



Furanocoumarins from the Roots of *Cachrys cristata* DC.: Isolation, Anticholinesterase Activity, and Molecular Docking Studies

✉ Fadıl Kaan KURAN^{1*}, ✉ Ceren KUŞ¹, ✉ Zehra Ezel DOLAP², ✉ Şule Nur KARAVUŞ KANTEMÜR³, ✉ Kübra Şeyma ÖZTÜRK¹,
✉ Gülsüm ALTIPARMAK ÜLBEGİ⁴, ✉ Mahmut MISKİ¹

¹Istanbul University Faculty of Pharmacy, Department of Pharmacognosy, İstanbul, Türkiye

²Istanbul University, Health Sciences Institute, Department of Pharmacognosy, İstanbul, Türkiye

³Istanbul Medipol University Faculty of Pharmacy, Department of Pharmacognosy, İstanbul, Türkiye

⁴Istanbul University Faculty of Pharmacy, Department of Biochemistry, İstanbul, Türkiye

ABSTRACT

Objectives: The present study aimed to investigate the phytochemical constituents of *Cachrys cristata* roots, to evaluate the cholinesterase-inhibitory activity of the isolated compounds, and to assess their possible binding interactions with acetylcholinesterase and butyrylcholinesterase through molecular docking studies.

Materials and Methods: The roots were extracted with dichloromethane by Soxhlet extraction, and a coumarin-rich fraction was obtained by precipitation from the crude extract. Chromatographic purification of this fraction using Sephadex LH-20, reversed-phase C₁₈ column chromatography, and preparative thin-layer chromatography afforded ten known furanocoumarins: oxypeucedanin hydrate, oxypeucedanin, bergapten, xanthotoxin, 3'-hydroxyprantschimgin, oxypeucedanin ethanolate (oxypeucedanin hydrate-3"-ethyl ether), pabularinone, marmesin, pabulenol and saxalin. The structures of the isolated compounds were identified by ¹H NMR spectroscopy and comparison with literature data. Selected isolated compounds were evaluated for inhibitory activity against acetylcholinesterase and butyrylcholinesterase, and the results were further assessed by molecular docking.

Results: The tested furanocoumarins showed weak cholinesterase inhibitory activity. Bergapten was the most active compound against acetylcholinesterase, with an IC₅₀ value of 92.15 µM. For butyrylcholinesterase, bergapten, and pabulenol exhibited measurable inhibition, with IC₅₀ values of 150.25 µM and 157.28 µM, respectively.

Conclusion: This study contributes to the phytochemical characterization of *Cachrys cristata* roots and provides the first phytochemical evidence for the occurrence of furanocoumarin-type constituents in the roots of this species. The isolated compounds exhibited weak cholinesterase inhibitory activity, suggesting that the furanocoumarin scaffold may serve as a preliminary framework for further structural optimization rather than as a source of potent cholinesterase inhibitors.

Keywords: *Cachrys cristata*, coumarins, molecular docking, cholinesterase inhibition

INTRODUCTION

The family Apiaceae (Umbelliferae) comprises 456 accepted genera and approximately 3,800 species and includes medicinal and aromatic plants widely used in traditional medicine.^{1,2}

Species in this family are rich in secondary metabolites, such as coumarins, terpenoids, flavonoids, steroids, and polyacetylenes. Owing to their diverse secondary metabolite profiles, these species exhibit various biological activities,

*Correspondence: kaankuran@istanbul.edu.tr, ORCID-ID: orcid.org/0000-0003-2793-0577

Received: 22.09.2026 Accepted: 14.10.2026 Publication Date: 27.10.2026

Cite this article as: Kuran KF, Kuş C, Dolap ZE, Karavuş Kantemür ŞN, Öztürk KŞ, Altıparmak Ülbegî G, Miskî M. Furanocoumarins from the roots of *Cachrys cristata* DC.: isolation, anticholinesterase activity, and molecular docking studies. Turk J Pharm Sci. 2026;23(3):165-173



Copyright© 2026 The Author(s). Published by Galenos Publishing House on behalf of Turkish Pharmacists' Association.
This is an open access article under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 (CC BY-NC-ND) International License.

including antimicrobial, cytotoxic, anti-inflammatory, and analgesic effects, and are therefore regarded as potential sources of drug candidates.³

Cachrys L. is a genus in the family Apiaceae, comprising seven accepted species and is distributed in Europe, North Africa, Western Asia, and adjacent regions.¹ There are differing opinions in the literature regarding the taxonomic circumscription of the genus. According to Tutin (1968), *Cachrys* also includes *Prangos* Lindl. and *Hippomarathrum* Link; however, Boissier classified these taxa as three distinct genera in 1872.⁴ In Türkiye, the genus is commonly known by vernacular names such as “Çaşır”, “Tarhana Otu”, “Çarşambaotu”, and is represented by two species: *Cachrys cristata* and *Cachrys crassiloba*.^{4,5} Although data on the traditional medicinal uses of *Cachrys* species remain limited, *Cachrys libanotis* L. has been reported for the treatment of rheumatism, whereas plant material historically reported as *Cachrys alpina* M. Bieb. has been used as an aphrodisiac.^{6,7}

Various coumarin derivatives, especially furanocoumarins, have been reported from *Cachrys* species. Coumarins such as isoimperatorin, imperatorin, bergapten, xanthotoxin, isopimpinellin, prantschimgin, spregelianin, ulopterol, and oxypeucedanin have been isolated from *Cachrys sicula*. Coumarin derivatives, including xanthotoxin, bergapten, isopimpinellin, psoralen, isooxypeucedanin, columbianetin, jatamansin, seselin, osthol, and suberosin, have also been detected in extracts and fractions obtained from *Cachrys sicula*, *Cachrys libanotis*, and *Cachrys pungens*.⁸⁻¹⁰ In addition to these phytochemical studies, investigations of *Cachrys* species have generally focused on their essential oil compositions.¹¹⁻¹⁵

Dementia is characterized by a decline in cognitive functions, including memory and thinking, which can interfere with everyday activities. Alzheimer's disease is the most common form of dementia.¹⁶ Impaired cholinergic neurotransmission plays an important role in the pathophysiology of Alzheimer's disease. The inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), which helps maintain acetylcholine levels, is considered one of the main symptomatic approaches to support learning and memory functions.¹⁷ To the best of our knowledge, no specific ethnobotanical record documents the use of *Cachrys cristata* for memory impairment, cognitive dysfunction, or neurodegenerative disorders. Therefore, the selection of cholinesterase inhibition as an endpoint in the present study was not based on a direct traditional claim for this species, but rather on the plant's phytochemical profile. Since the isolated constituents mainly belonged to the furanocoumarin class, and several coumarin/furanocoumarin derivatives, such as bergapten, xanthotoxin, and umbelliferone, have been reported to improve cognitive functions in experimental models through cholinergic, antioxidant, and anti-inflammatory mechanisms,^{18,19} AChE and BChE inhibition assays were selected as a phytochemistry-driven bioactivity screening approach. These findings suggest that coumarins may be promising candidates for biological activity studies targeting neurodegenerative diseases.

