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Research Article

Volatile profile and multitarget *in silico* evaluation of *Agrimonia eupatoria* L. essential oil and infusion*

Stoyan Stoyanov[1], Ivayla Dincheva[2], Iliyan Kolev[3], Halil Şenol[4], Momchil Barbolov[5], Pawel Olczyk[6], Galina Yaneva[1], Oskan Tasinov[5]

1 *Department of Biology, Faculty of Pharmacy, Medical University of Varna, 9002 Varna, Bulgaria*
2 *Department of Agrobiotechnologies, AgroBioInstitute, Agricultural Academy, 1164 Sofia, Bulgaria*
3 *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Varna, 9002 Varna, Bulgaria*
4 *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Bezmialem Vakif University, 34093 Fatih, İstanbul, Türkiye*
5 *Department of Biochemistry, Molecular Medicine and Nutrigenomics, Faculty of Pharmacy, Medical University of Varna, 9002 Varna, Bulgaria*
6 *Faculty of Medical Sciences and Health Sciences, Radom University, Chrobrego 27, 26-600 Radom, Poland*

Corresponding author: Oskan Tasinov (oskan.tasinov@gmail.com)

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Abstract

Agrimonia eupatoria L. is a medicinal plant used in traditional medicine in Bulgaria and other European countries. Recently, the volatile profile of the essential oil and infusion obtained from the dried aerial parts of *A. eupatoria* was analyzed, and their biological potential was determined through a multitarget *in silico* approach. Steam distillation, gas chromatography–mass spectrometry (GC-MS), and gas chromatography–flame ionization detection (GC-FID) were used to obtain and analyze substances. Molecular docking was performed on the major compounds from both extracts against cyclooxygenase-2 (COX-2), monoamine oxidase B (MAO-B), acetylcholinesterase (AChE), and butyrylcholinesterase (BChE)—enzymes associated with neuroinflammation. Seventeen volatile compounds were identified, with a significant difference between the two profiles. The extraction method influenced the composition of the preparations: nonpolar substances, such as hexahydrofarnesyl acetone and β-bisabolene, predominated in the oil, while more polar compounds, such as linalool, prevailed in the infusion. Isophytol and hexahydrofarnesyl acetone exhibited the best binding affinities against the target enzymes *in silico*, suggesting potential anti-inflammatory, antioxidant, and neuroprotective effects.

Keywords

Agrimony, COX-2, herbal medicine, isophytol, volatile compounds

Background

Agrimonia eupatoria L. (common agrimony, stickwort, liverwort, or church steeples) is a perennial herbaceous plant. It has a short root and a long, hairy stem that reaches a height of 50–150 cm (Paluch et al. 2020). Its serrated, pinnate leaves are covered with soft hairs. The small, yellow, hermaphroditic flowers grow in raceme-like clusters

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with a double perianth. The fruits are grooved and contain two seeds. Flowering occurs from June to September (Ivanova et al. 2011; Muruzović et al. 2016; Santos et al. 2017). Common agrimony grows in temperate zones of North America, Europe, Asia, and parts of North Africa and the Canary Islands (Kwon et al. 2005). It can be seen in bushes, grassy areas, around deciduous forests, and along roadsides (Neshev and Landzhev 1989).

The therapeutic effects of common agrimony are described in the scientific works of scholars who lived in the 4th and 5th centuries AD (Paluch et al. 2020). In folk medicine, the aerial parts of the plant are used (Ivanov et al. 1973). *A. eupatoria* infusions are widely used both externally (as gargles, compresses, and baths) and internally (as infusions) in Bulgarian traditional medicine. Some of the most common medical conditions that are treated include diarrhea, skin and oral cavity inflammation, and liver or gallbladder diseases (Yordanov et al. 1973; Malheiros et al. 2022)

There is still insufficient research data on the biomedical potential of the plant. Various studies have shown that extracts from *A. eupatoria* can capture exogenous or endogenous radicals and inhibit lipid peroxidation (Correia et al. 2006, 2007; Muruzović et al. 2016). A clinical trial has shown that consuming an infusion made from the plant for 30 days increases the total antioxidant capacity of blood plasma (Ivanova et al. 2013). In addition, lower levels of the pro-inflammatory cytokine interleukin-6 (IL-6) and increased high-density lipoprotein (HDL) levels were reported in the plasma of healthy volunteers. Dietary administration of the herb extract in animals with metabolic syndrome reduced levels of monocyte chemoattractant protein-1 (MCP-1) (Kiselova et al. 2011). The anti-inflammatory activity of common agrimony extracts has also been confirmed in experimental models using lipopolysaccharide-activated BV2 microglial cells and macrophages (Bae et al. 2010; Santos et al. 2017). Antimicrobial activity has been established against *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Helicobacter pylori*, and *Campylobacter jejuni* (Copland et al. 2003; Cwikla et al. 2010; Watkins et al. 2012; Ghaima 2013). Water extract from the aerial parts of the herb inhibits the secretion of hepatitis B viral surface antigen (Kwon et al. 2005). Fungicidal activity against *Candida albicans* has also been established (Muruzović et al. 2016). The results of Ad'hiah et al. (2013) demonstrated a possible concentration-dependent antitumor effect against the HeLa cell line and rhabdomyosarcoma (RD) cells. In models of streptozotocin-induced diabetes in rats, decoctions of *A. eupatoria* leaves have been reported to reduce diabetes-associated symptoms such as weight loss, thirst, and increased hunger (Swanston-Flatt et al. 1990; Gray and Flatt 1998). In addition, vasodilatory, anticoagulant, hepatoprotective, and wound-healing effects have also been reported in the literature (Kuczmannová et al. 2016; Cho et al. 2018; Tsirigotis-Maniecka et al. 2019; Vasilenko et al. 2022). In a model of glutamate-induced oxidative damage in HT22 cells, methanol extract of the aerial parts reduced oxidative stress and had a neuroprotective effect (Lee et al. 2010).

