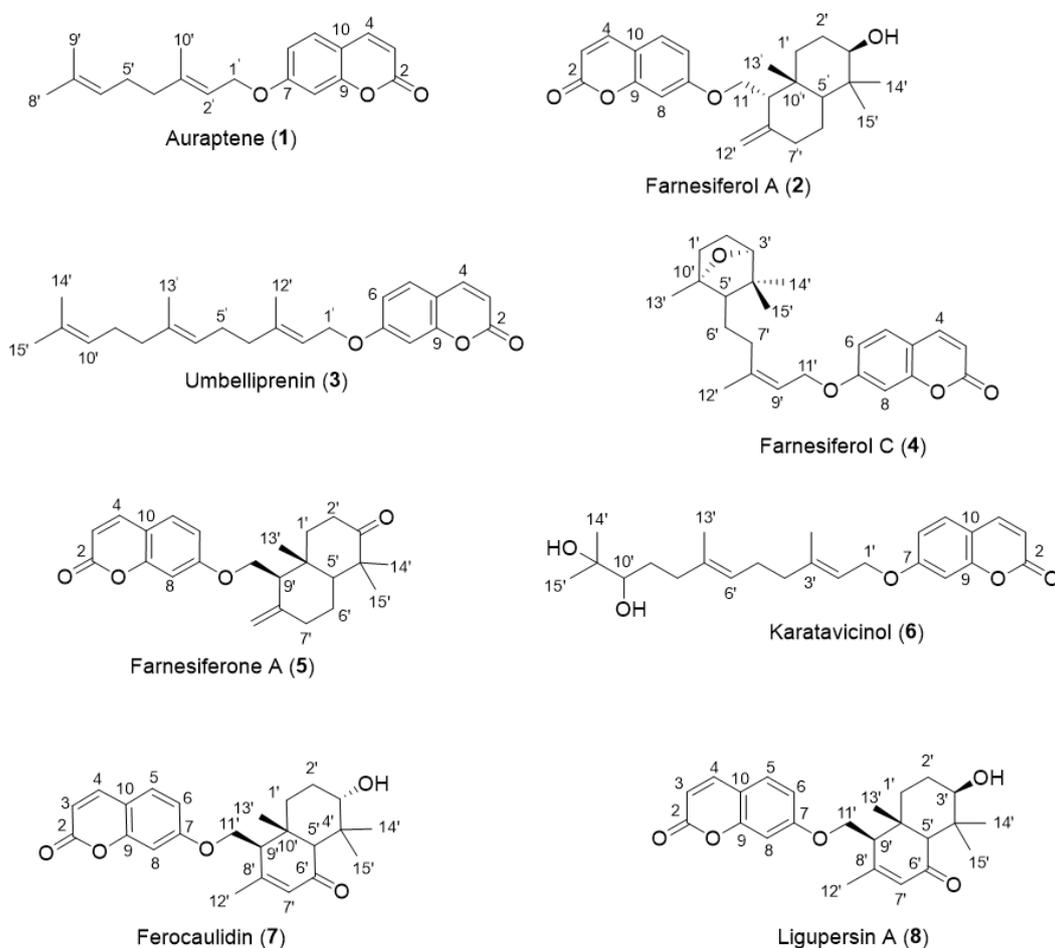


Table 2. Cholinesterase blockade by compounds 1–8

Compound	AChE Inhibition (%) ^a	AChE IC ₅₀ (μg/mL)	BuChE Inhibition (%) ^a	BuChE IC ₅₀ (μg/mL)
Auraptene (1)	NA	>40	NA	>40
Farnesiferol A (2)	7.34 ± 0.42	>40	51.86 ± 0.95	31.46 ± 0.37 (82.36 μM)
Umbelliprenin (3)	NA	>40	NA	>40
Farnesiferol C (4)	NA	>40	17.54 ± 0.57	>40
Farnesiferone A (5)	11.38 ± 0.37	>40	30.75 ± 0.96	>40
Karatavicinol (6)	14.93 ± 0.22	>40	35.62 ± 0.83	>40
Ferocaulidin (7)	14.01 ± 0.71	>40	24.24 ± 0.12	>40
Ligupersin A (8)	26.38 ± 0.54	>40	43.01 ± 0.29	>40
Donepezil (reference)	-	0.020 ± 0.002	-	1.50 ± 0.26