Within this context, the present study evaluated the inhibitory

effects of furanocoumarin-type compounds isolated from *Cachrys cristata* on AChE and BChE. The possible interactions of these compounds with the active sites of the enzymes were further investigated using molecular docking analyses.

MATERIALS AND METHODS

Plant material

The plant material was collected by Prof. Dr. Mahmut Miski and Ersan Pütün from the Alaşehir-Buldan road in Denizli Province, Türkiye, at an altitude of 1650 m on 9 June 1982. The plant was identified by Prof. Dr. Mine Koçyiğit Avcı as *Hippomarathrum cristatum* (DC.) Boiss. (syn. *Cachrys cristata* DC.). A voucher specimen prepared from the aerial parts of the plant was deposited in the Herbarium of the Faculty of Pharmacy, İstanbul University, under the accession number ISTE 48929.

General experimental procedures

Hexane, dichloromethane, methanol (MeOH), ethyl acetate (EtOAc), acetonitrile (ACN), toluene, and sulfuric acid (H₂SO₄) were used for extraction, chromatographic separation, and thin-layer chromatographic analysis. DCM, EtOAc, and *p*-anisaldehyde were purchased from Sigma-Aldrich (St. Louis, MO, USA), while MeOH, ACN, toluene, and H₂SO₄ were purchased from Merck (Darmstadt, Germany). Sephadex LH-20 was purchased from GE Healthcare (Chicago, IL, USA). LiChroprep RP-18 (40–63 µm) was purchased from Merck and used for reversed-phase column chromatography. Silica gel 60 F₂₅₄ TLC plates (0.25 mm; Merck, Darmstadt, Germany) were used for analytical thin-layer chromatography, and Supelco silica gel 60 F₂₅₄ preparative TLC plates (0.5, 1, and 2 mm; Merck, Darmstadt, Germany) were used for preparative separations. TLC plates were visualized under UV light at 254 and 366 nm using a UV lamp (Camag, Muttens, Switzerland). When necessary, the plates were sprayed with freshly prepared anisaldehyde-H₂SO₄ reagent, followed by heating. Chromatographic separations were carried out using Sephadex LH-20 column chromatography, reversed-phase C₁₈ column chromatography, and preparative thin-layer chromatography. Fractions showing similar TLC profiles were combined and further purified according to their chromatographic behavior. The structures of the isolated compounds were elucidated mainly by (1)H NMR spectroscopy. NMR spectra were recorded on a Bruker 500 MHz spectrometer using CDCl₃ as the solvent.

Extraction and isolation

The roots used in the present study originated from the same collection as the voucher specimen. The root material was extracted shortly after collection in 1982, and the resulting extract was stored under deep-freeze conditions until the phytochemical investigation was performed.

The dried and coarsely powdered roots of *Cachrys cristata* DC. (280.0 g) were extracted with dichloromethane using a Soxhlet apparatus. The solvent was evaporated under reduced pressure to ca. 100 mL and kept refrigerated overnight. The resulting white crystalline precipitate was collected as a coumarin-rich fraction (4.0 g). This fraction (4.0 g) was initially

subjected to Sephadex LH-20 chromatography using a glass column measuring 50 cm in length and 5 cm in diameter. The column was eluted with a mixture of hexane–dichloromethane–MeOH (7:4:1, v/v/v) to obtain preliminary fractions. A total of 243 fractions were collected and monitored by TLC under UV light at 254 and 366 nm. Fractions showing similar TLC profiles were then combined and further purified according to their chromatographic behavior. Sephadex LH-20 fractions 223–242 directly afforded oxypeucedanin hydrate (58 mg). Sephadex LH-20 fractions 37–42 were further separated by reversed-phase C_{18} column chromatography. Subfractions 21–22, obtained from this column, afforded oxypeucedanin (8.4 mg). Sephadex LH-20 fractions 43–50 were fractionated by reversed-phase C_{18} column chromatography. Subfractions 27–32 afforded xanthotoxin (2.0 mg), while subfractions 34–43 yielded bergapten (3.8 mg). Subfractions 48–53 were further purified by preparative TLC using silica gel plates (0.5 mm thick) and toluene–dichloromethane–EtOAc–ACN (4:4:2:1, v/v/v/v) as the mobile phase, yielding oxypeucedanin ethanolate (2.2 mg) and pabularinone (0.8 mg). Sephadex LH-20 fractions 63–84 were further separated by reversed-phase C_{18} column chromatography, and subfractions 39–45 afforded 3'-hydroxyprantschimgin (3.6 mg). Sephadex LH-20 fractions 85–105 were also separated by reversed-phase C_{18} column chromatography. Subfractions 12–17 and 24–29 were purified by preparative TLC under the same conditions to yield marmesin (3.9 mg) and pabulenol (1.3 mg), respectively. Subfractions 30–38 were purified by preparative TLC using 1-mm silica gel plates and the same mobile phase to obtain saxalin (1.2 mg).

Determination of cholinesterase inhibitory activities

Compounds 1, 2, 3, 5, 7, and 9 were selected from the isolated compounds for cholinesterase inhibition assays based on the availability of sufficient material and acceptable purity following final purification, as assessed by TLC and ^1H NMR. The inhibitory activities against AChE (Sigma-Aldrich, Cat. No. C3389) and BChE (Sigma-Aldrich, Cat. No. C7512) were determined using the Ellman method,²⁰ with modifications based on Sevinçli et al.²¹ The compounds and donepezil, used as the reference inhibitor, were dissolved in dimethyl sulfoxide (DMSO) and serially diluted to obtain final assay concentrations of 20, 40, 80, 120, and 160 μM for the isolated compounds; 1.2, 2.4, 4.8, and 9.6 μM for donepezil in the BChE assay; and 0.12, 0.24, 0.48, and 0.96 μM for donepezil in the AChE assay. Ten microliters of the isolated compound or donepezil solution was mixed with 220 μL of Ellman reagent (95.5 mM sodium phosphate buffer, pH 7.5, containing 955 μM ATChI or BTChI and 318 μM DTNB). For the control, an equal volume of DMSO was used in place of the inhibitor solution. Subsequently, 20 μL of AChE (0.4 U/mL) or BChE (0.3 U/mL) was added, and the absorbance was recorded at 412 nm at 1-min intervals for 10 min. The percentage inhibition was calculated using the following equation:

Inhibition (%) = $[1 - (\Delta A_{\text{sample}}/\text{min} / \Delta A_{\text{control}}/\text{min})] \times 100$
 where $\Delta A_{\text{sample}}/\text{min}$ and $\Delta A_{\text{control}}/\text{min}$ represent the rates of absorbance increase in the presence and absence of the inhibitor, respectively.