The aerial parts of common agrimony contain a number of biologically active substances, with polyphenolic compounds being the main constituents (Paluch et al. 2020). Phenolic acids include vanillic acid, gentisic acid, ellagic acid, chlorogenic acid, neochlorogenic acid, and others (Shabana et al. 2003; Correia et al. 2006; Granica et al. 2015; Pukalskienė et al. 2018; Paluch et al. 2020). Flavones comprise derivatives of apigenin (isovitexin, vitexin, apigetrin) and luteolin (Kubinova et al. 2012; Granica et al. 2013, 2015), while flavonols include quercetin (rutin, hyperoside, isoquercitrin) and kaempferol (astragalin) derivatives (Correia et al. 2007; Venskutonis et al. 2008; Lee et al. 2010; Kubinova et al. 2012; Granica et al. 2013, 2015). It has been proven that *A. eupatoria* contains at least 2% tannins (Paluch et al. 2020). Most of them are condensed tannins, particularly proanthocyanidins (Paluch et al. 2020). Agrimoniin and pedunculagin have been reported from the hydrolyzable tannins (Granica et al. 2013, 2015; Santos et al. 2017; Pukalskienė et al. 2018). Triterpenoids such as ursolic acid, α-amyrin, and euscaphic acid have also been identified in the plant (Paluch et al. 2020).

There are a few studies investigating the volatile organic compounds in *A. eupatoria* (Table 1). Only one report could be identified in which essential oil from dried plant material collected in Bulgaria was studied (Doğan et al. 2023). Furthermore, previous studies have not examined volatile components in herbal infusion. The profile of volatile organic compounds in the plant is characterized by a strong predominance of the monoterpene (+)-α-pinene (Navaei and Mirza 2009; Feng et al. 2013; Doğan et al. 2023; Iqbal et al. 2023; Milani et al. 2025). In addition to monoterpenes, essential oils also contain sesquiterpenes, their oxygen-containing derivatives (alcohols, ketones, oxides), aliphatic aldehydes, fatty acids, and their esters. Some of these substances have biological activity. For example, α-pinene and eucalyptol exhibit anti-inflammatory effects (Juergens et al. 2003; Tümen et al. 2018). However, the mechanisms of action of many of the constituents remain unclear.

It is now widely recognized that oxidative stress and inflammation reinforce each other (Bellanti et al. 2025). The pathogenesis of Alzheimer's disease is strongly associated with chronic inflammation and oxidative stress, which promote neuronal damage, synaptic dysfunction, and gradual cognitive decline (Kurban et al. 2024; Farooq et al. 2025). There are currently very few drugs approved for Alzheimer's disease, which is why research efforts are focused on developing new avenues of treatment (Almaghrabi 2025). Research highlights the coordinated involvement of key enzymes, including COX-2, MAO-B, AChE, and BChE, in mechanisms underlying the development of Alzheimer's disease (Asghar et al. 2024). The interconnected roles of these enzymes make them key therapeutic targets in combating inflammation, oxidative stress, and neurodegenerative diseases (Kurban et al. 2024; Farooq et al. 2025). COX-2, overexpressed during inflammation, leads to oxidative damage and neuronal injury; thus, its inhibition helps reduce neuroinflammation and oxidative imbalance (Moussa and Dayoub 2023; Şenol et al.

Table 1. Volatile organic compounds found in different parts of *A. eupatoria*.

Part of the plant	State of material	Method of obtaining and analysis of essential oil	Total number of isolated volatile compounds	Major isolated substances
Wild growing leaves	n.s	hydrodistillation, GC-MS[1], GC-FID[2]	21	β-caryophyllene, caryophyllene oxide, α-humulene, *E*-β-farnesene (Navaei and Mirza 2009)
Cultivated leaves			21	α-pinene, β-caryophyllene, *E*-α-farnesene, cumine aldehyde (Navaei and Mirza 2009)
Wild growing flowers			27	β-caryophyllene, *E*-β-farnesene, α-humulene (Navaei and Mirza 2009)
Cultivated growing flowers			25	β-caryophyllene, caryophyllene oxide, α-copaene (Navaei and Mirza 2009)
Roots and leaves	Fresh	hydrodestillation, GC-MS, SWFA[3], O-PLS[4]	68 (main root), 65 (leaves)	cedrol, *α*-pinene, linalool, *α*-terpineol, *α*-eudesmol, eucalyptol (Feng et al. 2013)
Leaves	Fresh	HS-SPME-GC-MS[5]	44	α-pinene, (−)limonene, β-thujene (Milani et al. 2025)
Aerial parts	Fresh	steam distillation, GC-MS	24	α-pinene, β-pinene, isophene, α-caryophyllene, carvone (Iqbal et al. 2023)
Aerial parts	Dried	hydrodistillation, GC-MS	20	α-pinene, *n*-hexadecanoic acid, (5*E*,9*E*)-farnesyl acetone, (5*E*,9*Z*)-farnesyl acetone (Doğan et al. 2023)