^a Percentage inhibition assessed at 40 μg/mL; ^bNA: inactive

Within the broad spectrum of medicinal flora, the *Ferula* genus features diverse species renowned for therapeutic benefits and high levels of coumarins [44]. Many *Ferula* varieties impact neural pathways [45], and anti-AD potentials for several have appeared in reports. As an example, the chloroform fraction of *Ferula halophila* strongly suppressed AChE and BuChE (equivalent to 4.86 and 6.13 mg galantamine per g extract; GALAE units) [46]. Plant exudates known as oleo-gum-resins incorporate volatiles, gums, and resins, delivering noteworthy pharmacological effects [47]. Essential oil from frankincense (*Boswellia sacra* resin) exerted strong AChE suppression (IC₅₀ = 0.043 mg/mL), outperforming galantamine (IC₅₀ = 98.0 mg/mL) [48]. Water-based resin extract from *Ferula asafoetida* boosted cognition and recall in rodent trials using elevated plus maze and passive

avoidance tasks, with oral doses of 200 mg/kg and 400 mg/kg producing dose-linked improvements in retention and delay times [49].

Previous works have outlined the chemical profile of *Ferula persica* [50], yet its resin (sagapenum) lacked such documentation. In this investigation, the dichloromethane fraction revealed strong targeted BuChE suppression ($IC_{50} = 23.41 \pm 0.58 \mu\text{g/mL}$), justifying deeper separation that produced eight pure isolates: auraptin (1), farnesiferol A (2), umbelliprenin (3), farnesiferol C (4), farnesiferone A (5), karatavicinol (6), ferrocaulidin (7), and ligupersin A (8). Screening against cholinesterases highlighted farnesiferol A (2) as the leading targeted BuChE suppressor ($IC_{50} = 82.36 \mu\text{M}$) (**Table 2**). In pursuit of agents hitting multiple targets, BACE1 testing showed promising blockade ($IC_{50} = 5.14 \mu\text{M}$).

Coumarins represent a vast group of oxygenated heterocycles recognized for substantial cholinesterase suppression [51, 52]. Naturally occurring coumarins have especially captured interest. Isolates from *Angelica decursiva* and *Artemisia capillaris* encompassed umbelliferone, umbelliferone 6-carboxylic acid, esculetin, daphnetin, scopoletin, isoscapoletin, 7-methoxy coumarin, scoparone, scopolin, and 2'-isopropyl psoralene. Several blocked AChE, BuChE, and BACE1 pathways. Esculetin ($IC_{50} = 6.13 \mu\text{M}$) and daphnetin ($IC_{50} = 8.66 \mu\text{M}$) performed best against AChE and BuChE (relative to berberine: $IC_{50} = 0.72$ and $7.01 \mu\text{M}$), whereas umbelliferone 6-carboxylic acid excelled in BACE1 suppression ($IC_{50} = 0.34 \mu\text{M}$) against quercetin ($IC_{50} = 13.98 \mu\text{M}$) [53].

Auraptin (1), farnesiferol A (2), and umbelliprenin (3), previously obtained from *Ferula* species, exhibited only mild AChE inhibition, aligning closely with the findings presented here [54]. In contrast, auraptin and farnesiferol A derived from the oleo-gum-resin of *Ferula gummosa* Boiss [28] displayed varying AChE inhibition strengths, recording IC_{50} values of $159.3 \mu\text{M}$ and $46.2 \mu\text{M}$, respectively. Assessment of cholinesterase blockade by karatavicinol, purified from *Heptaptera anatolica* (Boiss.) Tutin, revealed 17.94% inhibition against AChE at $100 \mu\text{g/mL}$ alongside notable BuChE suppression ($IC_{50} = 37.2 \mu\text{M}$) [55]. The BuChE outcome for the latter isolate diverged from our data (**Table 2**), (compound 8). No prior reports exist on BuChE or BACE1 inhibition by farnesiferol A (2), and the current findings highlight its considerable effectiveness. *Ferula persica* itself has exhibited low toxicity. Sub-chronic administration of its methanol extract in female mice yielded an LD_{50} exceeding 2000 mg/kg with no fatalities recorded [56]. Furthermore, the hexane fraction proved non-toxic toward the normal MDBK cell line ($IC_{50} > 100 \mu\text{g/mL}$) [57, 58]. That said, toxicity data for the oleo-gum-resin remain unavailable. Overall, these observations position sagapenum as a promising and safe option for additional research into anti-AD dietary supplements.

Conclusion

For the first time, the phytochemical profile of sagapenum—the oleo-gum-resin from *F. persica*—was explored, resulting in the purification and characterization of auraptin, farnesiferol A, umbelliprenin, farnesiferol C, farnesiferone A, karatavicinol, ferrocaulidin, and ligupersin A. Guided by traditional Persian medicine's endorsement of oleo-gum-resins for enhancing memory, sagapenum was screened for inhibitory effects on AChE, BuChE, and BACE1. Farnesiferol A emerged as a highly effective blocker of both BuChE and BACE1, suggesting it warrants further evaluation in preclinical animal models or human clinical trials.

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