The cholinesterase inhibition assays were performed in three to four independent experiments, each with two to four technical replicates. IC_{50} values were calculated by non-linear regression analysis using GraphPad Prism (version 11.0.2; San Diego, CA, USA). Inhibition percentages and IC_{50} values are expressed as mean $\pm$ standard deviation.

Molecular docking

Molecular docking studies were performed using the Schrödinger software package (Schrödinger Release 2017-3, Schrödinger, LLC, New York, NY, USA). The crystal structures of human AChE in complex with donepezil (PDB ID: 4EY7)²² and human BChE in complex with tacrine (PDB ID: 4BDS)²³ were retrieved from the Protein Data Bank and prepared using the Protein Preparation Wizard. Hydrogen atoms were added, bond orders were assigned, non-essential water molecules and heteroatoms were removed, and the structures were minimized using the OPLS3 force field.²⁴ The isolated furanocoumarins were prepared using LigPrep with default settings, including Epik-based ionization-state generation, and were minimized with the OPLS3 force field. Receptor grids were generated around the binding sites of the co-crystallized ligands: donepezil for AChE and tacrine for BChE. Docking calculations were performed using Glide SP with flexible ligand sampling.^{25,26}

The docking protocols were validated by redocking the co-crystallized ligands into their respective active sites. Although electric eel AChE and equine serum BChE were used in *in vitro* enzyme inhibition assays, human cholinesterase crystal structures were selected for docking because they provide experimentally determined, ligand-bound models suitable for grid generation and redocking validation. In this context, the human BChE–tacrine complex 4BDS provides a ligand-bound model suitable for validating the docking protocol for BChE. A similar methodological approach was reported previously in a furanocoumarin-related cholinesterase study, in which electric eel AChE and horse serum BChE were used in enzymatic assays, and docking analyses were performed using a human BChE crystal structure.²⁷ Since the enzyme sources used in the *in vitro* assays and the docking models were not identical, the docking results were interpreted only as qualitative structural support for the possible binding poses and interaction patterns of the isolated furanocoumarins, rather than as species-specific quantitative predictions of inhibitory potency.

Ethics approval and informed consent

Ethics committee approval and informed consent were not required for this study, as it did not involve human participants or experimental animals.

RESULTS

Phytochemical results

Ten furanocoumarin-type compounds were isolated from the coumarin-rich fraction of the dichloromethane extract obtained from the roots of *Cachrys cristata*. Their structures were identified by comparison of their ^1H NMR spectroscopic data with those reported in the literature as bergapten²⁸ (1) (3.8 mg),

xanthotoxin²⁸ (2) (2 mg), marmesin²⁹ (3) (3.9 mg), pabularinone³⁰ (4) (0.8 mg), pabulenol³¹ (5) (1.3 mg), 3'-hydroxyprantschimgin³² (6) (3.6 mg), oxypeucedanin³³ (7) (8.4 mg), oxypeucedanin ethanolate/oxypeucedanin hydrate-3"-ethyl ether³⁴ (8) (2.2 mg), oxypeucedanin hydrate³³ (9) (58 mg), and saxalin³⁴ (10) (1.2 mg). For compounds containing stereogenic centers, stereochemical assignments were made by comparison with reported data for the corresponding known compounds, and the measured optical rotation values were consistent with literature values. According to the available literature, furanocoumarins have not previously been reported from the roots of *Cachrys cristata*. The ¹H NMR spectra of the isolated compounds are provided in the Supplementary Figures S1-13. The molecular structures of the compounds are presented in Figure 1.

Cholinesterase inhibitory activity

The isolated compounds exhibited different inhibitory profiles

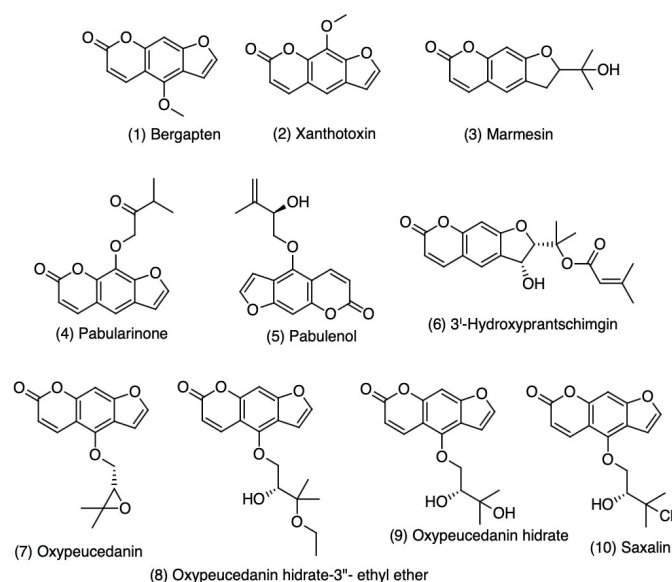


Figure 1. Structures of compounds isolated from *Cachrys cristata*.

against AChE and BChE (Table 1). Regarding AChE inhibition, bergapten (1) was the most active compound, with an IC_{50} of 92.15 μ M. Pabulenol (5) exhibited 31.71% inhibition at the highest tested concentration, 160 μ M, whereas xanthotoxin (2) and marmesin (3) exhibited only limited activity. Oxypeucedanin (7) and oxypeucedanin hydrate (9) showed no significant AChE inhibitory activity at 160 μ M.

For BChE, pabulenol (5) and bergapten (1) reached approximately 50% inhibition, displaying IC_{50} values of 157.28 μ M and 150.25 μ M, respectively. The remaining compounds showed only weak BChE inhibitory activity and did not reach 50% inhibition within the tested concentration range. Although bergapten was the most active molecule against AChE and BChE, its inhibitory potency remained markedly lower than that of donepezil (IC_{50} = 0.40 μ M for AChE; 3.38 μ M for BChE).