[1] Gas chromatography–mass spectrometry, [2] gas chromatography–flame ionization detector,
[3] subwindow factor analysis, [4] orthogonal projections to latent structures, [5] headspace–solid phase microextraction–gas chromatography–mass spectrometry; n.s = not specified

2023). MAO-B, which metabolizes dopamine and generates reactive oxygen species, contributes to oxidative stress and neuronal apoptosis when overactive, making its inhibition crucial for neuroprotection (Behl et al. 2021). Meanwhile, AChE and BChE regulate acetylcholine levels essential for memory and cognition. Their excessive activity in Alzheimer's disease accelerates neurotransmitter depletion and cognitive decline (Banik et al. 2021; Demir et al. 2026). Therefore, the simultaneous inhibition of these enzymes could, in principle, restore redox and neurotransmitter balance, alleviate inflammation, and protect against neurodegeneration, supporting their selection as primary docking targets for evaluating the therapeutic potential of bioactive compounds (Senol et al. 2023; Naz et al. 2024; Toraman et al. 2024). In recent years, numerous plant-derived compounds have been reported to inhibit these enzymes (Chaurasiya et al. 2022; Ju et al. 2022; Dimitrova et al. 2025). However, the potential of these volatile phytochemicals remains largely underexplored in this context.

This study aimed to analyze the volatile profile of the essential oil and infusion obtained from the dried aerial parts of *A. eupatoria*. In addition, the main volatile constituents found were subjected to multitarget *in silico* analysis against COX-2, MAO-B, AChE, and BChE to evaluate their anti-inflammatory, antioxidant, and neuroprotective effects.

Materials and methods

Plant material

The plant material was purchased from a retail outlet in Varna, Bulgaria. The product was packaged by "Bilki" EOOD, Sinitovo Village, Industrial Zone. As per the manufacturer's specification, the material was procured during the anthesis phenophase, specifically spanning the months of July to August, within the Eastern Rhodopes region. After harvesting, the aerial parts were subjected to coarse crushing and dried in the shade.

Preparation of aqueous infusion

The preparation protocol for the infusion was adapted from the European Medicines Agency's monograph on *A. eupatoria* (Committee on Herbal Medicinal Products [HMPC] 2015). In brief, a quantity of 2.5 g of the specified medicinal plant material was infused with 200 mL of boiling water, followed by a 10-min incubation period within a closed vessel.

Extraction of volatile compounds

Volatile (essential oil) components were extracted via steam distillation from the air-dried aerial tissues of *A. eupatoria* utilizing a laboratory-scale distillation apparatus with a 0.01 m^3 capacity. The resultant hydrolate (floral water) was subjected to liquid–liquid extraction, performed in duplicate using 10 mL aliquots of pentane, for the isolation of the volatile oil fraction. The combined organic phases were concentrated *in vacuo* under a gentle argon stream (5 mL/min). Fifty μL aliquots of the thus obtained concentrated solution were subjected to GC-MS analysis.

Parallel extraction methodology, employing an analogous organic solvent, was implemented for the isolation of volatile constituents from the prepared infusion.

Analysis of volatile compounds

To evaluate analytical repeatability, four independent replicates were prepared and measured for each preparation (essential oil and infusion). The chemical profile was determined using a 7890A gas chromatograph coupled to a 5975C mass selective detector (Agilent Technologies Inc., Santa Clara, CA,

USA). Separation was achieved on an HP-5 ms fused-silica capillary column (30 m × 0.32 mm i.d., 0.25 µm film thickness). The GC oven conditions were as follows: initial temperature was 60 °C (3 min), increased to 80 °C at 1 °C/min and held for 3 min, then ramped to 300 °C at 5 °C/min and held for 5 min. Helium was used as the carrier gas at 1.0 mL/min. A 1.0 µL aliquot was injected in split mode 20:1. The injector, ion source, and quadrupole temperatures were set to 250 °C, 230 °C, and 150 °C, respectively. Mass spectra were acquired in full-scan EI mode at 70 eV. Compound identification was performed by comparing linear retention indices (LRIs) and mass fragmentation patterns with the NIST'08 and Adams libraries. LRIs were calculated using a homologous *n*-alkane series (C8–C36) analyzed under identical conditions.

Quantitative analysis was carried out using GC-FID on the same chromatographic system and column. The oven temperature program followed the conditions described above. The injector and detector temperatures were set at 220 °C and 280 °C, respectively. Helium served as the carrier gas at 1.0 mL/min, and 1.0 µL of each sample was injected in split mode. Relative proportions of volatile constituents were calculated by peak-area normalization based on total ion current.

Statistics

Microsoft Excel 2016 was used to process the data. The following functions were applied sequentially to analyze the data. First, AVERAGE was used to calculate the mean concentration of the compounds in replicate samples. Next, STDEV.S was used to determine the standard deviation. This was followed by F.TEST to compare variances. Finally, T.TEST (Welch's *t*-test) was conducted to assess statistical significance. A *p*-value of less than 0.05 was considered statistically significant.

Computational studies

Molecular docking and dynamics simulations were performed using Schrödinger Molecular Modeling Software (2025-1) with the Maestro interface (version 14.3) and Desmond (D. E. Shaw Research 2024-4). Target proteins, including COX-2 (PDB ID: 4PH9, resolution 1.81 Å), MAO-B (PDB ID: 4A79, resolution 1.89 Å), AChE (PDB ID: 4EY7, resolution 2.35 Å), and BChE (PDB ID: 6EP4, resolution 2.30 Å), were retrieved from the Protein Data Bank. Protein structures were prepared using the Protein Preparation Wizard, including the addition of missing hydrogens, optimization of protonation states, and energy minimization to remove steric clashes. Ligands were prepared with the LigPrep module, generating all possible ionization and tautomeric states at pH 7.4 ± 2, followed by geometry optimization and energy minimization using the OPLS4 force field. Docking was performed using Glide and Induced Fit Docking (IFD). For Glide docking, a cubic grid box of 20 × 20 × 20 Å was centered on the active site to fully encompass the binding pocket, ensuring accurate evaluation of ligand placement. The best docking poses were selected based on IFD docking scores, and binding free energies were estimated using Prime MM-GBSA with the VSGB solvation model, capturing both polar and hydrophobic contributions (Demir et al. 2026; Tokalı et al. 2026).

To assess the dynamic stability of the complexes, 250 ns molecular dynamics (MD) simulations were carried out using Desmond. Each complex was solvated in a TIP4P water box with Na- and Cl- ions to neutralize the system. After energy minimization, the systems were equilibrated in the NPT ensemble at 300 K and 1 atm, followed by 250 ns production runs. Stability analyses included RMSD of protein Cα and ligand atoms, RMSF of residues and ligand atoms, and monitoring of hydrogen bonds, hydrophobic contacts, and other key interactions throughout the simulation (Tokalı et al. 2025). These computational studies provide a comprehensive understanding of ligand binding modes, stability, and dynamic behavior of isophytol complexes with COX-2, MAO-B, AChE, and BChE.

Results

Isolated volatile organic compounds

Analysis of the essential oil and herbal infusion samples revealed the presence of 17 distinct volatile organic compounds, as delineated in Table 2. Classification based on data retrieved from the PubChem database indicated the following compositional distribution: alcohols ($n = 5$), terpenes ($n = 4$), ketones ($n = 2$), aldehydes ($n = 2$), phenylpropanoids ($n = 1$), and alkanes ($n = 1$), as visually represented in Fig. 1. Principal constituents were operationalized as those exhibiting a relative abundance exceeding 5% of the total content. Welch's *t*-test, implemented to evaluate disparities between the essential oil and infusion volatile profiles, revealed statistically significant differentiation across all volatile compounds investigated ($p < 0.05$). Total ion chromatograms for both sample types are shown in Suppl. material 1: fig. S1A, B.

Volatile profile of the essential oil

Analysis of the composition of the essential oil revealed the predominant presence of ketones (40%), followed by alcohols (21%), sesquiterpenes (15%), aldehydes (14%), and monoterpenes (7%) (Fig. 2). Quantitative analysis identified hexahydrofarnesyl acetone (26.64 ± 1.28) as the most abundant volatile organic compound, succeeded by β-bisabolene (10.78 ± 1.24), isophytol (7.29 ± 0.53), *n*-hexadecanal (6.07 ± 0.43), and α-thujene (4.83 ± 0.49). Minor constituents, listed in Table 2, included (5*Z*,9*E*)-farnesyl acetone (3.27 ± 0.43), *n*-tridecanal (2.89 ± 0.43), linalool (2.47 ± 0.37), and *n*-hexadecanol (2.44 ± 0.36).

Volatile profile of the herbal infusion

Analysis of the infusion's volatile composition revealed a significantly divergent profile compared to the essential oil. The infusate presented an elevated concentration of

Table 2. Isolated volatile organic compounds after GC analysis of essential oil and infusion from dried aerial parts of *A. eupatoria*. The concentrations are given in mg/mL extract. Results are presented as mean ± SD.