Molecular docking results

The AChE docking protocol was evaluated by redocking the co-crystallized ligand donepezil into the active site of the human AChE structure 4EY7. The redocked pose yielded an RMSD of 2.11 Å relative to the crystallographic pose, which is slightly above, but close to, the commonly used 2.0 Å threshold for docking validation. However, visual inspection of the superposed crystallographic and redocked poses showed that donepezil occupied the same active-site groove and largely preserved the overall orientation of the native ligand. The redocked pose also retained the principal interaction regions reported for the 4EY7 complex, particularly the aromatic region involving Trp86 and Trp286. Therefore, given the flexible and elongated structure of donepezil, the docking setup was acceptable for qualitative interpretation of the binding poses of the isolated furanocoumarins. The superposition of the crystallographic and redocked donepezil poses is provided in the Supplementary Figure S11. The 2D and 3D interaction diagrams of redocked donepezil are shown in Figure 2.

In the AChE docking analysis, the isolated furanocoumarins showed Glide SP scores ranging from -6.935 to -7.474 kcal/mol. Bergapten (1) exhibited the best docking score (-7.474 kcal/

Table 1. AChE and BChE inhibitory activities of the isolated compounds.

Compound	AChE		BChE	
	Inhibition (%) at 160 μ M	IC_{50} (μ M)	Inhibition (%) at 160 μ M	IC_{50} (μ M)
Oxypeucedanin	–		22.21 $\pm$ 2.47	
Pabulenol	31.71 $\pm$ 2.62		50.61 $\pm$ 1.55	157.28 $\pm$ 9.68
Bergapten	65.87 $\pm$ 2.69	92.15 $\pm$ 1.73	53.28 $\pm$ 3.74	150.25 $\pm$ 6.63
Xanthotoxin	13.43 $\pm$ 7.94		34.62 $\pm$ 8.07	
Marmesin	12.37 $\pm$ 7.98		14.92 $\pm$ 6.74	
Oxypeucedanin hydrate	–		20.65 $\pm$ 6.07	
Donepezil		0.40 $\pm$ 0.03		3.38 $\pm$ 0.45

Values are expressed as mean $\pm$ SD (n = 3). IC_{50} values were not calculated for compounds showing less than 50% inhibition at 160 μ M (the highest tested concentration). "–" indicates no significant inhibitory activity.

AChE, acetylcholinesterase; BChE, butyrylcholinesterase; SD, standard deviation.

mol), followed by marmesin (3), which had a score of -7.381 kcal/mol. The 2D and 3D docking poses of bergapten are shown in Figure 3.

The BChE docking protocol was evaluated by redocking the co-crystallized ligand tacrine into the active site of the human BChE structure 4BDS. The redocked pose showed an RMSD value of 1.44 Å compared with the crystallographic pose. The superposition of the crystallographic and redocked poses of tacrine is provided in the Supplementary Figure S12. The 2D and 3D interaction diagrams of redocked tacrine are shown in Figure 4.

In the BChE docking analysis, the isolated furanocoumarins showed Glide SP scores ranging from -6.165 to -7.471 kcal/mol.

Pabulenol (5) exhibited the best docking score (-7.471 kcal/mol), followed by oxypeucedanin hydrate (9) (-7.387 kcal/mol), bergapten (1) (-6.869 kcal/mol), oxypeucedanin (7) (-6.728 kcal/mol), and xanthotoxin (2) (-6.630 kcal/mol). The 2D and 3D docking poses of pabulenol, the compound with the best BChE docking score, are shown in Figure 5.

The Glide SP docking scores of the investigated compounds and reference ligands are summarized in Table 2.

DISCUSSION

The phytochemical investigation of the roots of *Cachrys cristata* led to the isolation of ten furanocoumarin-type compounds from the coumarin-rich fraction of the dichloromethane extract. The

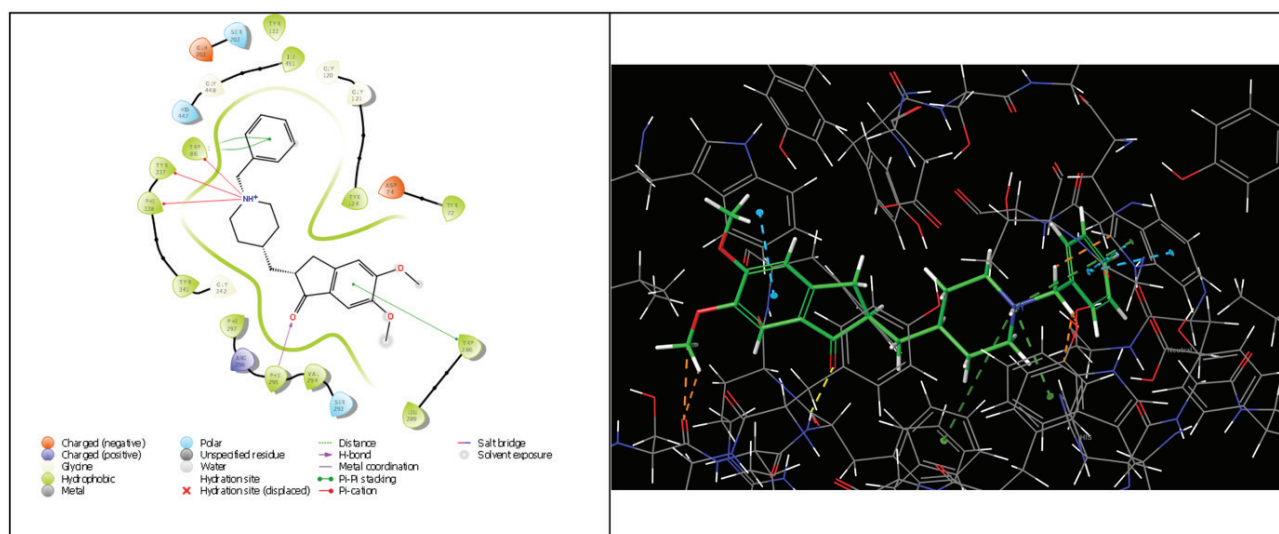


Figure 2. 2D and 3D redocked pose of donepezil in the active site of AChE (PDB ID: 4EY7).

AChE, acetylcholinesterase.