Peak	RT[a] (min.)	RI[b]	Name	Content in essential oil (mg/mL)	Content in infusion (mg/mL)
1	9.79	927	α-Thujene	4.83 ± 0.49	0.82 ± 0.06
2	15.14	1097	Linalool	2.47 ± 0.37	48.73 ± 0.66
3	20.21	1266	Anethol	0.12 ± 0.02	1.04 ± 0.28
4	24.82	1439	(*Z*)-β-Farnesene	0.43 ± 0.06	1.42 ± 0.11
5	24.95	1455	(*E*)-β-Farnesene	0.29 ± 0.05	1.23 ± 0.09
6	26.34	1508	β-Bisabolene	10.78 ± 1.24	4.18 ± 0.32
7	26.41	1556	*n*-Tridecanal	2.89 ± 0.43	0.93 ± 0.07
8	27.62	1609	*n*-Tetradecanal	0.60 ± 0.09	1.32 ± 0.12
9	29.03	1690	*n*-Pentadecanal	0.67 ± 0.11	1.11 ± 0.10
10	31.09	1795	*n*-Hexadecanal	6.07 ± 0.43	2.31 ± 0.25
11	33.99	1852	Hexahydrofarnesyl acetone	26.64 ± 1.28	4.63 ± 0.35
12	34,39	1873	*n*-Hexadecanol	2.44 ± 0.36	0.71 ± 0.05
13	35.15	1897	(5*Z*,9*E*)-farnesyl acetone	3.27 ± 0.43	0.68 ± 0.05
14	35.86	1940	Phytol	1.93 ± 0.30	0.79 ± 0.06
15	35.98	1948	Isophytol	7.29 ± 0.53	1.90 ± 0.14
16	36.57	2071	*n*-Octadecanol	1.64 ± 0.24	0.53 ± 0.04
17	39.48	2298	*n*-Tricosane	1.83 ± 0.27	0.50 ± 0.04

[a] RT – retention time; [b] RT – retention index.

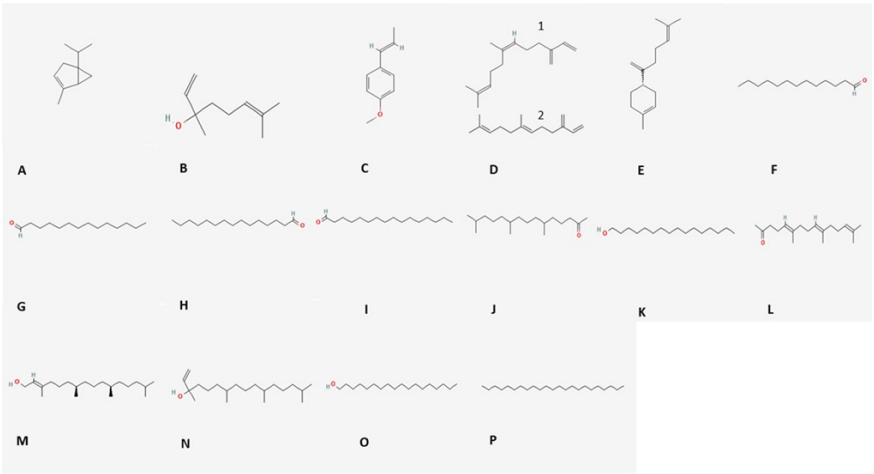


Figure 1. Chemical structure of α-thujene **(A)**, linalool **(B)**, anethole **(C)**, (*Z*)-β-farnesene **(D1)**, (*E*)-β-farnesene **(D2)**, β-bisabolene **(E)**, *n*-tridecanal **(F)**, *n*-tetradecanal **(G)**, *n*-pentadecanal **(H)**, *n*-hexadecanal **(I)**, hexahydrofarnesyl acetone **(J)**, *n*-hexadecanol **(K)**, (5*Z*,9*E*)-farnesyl acetone **(L)**, phytol **(M)**, isophytol **(N)**, *n*-octadecanol **(O)**, and *n*-tricosane **(P)**. Available from: https://pubchem.ncbi.nlm.nih.gov/ [Accessed on 27 October 2025].

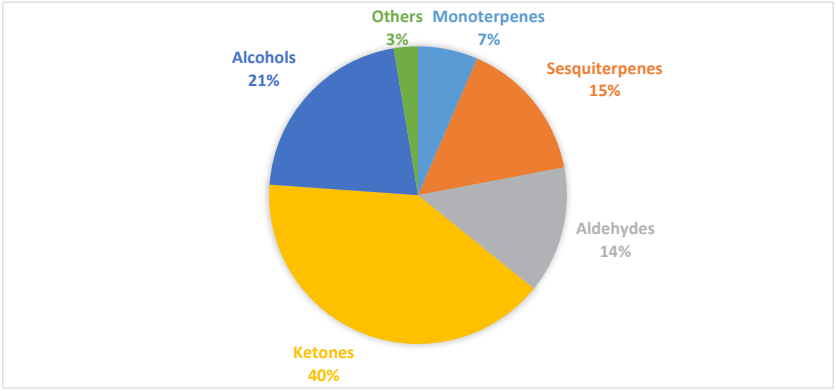


Figure 2. Percentage content of volatile organic compounds in essential oil from dried aerial parts of *A. eupatoria*. Ketones (40%) prevailed in the essential oil profile, followed by alcohols (21%), sesquiterpenes (15%), aldehydes (14%), and monoterpenes (7%). Others include phenylpropanoids and alkanes.

alcoholic compounds; gas chromatographic analysis identified linalool (48.73 ± 0.66) as the primary volatile organic compound, comprising approximately two-thirds of the total volatile fraction (Fig. 3). Further prominent constituents of the infusion included hexahydrofarnesyl acetone (4.63 ± 0.35) and β-bisabolene (4.18 ± 0.32). With the exception of anethole (1.04 ± 0.28), (*E*)-β-farnesene (1.23 ± 0.09), (*Z*)-β-farnesene (1.42 ± 0.11), *n*-tetradecanal (1.32 ± 0.12), and *n*-pentadecanal (1.11 ± 0.10) (Table 2), the concentrations of the remaining compounds in the infusion were determined to be lower relative to those in the essential oil.

Molecular docking studies

In the present study, molecular docking analyses were conducted to predict the possible molecular targets and binding affinities of the most abundant phytochemical constituents identified in the extracts. The selected compounds, hexahydrofarnesyl acetone, β-bisabolene, linalool, *n*-hexadecanal, α-thujene, isophytol, and (5*Z*,9*E*)-farnesyl acetone, were docked into the active sites of selected target proteins to evaluate their interaction patterns and potential biological relevance. The results are given in Table 3. To achieve accurate binding conformations and affinity estimations, Induced Fit Docking (IFD) simulations were performed, allowing both ligand and receptor flexibility during the docking process. Furthermore, MM-GBSA binding free energy (Δ*G* bind) calculations were applied to refine the docking results and provide a more reliable estimation of the ligand–protein binding strength (Şenol 2024).

Among the tested compounds, isophytol and hexahydrofarnesyl acetone exhibited the most favorable binding affinities across multiple targets. Isophytol demonstrated remarkably strong IFD docking scores with COX-2 (−10.302 kcal/mol) and MAO-B (−10.222 kcal/mol), accompanied by highly negative MM-GBSA energies (−74.61 and −74.17 kcal/mol, respectively). The 2D and 3D ligand–protein interaction analysis of related complexes obtained from isophytol with COX-2, MAO-B, AChE, and BChE is given in Figs 2, 3. Similarly, hexahydrofarnesyl acetone showed consistently high affinities toward MAO-B (−9.787 kcal/mol) and AChE (−9.166 kcal/mol), with corresponding Δ*G* bind values of −63.22 and −53.99 kcal/mol. (5*Z*,9*E*)-Farnesyl acetone also displayed favorable docking to MAO-B and AChE (−9.584 and −8.892 kcal/mol, respectively), with Δ*G* bind values approximately −63 to −59 kcal/mol. *n*-Hexadecanal, though slightly lower in docking scores, exhibited balanced affinities toward COX-2 (−7.969 kcal/mol) and BChE (−7.311 kcal/mol), with moderate Δ*G* binding values.

Molecular dynamics simulations

To further evaluate the stability and dynamic behavior of the isophytol complexes with COX-2, MAO-B, AChE, and BChE, each complex was subjected to 250 ns molecular dynamics (MD) simulations. Key parameters, including root mean square deviation (RMSD), root mean square fluctuation (RMSF), and interaction histograms, were analyzed to assess structural stability and ligand–protein

Table 3. IFD docking scores and MM-GBSA Δ*G* binding free energies of the most abundant compounds in the extracts.

№	Compounds	IFD Docking Scores (kcal/mol)				MM-GBSA ΔG bind. (kcal/mol)			
		COX-2	MAO-B	AChE	BChE	COX-2	MAO-B	AChE	BChE
1	Hexahydrofarnesylacetone	-8,786	−9,787	−9,166	−6,572	−76,63	−63,22	−53,99	−44,39
2	β-Bisabolene	−8,669	−8,432	−7,673	−7,961	−55,61	−42,74	−42,99	−34,47
3	Linalool	−6,175	−6,835	−6,900	−5,859	−28,19	−46,37	−33,36	−30,15
4	*n*-Hexadecanal	−7,969	−8,763	−6,911	−7,311	−54,88	−62,69	−33,54	−48,96
5	α-Thujene	−7,249	−7,434	−6,418	−6,731	−29,75	−38,14	−17,59	−30,59
6	Isophytol	−10,302	−10,222	−9,460	−7,291	−74,61	−74,17	−65,47	−56,67
7	(5*Z*,9*E*)-Farnesylacetone	−9,413	−9,584	−8,892	−7,300	−68,54	−63,26	−58,75	−52,52

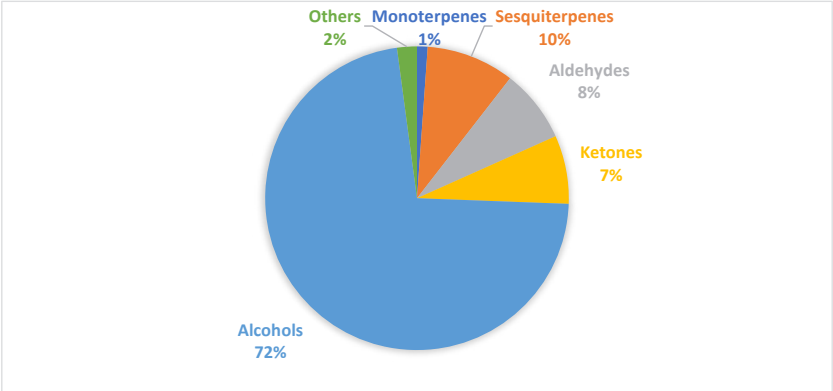


Figure 3. Percentage content of volatile organic compounds in infusion from dried aerial parts of *A. eupatoria*. In comparison with the essential oil, the levels of ketones (7%), sesquiterpenes (10%), aldehydes (8%), and monoterpenes (1%) were much lower. Alcohols made up the majority, accounting for over 70%. Others include phenylpropanoids and alkanes.