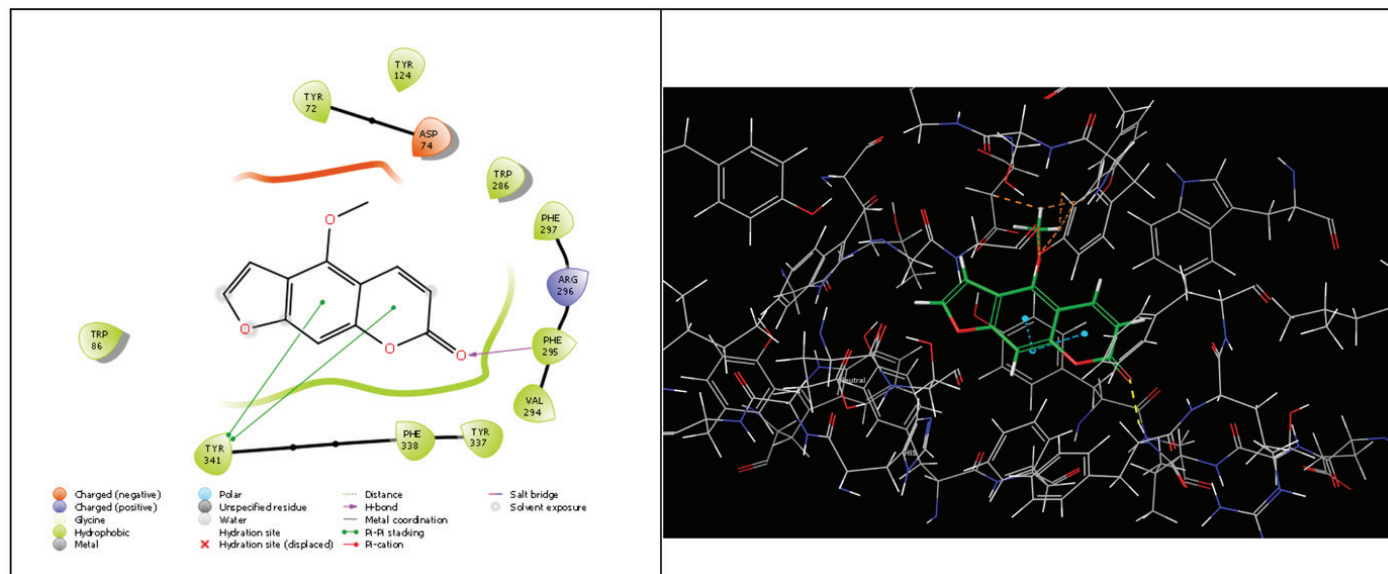


Figure 3. 2D and 3D docking pose of bergapten in the active site of AChE (PDB ID: 4EY7).

AChE, acetylcholinesterase.

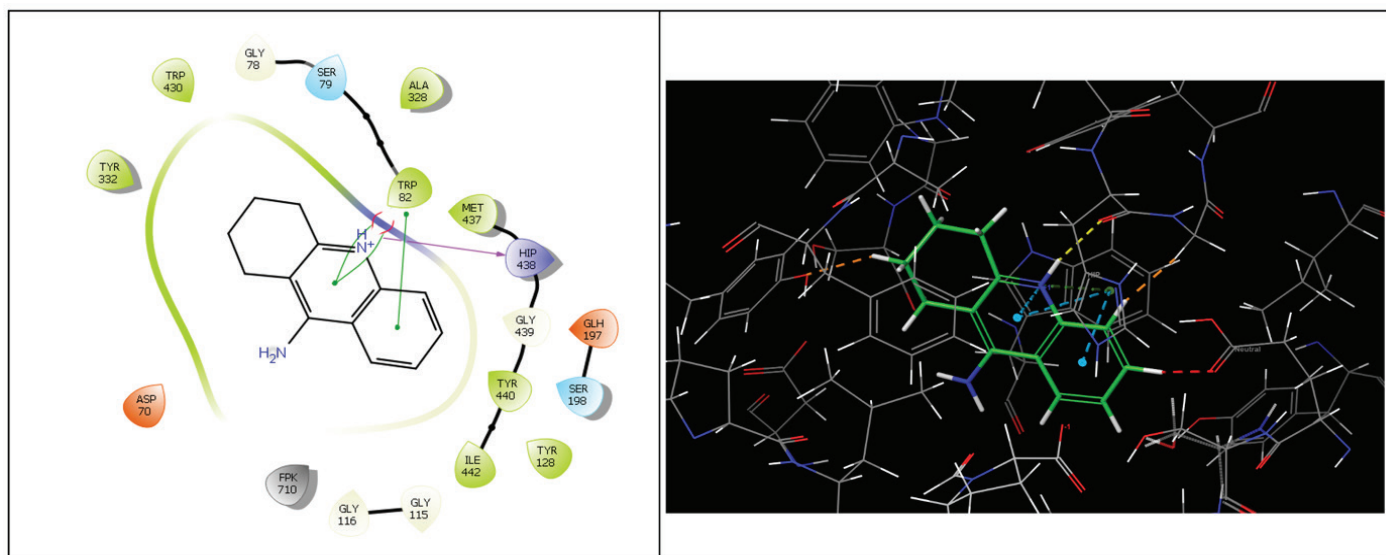


Figure 4. 2D and 3D redocked pose of tacrine in the active site of BChE (PDB ID: 4BDS). BChE, butyrylcholinesterase.