interactions. RMSD measures the overall structural deviation of the protein backbone over time, providing insight into system equilibration and stability. RMSF indicates the flexibility of individual residues, highlighting dynamic regions of the protein. Interaction histograms summarize the frequency and type of contacts, such as hydrogen bonds or hydrophobic interactions, between the ligand and surrounding amino acids throughout the simulation (Tokalı et al. 2026). Suppl. material 1: figs S4–S7 show the results of the 250 ns MD simulations of the isophytol–COX-2, isophytol–MAO-B, isophytol–AChE, and isophytol–BChE complexes. The 250 ns MD simulations of isophytol with COX-2, MAO-B, AChE, and BChE provide comprehensive insights into the dynamic stability and binding behavior of the ligand across multiple targets. Throughout the simulations, isophytol maintained stable hydrogen-bond interactions with key residues in all complexes, including Tyr-326 (COX-2), Ser-531 (MAO-B), Arg-296 and Phe-295 (AChE), and Gly-115 and Tyr-128 (BChE). These persistent interactions, often exceeding 70–90% occupancy during the simulations, indicate strong anchoring of the ligand in the respective binding pockets.

RMSD analyses revealed that the protein backbones remained stable in all cases (average ~1.2–1.5 Å), confirming the structural integrity of the targets during ligand binding. Ligand RMSD values varied slightly between complexes, with the lowest fluctuations observed in AChE (~0.9–2.0 Å) and somewhat higher mobility in BChE (~1.6–3.0 Å), suggesting that the ligand adapts subtly to each binding site while remaining stably accommodated. RMSF profiles further confirmed that residues directly interacting with isophytol exhibited reduced flexibility, whereas other protein regions retained normal fluctuations, highlighting the stabilizing effect of ligand binding on key active site residues.

Fractional interaction analyses demonstrated that isophytol forms both hydrogen-bond and hydrophobic interactions with multiple residues in each site. COX-2 and AChE complexes showed the most extensive and persistent interactions, suggesting highly favorable binding. MAO-B also exhibited stable polar and hydrophobic contacts, while BChE, despite slightly higher ligand mobility, maintained strong anchoring through Gly-115 and Tyr-128, complemented by hydrophobic stabilization from Leu-125 and Trp-82.

Discussion

The present study expands what is known about the essential oil composition of *A. eupatoria*, as most research has focused on its polyphenolic profile (Malheiros et al. 2022). Some of the compounds identified have been reported by other authors (Table 1)—for example, linalool has been detected in essential oil from fresh aerial parts (Iqbal et al. 2023). Feng et al. (2013) also identified linalool and anethole in fresh leaves and roots. α-Thujene was also present in fresh leaves of the herb (Milani et al. 2025). (*E*)-β-Farnesene, detected by Navaei and Mirza (2009), was one of the major compounds in wild leaves. The present study builds upon the findings of Doğan et al. (2023), who analyzed essential oil extracted from dried aerial parts collected in southern Bulgaria. The authors identified 20 compounds, with monoterpene hydrocarbons prevailing. For comparison, in the present study, terpene ketones had the largest relative share. Doğan et al. also reported *n*-tridecanal in the essential oil. The possible differences between the present findings and those of previous studies can be explained by the cumulative effect of several factors. The main ones include the genetic differences of plants and various environmental factors, such as sunlight, temperature, air, and soil (Dimova and Zhelev 2022; Kozuharova et al. 2023). The period of herb collection is also of importance. The aerial parts in the present study were collected in July or August, coinciding with the plant's flowering period. The role of plant material preparation and the methodology used should not be overlooked when discussing the results. A limitation of the present study is the unspecified drying temperature of the herbs. Another research group used hydrodistillation with a Clevenger apparatus to obtain essential oil. A disadvantage of hydrodistillation is that it can lead to the loss of some volatile components and the degradation of sensitive compounds after contact with boiling water (Pheko-Ofitlhile and Makhzoum 2024). According to Řebíčková et al. (2020), steam distillation produces higher quality oils with stronger biological properties. These factors, along with the specific plant part used, its condition (fresh or dried), and the growth environment, likely explain why results vary worldwide, as shown in Table 1. In addition, in Europe, *A. eupatoria* plants are found with diploid cytotypes ($2n = 28$), while in Asia, they are polyploid (Kumar et al. 2014). Published data on the volatile composition of *A. procera*, a species also occurring in Bulgaria, were not found, which would allow for a direct comparison with *A. eupatoria*. Although information was available for *A. asiatica* and *A. pilosa*, their reported volatile profiles did not reveal compounds in common with those identified in *A. eupatoria* (Wang et al. 2012; Kozykeyeva et al. 2020).