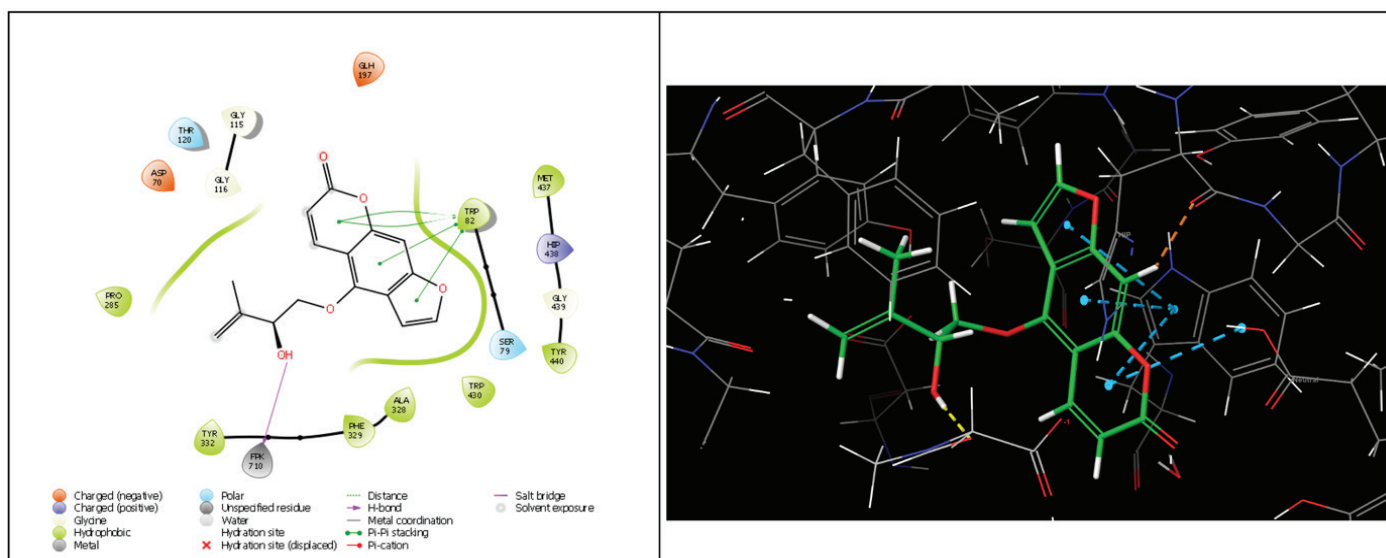


Figure 5. 2D and 3D docking pose of pabulenol in the active site of BChE (PDB ID: 4BDS). BChE, butyrylcholinesterase.

Table 2. Glide SP docking scores of the investigated furanocoumarins and reference ligands against AChE and BChE.

Compound	AChE Glide SP docking score	BChE Glide SP docking score
Bergapten (1)	-7.474	-6.869
Xanthotoxin (2)	-6.995	-6.630
Marmesin (3)	-7.381	-6.165
Pabulenol (5)	-7.183	-7.471
Oxypeucedanin (7)	-6.935	-6.728
Oxypeucedanin hydrate (9)	-7.166	-7.387
Donepezil	-12.798	-
Tacrine	-	-7.793

Values are reported as Glide SP docking scores.

AChE, acetylcholinesterase; BChE, butyrylcholinesterase.

predominance of furanocoumarins in this fraction is consistent with the known chemical profile of Apiaceae taxa, particularly in genera that are rich in linear and angular furanocoumarin derivatives. This finding is noteworthy from a phytochemical perspective, since, according to the available literature, furanocoumarins have not previously been reported from the roots of *Cachrys cristata*.

In the cholinesterase inhibition assays, the isolated compounds generally showed weak inhibitory activity compared with donepezil. Among the tested compounds, bergapten (1) was the most notable AChE inhibitor, with an IC_{50} value of 92.15 μ M. In contrast, pabulenol (5) and bergapten (1) showed measurable but weak BChE inhibition, with IC_{50} values of 157.28 μ M and 150.25 μ M, respectively. These findings suggest that the furanocoumarin scaffold alone is insufficient to confer strong cholinesterase inhibition, but may provide simple, natural framework for further structural optimization.

However, the estimated BChE IC_{50} values for pabulenol and bergapten were close to the upper limit of the investigated concentration range (160 μ M). Given that the inhibition percentages at the highest tested concentration were only slightly above 50%, these estimates should be interpreted with caution. The absence of measurements above 160 μ M may introduce uncertainty into IC_{50} estimates, and further evaluation over an extended concentration range would improve their precision.

Previous studies on Apiaceae furanocoumarins have reported variable cholinesterase inhibitory profiles depending on compound structure, enzyme source, and assay conditions. In *Angelica dahurica*, isoimperatorin, imperatorin, and oxypeucedanin were reported to be AChE-inhibitory constituents with IC_{50} values of 74.6, 63.7, and 89.1 μ M, respectively, whereas the reference inhibitor, berberine, showed an IC_{50} value of 2.9 μ M.³⁵ In another study on *Angelica dahurica* roots, oxypeucedanin and oxypeucedanin hydrate showed measurable but weaker cholinesterase inhibition than eserine, with IC_{50} values of 69.3 and 136.4 μ M against AChE and 65.6 and 71.2 μ M against BChE, respectively.³⁶ Studies on *Angelica officinalis* and *Angelica archangelica* further indicated that some furanocoumarins may inhibit BChE more strongly than AChE; however, simple furanocoumarins such as bergapten, xanthotoxin, and isoimperatorin may be weak inhibitors or inactive depending on the assay system.^{37,38} Therefore, the weak cholinesterase-inhibitory activity observed in the present study is generally consistent with previous reports and supports the view that the furanocoumarin scaffold alone is not sufficient for potent cholinesterase inhibition; rather, substitution patterns, side-chain features, and enzyme-specific interactions appear to be critical determinants of activity.

The AChE docking results partially supported the experimental activity profile. Bergapten (1) exhibited the most favorable docking score among the isolated compounds and was also the only compound with a measured AChE IC_{50} value. Its docking pose indicated accommodation in the aromatic region of the AChE active-site groove, mainly via hydrophobic and π -type interactions with residues such as Tyr341 and Phe295 and

other surrounding aromatic residues. However, the remaining compounds showed weak or negligible AChE inhibition despite comparable docking scores. This discrepancy indicates that the docking scores should be interpreted qualitatively rather than as direct predictors of inhibitory potency.

Comparison with donepezil further supports the observed weak AChE-inhibitory activity of the isolated furanocoumarins. Donepezil extends along the active-site groove and interacts with multiple binding regions, including aromatic contacts with Trp86 and Trp286. In contrast, the isolated furanocoumarins are smaller, mostly planar, and lack an extended linker or a cationic center. Although their fused coumarin-furan scaffold can be accommodated within the AChE active site, their limited interaction profile may preclude stronger binding and result in weaker inhibitory activity.

For BChE, pabulenol (5) showed the best docking score and displayed measurable inhibition. Its predicted pose suggested interactions with Trp82 and neighboring hydrophobic or aromatic residues such as Tyr332, Ala328, Met437, and Tyr440. The additional polar contact formed through its oxygenated side chain may contribute to its relatively favorable docking score. Bergapten (1), despite its lower docking score, also showed measurable BChE inhibition and was positioned near Trp82, where it maintained aromatic and hydrophobic contacts within the catalytic cavity.

Nevertheless, the BChE docking scores did not fully parallel the experimental results. Oxypeucedanin hydrate (9) showed a relatively favorable docking score but did not display comparable inhibitory activity. This suggests that factors such as ligand orientation, desolvation, conformational effects, and the absence of stronger catalytic-site interactions may influence the observed activity. Compared with tacrine, the tested furanocoumarins lacked a cationic center and did not reproduce the same strong interaction pattern with His438 and the active-site water network. This limited interaction profile may explain their weak inhibitory effects on BChE. Taken together, the AChE and BChE docking results should be interpreted cautiously, since the Glide SP docking scores of the isolated furanocoumarins were distributed within a relatively narrow range and did not fully parallel the experimental inhibition data. Therefore, the docking analysis was used only as qualitative structural support for possible binding orientations and interaction patterns, rather than as a quantitative predictor of cholinesterase inhibitory potency.

Overall, the biological and docking results indicate that the furanocoumarins isolated from *Cachrys cristata* exhibit only weak cholinesterase-inhibitory activity. However, the observed accommodation of these compounds within the AChE and BChE active-site cavities suggests that the furanocoumarin scaffold may serve as a starting point for designing semi-synthetic derivatives with enhanced binding interactions and stronger inhibitory activity.

Study limitations

The present study has some limitations. First, some isolated compounds could not be evaluated in the cholinesterase

inhibition assays because they were obtained in limited quantities. Therefore, biological evaluation was restricted to compounds available in sufficient quantities. Second, enzyme inhibition assays were performed as preliminary endpoint screening experiments; detailed kinetic studies were not conducted to determine the mechanism of inhibition. The molecular docking studies served only as qualitative structural support for possible binding orientations and interaction patterns; docking scores should not be interpreted as quantitative predictors of inhibitory potency.

CONCLUSION

Furanocoumarins isolated from *Cachrys cristata* exhibited weak cholinesterase- inhibitory activity. Bergapten was the most active compound against AChE, while only bergapten and pabulenol reached sufficient BChE inhibition to allow IC₅₀ determination. Molecular docking studies supported the experimental findings qualitatively, indicating that these compounds can be accommodated within the active-site cavities mainly through hydrophobic and π -type interactions with key aromatic residues. However, their limited interaction profiles, lack of a cationic center, and absence of extended binding features likely account for their markedly weaker activity compared with donepezil. Overall, these results suggest that the furanocoumarin scaffold may serve as a simple natural framework for the design of more potent semi-synthetic cholinesterase inhibitors.

Ethics

Ethics Committee Approval: Ethics committee approval was not required for this study, as it did not involve human participants or experimental animals.

Informed Consent: Informed consent was not required for this study, as it did not involve human participants or experimental animals.

Footnotes

Authorship Contributions

Concept: F.K.K., M.M., Design: F.K.K., Data Collection or Processing: F.K.K., C.K., Z.E.D., Ş.N.K.K., K.Ş.Ö., G.A.Ü., Analysis or Interpretation: F.K.K., G.A.Ü., M.M., Literature Search: F.K.K., C.K., Z.E.D., Writing: F.K.K., Z.E.D., G.A.Ü.

Conflict of Interest: The authors declare no conflicts of interest.

Financial Disclosure: This study was funded by the Scientific Research Projects Coordination Unit of İstanbul University. Project number: TLO-2026-42628.

Supplementary Figures S1-13: <https://d2v96fxpocvxx.cloudfront.net/bd1986e1-0bc1-4f4d-af66-3a184850a065/content-images/9b4a7fb8-bbb0-4dec-b556-84da3b5feff1.pdf>

REFERENCES

1. POWO. Plants of the World Online [Internet]. Royal Botanic Gardens, Kew; 2026. Available from: <https://powo.science.kew.org/>. Accessed 4 July 2026.

2. Wang XJ, Luo Q, Li T, Meng PH, Pu YT, Liu JX, Zhang J, Liu H, Tan GF, Xiong AS. Origin, evolution, breeding, and omics of Apiaceae: a family of vegetables and medicinal plants. *Hortic Res*. 2022;9:uhac076.
3. Sayed-Ahmad B, Talou T, Saad Z, Hijazi A, Merah O. The Apiaceae: Ethnomedicinal family as source for industrial uses. *Ind Crops Prod*. 2017;109:661-671.
4. Musolino V, Perri MR, Conforti F, Gliozzi M, Marrelli M, Mollace V. *Cachrys* L. Genus: a comprehensive review on botany, phytochemistry and biological properties. *Plants (Basel)*. 2023;12:565.
5. Nezahat Gökyiğit Botanik Bahçesi. Türkiye Bitkileri Listesi [Internet]. Available from: <https://www.bizimbitkiler.org.tr/list.html>. Accessed 4 July 2026.
6. Aouachria S, Boumerfeg S, Benslama A, Boussoulaim N, Trabsa H, Baghiani A. Phenolics contents, xanthine oxidoreductase inhibitory potential, antibacterial and antioxidant activities of *Cachrys libanotis* L. Root Extracts. *J Drug Deliv Ther*. 2020;10:71-79.
7. Baser KHC, Demirci B, Akalin E, Özhatay N. Composition of the essential oil of *Cachrys alpina* Bieb. *J Essent Oil Res*. 2004;16:167-168.
8. Grande M, Aguado MT, Mancheño B, Piera F. Coumarins and ferulol esters from *Cachrys sicula*. *Phytochemistry*. 1986;25:505-508.
9. Marrelli M, Perri MR, Amodeo V, Giordano F, Statti GA, Panno ML, Conforti F. Assessment of photo-induced cytotoxic activity of *Cachrys sicula* and *Cachrys libanotis* enriched-coumarin extracts against human melanoma cells. *Plants (Basel)*. 2021;10:123.
10. Menichini G, Alfano C, Provenzano E, Marrelli M, Statti GA, Menichini F, Conforti F. *Cachrys pungens* Jan inhibits human melanoma cell proliferation through photo-induced cytotoxic activity. *Cell Prolif*. 2012;45:39-47.
11. Maresca V, Badalamenti N, Ilardi V, Bruno M, Basile A. The antioxidant properties and protective capacity of *Prangos trifida* and *Cachrys cristata* essential oils against Cd stress in *Lunularia cruciata* and *Brassica napus*. *Antioxidants (Basel)*. 2023;12:793.
12. Sgadari F, Vaglica A, Porrello A, Crisafulli A, Schicchi R, Bruno M. *Cachrys pungens* Jan ex Guss.: the essential oil chemical composition of the accession growing wild in Sicily (Italy). *Nat Prod Res*. 2025;39:5996-6001.
13. Koutsaviti A, Georgiou C, Milenković M, Tzakou O. Composition and antimicrobial activity of the essential oils from different parts of *Cachrys cristata* DC. from Greece. *Rec Nat Prod*. 2015;9:436-440.
14. Smaili T, Bendif H, Flamini G, Öztürk M, Duru ME, Bruno M, Maggi F. Chemical composition, antioxidant and anticholinesterase activity of the essential oil of Algerian *Cachrys sicula* L. *Nat Prod Res*. 2022;36:4094-4102.
15. Matejić JS, Džamić AM, Ristić MS, Randelović VN, Marin PD. Essential oil composition of *Cachrys cristata* – a rare and endangered species in the flora of Serbia. *Nat Prod Commun*. 2012;7:235-236.
16. World Health Organization. Dementia [Internet]. Available from: <https://www.who.int/news-room/fact-sheets/detail/dementia>. Accessed 4 July 2026.
17. Hampel H, Mesulam MM, Cuello AC, Farlow MR, Giacobini E, Grossberg GT, Khachaturian AS, Vergallo A, Cavado E, Snyder PJ, Khachaturian ZS. The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain*. 2018;141:1917-1933.

18. Kowalczyk J, Kurach Ł, Boguszewska-Czubara A, Skalicka-Woźniak K, Kruk-Słomka M, Kurzepa J, Wydrzynska-Kuźma M, Biała G, Skiba A, Budzyńska B. Bergapten improves scopolamine-induced memory impairment in mice via cholinergic and antioxidative mechanisms. *Front Neurosci.* 2020;14:730.
19. Kurach Ł, Kulczycka-Mamona S, Kowalczyk J, Skalicka-Woźniak K, Boguszewska-Czubara A, El Sayed N, Osmani M, Iwaniak K, Budzyńska B. Mechanisms of the procognitive effects of xanthotoxin and umbelliferone on LPS-induced amnesia in mice. *Int J Mol Sci.* 2021;22:1779.
20. Ellman GL, Courtney KD, Andres V Jr, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol.* 1961;7:88-95.
21. Sevinçli ZŞ, Soylu-Eter Ö, Hasbal Celikok G, Altiparmak Ulbegi G, Bayır E, Şendimir A, Aksoy Sagirli P, Karalı N. Novel 2-indolinone benzylpiperidine-thiosemicarbazone hybrids as cholinesterase inhibitors: design, synthesis and biological evaluation. *Bioorg Chem.* 2026;180:110150.
22. Cheung J, Rudolph MJ, Burshteyn F, Cassidy MS, Gary EN, Love J, Franklin MC, Height JJ. Structures of human acetylcholinesterase in complex with pharmacologically important ligands. *J Med Chem.* 2012;55:10282-10286.
23. Nachon F, Carletti E, Ronco C, Trovaslet M, Nicolet Y, Jean L, Renard PY. Crystal structures of human cholinesterases in complex with huprine W and tacrine: elements of specificity for anti-Alzheimer's drugs targeting acetyl- and butyryl-cholinesterase. *Biochem J.* 2013;453:393-399.
24. Sastry GM, Adzhigirey M, Day T, Annabhimoju R, Sherman W. Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. *J Comput Aided Mol Des.* 2013;27:221-234.
25. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, Repasky MP, Knoll EH, Shelley M, Perry JK, Shaw DE, Francis P, Shenkin PS. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J Med Chem.* 2004;47:1739-1749.
26. Halgren TA, Murphy RB, Friesner RA, Beard HS, Frye LL, Pollard WT, Banks JL. Glide: a new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening. *J Med Chem.* 2004;47:1750-1759.
27. Senol FS, Orhan IE, Yilmaz G, Cicek M, Sener B. An in vitro and in silico approach to cholinesterase inhibitory and antioxidant effects of furanocoumarins from *Angelica officinalis* L. roots. *Food and Chemical Toxicology.* 2011;49:3350-3357.
28. Yassaa N, Hadjiaghaee R, Delnavazi MR, Mofasseri M, Ghafarzadegan R, Heydari F, Tavakoli S. Evaluation of antioxidant and antibacterial activities of the bioactive compounds from the leaves of *Ferulago trifida* Boiss. *Iran J Chem Chem Eng.* 2024;43:2855-2865.
29. Lee S, Shin DS, Kim JS, Oh KB, Kang SS. Antibacterial coumarins from *Angelica gigas* roots. *Arch Pharm Res.* 2003;26:449-452.
30. Chatterjee A, Banerji J, Basa SC. Lactonic constituents of *Prangos pabularia* Lindl. (Umbelliferae). *Tetrahedron.* 1972;28:5175-5182.
31. Lee YY, Lee S, Jin JL, Yun Choi HS. Platelet anti-aggregatory effects of coumarins from the roots of *Angelica genuflexa* and *A. gigas*. *Arch Pharm Res.* 2003;26:723-726.
32. Jiménez B, Grande MC, Anaya J, Torres P, Grande M. Coumarins from *Ferulago capillaris* and *F. brachyloba*. *Phytochemistry.* 2000;53:1025-1031.
33. Thanh PN, Jin WY, Song GY, Bae KH, Kang SS. Cytotoxic coumarins from the root of *Angelica dahurica*. *Arch Pharm Res.* 2004;27:1211-1215.
34. Harker S, Razdan TK, Waight ES. Steroids, chromone and coumarins from *Angelica officinalis*. *Phytochemistry.* 1984;23:419-426.
35. Kim DK, Lim JP, Yang JH, Eom DO, Eun JS, Leem KH. Acetylcholinesterase inhibitors from the roots of *Angelica dahurica*. *Arch Pharm Res.* 2002;25:856-859.
36. Seo WD, Kim JY, Ryu HW, Kim JH, Han SI, Ra JE, Seo KH, Jang KC, Lee JH. Identification and characterisation of coumarins from the roots of *Angelica dahurica* and their inhibitory effects against cholinesterase. *J Funct Foods.* 2013;5:1421-1431.
37. Senol FS, Skalicka-Woźniak K, Khan MTH, Orhan IE, Sener B, Głowniak K. An in vitro and in silico approach to cholinesterase inhibitory and antioxidant effects of the methanol extract, furanocoumarin fraction, and major coumarins of *Angelica officinalis* L. fruits. *Phytochem Lett.* 2011;4:462-467.
38. Wszelaki N, Paradowska K, Jamróz MK, Granica S, Kiss AK. Bioactivity-guided fractionation for the butyrylcholinesterase inhibitory activity of furanocoumarins from *Angelica archangelica* L. roots and fruits. *J Agric Food Chem.* 2011;59:9186-9193.