The observed differences in the concentration of volatile organic compounds in the essential oil and infusion can be explained by the polarity of the compounds, their solubility in water, and the extraction method. Nonpolar and weakly polar compounds, such as hydrocarbons (*n*-tricosane), aliphatic aldehydes (*n*-tridecanal and *n*-hexadecanal), fatty alcohols (*n*-hexadecanol and *n*-octadecanol), and terpenes with their oxygen derivatives (α-thujene, β-bisabolene, hexahydrofarnesyl acetone, (5*Z*,9*E*)-farnesyl acetone, phytol, and isophytol), are retained in the oil. In contrast, more polar molecules are extracted more efficiently in water (Siddiqui et al. 2024). Although β-farnesene, like β-bisabolene, is a sesquiterpene, its concentration is expected to be higher in the oil. However, the opposite result was obtained, suggesting that the observed contradiction may be due to prolonged steam distillation, as a study by Gawde et al. (2014) showed that β-farnesene content decreased with increasing distillation time. Phytol (C20H40O) and its derivative isophytol are diterpene alcohols with a very long

hydrocarbon chain, which makes them almost insoluble in water (Saha and Bandyopadhyay 2020). Similar to phytol, linalool is also a terpene alcohol, but it has a shorter hydrocarbon chain, and its hydroxyl group gives it some polarity, which explains its better solubility in water than diterpene alcohols (Pereira et al. 2018). This may explain the observed difference in the percentage of alcohols in essential oil and infusion. The aliphatic aldehydes *n*-tridecanal, *n*-tetradecanal, *n*-pentadecanal, and *n*-hexadecanal have 13, 14, 15, and 16 carbon atoms, respectively. They are weakly polar compounds. Logically, they would be more concentrated in the oil, as observed for *n*-tridecanal and *n*-hexadecanal. Although the content of *n*-tetradecanal and *n*-pentadecanal in the oil and infusion is insignificant, their amount is higher in the latter. Based on a study by Krakowska-Sieprawska et al. (2022), this may be a consequence of the destruction of the cell walls during water infusion, which led to the release of these compounds into the solution. A similar case is that of anethole, which is poorly water-soluble. Its content in the infusion was also slightly higher, most likely due to the molecule's partially polar nature, higher vapor pressure, and the effect of hot water on plant cells (Raposo et al. 2024).

The refined IFD and MM-GBSA Δ*G* binding results provided a deeper understanding of the potential interactions of the dominant compounds in the extract, particularly in relation to anti-inflammatory, antioxidant, and neuroprotective effects. Molecular docking analysis showed that hexahydrofarnesyl acetone exhibited consistently high affinities toward MAO-B and AChE, reinforcing its role as a strong anti-Alzheimer's candidate capable of regulating both monoaminergic and cholinergic pathways. Isophytol demonstrated remarkably strong IFD docking scores with COX-2 and MAO-B, accompanied by highly negative MM-GBSA energies. These results indicate a stable and energetically favorable interaction profile, suggesting potent anti-inflammatory activity via COX-2 inhibition and neuroprotective potential through MAO-B modulation. (5*Z*,9*E*)-Farnesyl acetone also displayed favorable docking to MAO-B and AChE, supporting a synergistic contribution to the neuroprotective profile of the extract. *n*-Hexadecanal, though slightly lower in docking scores, exhibited balanced affinities toward COX-2 and BChE with moderate Δ*G* binding values, implying additional anti-inflammatory and anticholinesterase support. In contrast, linalool, β-bisabolene, and α-thujene showed comparatively weaker binding, consistent with their known antioxidant and mild anti-inflammatory effects rather than direct enzyme inhibition. A limitation of the present study is that the results are based solely on *in silico* analysis. Therefore, *in vitro* and *in vivo* experiments are required to confirm these findings and validate the predicted biological activities.

Essential oils are known to have various applications in aromatherapy, medicine, the food industry, and other fields (Pezantes-Orellana et al. 2024). Among the main compounds identified in the essential oil (hexahydrofarnesyl acetone, β-bisabolene, isophytol, *n*-hexadecanal, and α-thujene), only β-bisabolene and hexahydrofarnesyl acetone have been reported to exhibit biological activity. β-Bisabolene has been shown to exhibit anti-adipogenic activity in the nematode *Caenorhabditis elegans*, as well as activity against Gram-positive bacteria (Li et al. 2023). The terpene has shown selective cytotoxic effects against human and mouse breast cancer cells *in vivo* and *in vitro* (Yeo et al. 2016). β-Bisabolene, isolated from the essential oil of *Psammogeton canescens*, has demonstrated strong antioxidant activity (Kazemi and Rostami 2015). There are reports of antimicrobial, anti-inflammatory, antinociceptive, and antioxidant effects of hexahydrofarnesyl acetone (Radulović et al. 2006; Szewczyk et al. 2016; Avoseh et al. 2021). Direct *in vivo* or *in vitro* evidence for the neuroprotective effects of hexahydrofarnesyl acetone is limited. The most common form of application of the herb in folk medicine is infusion. A clinical study among healthy volunteers reported an increase in the total antioxidant capacity of blood plasma and an improvement in the lipid profile after 1 month of *A. eupatoria* infusion administration (Ivanova et al. 2013). To the best of our knowledge, the volatile compounds in the infusion from this herb have not been investigated. It is possible that, in addition to polyphenols (the dominant compounds with well-established health benefits), volatile compounds may also make a minor contribution (Paluch et al. 2020). Alcohols, particularly linalool, had the largest relative share in the infusion profile, followed by hexahydrofarnesyl acetone and β-bisabolene. The literature review revealed a variety of proven biological properties for linalool, such as antioxidant, anti-inflammatory, analgesic, antifungal, antibacterial, antiparasitic, and anticancer properties (Katsuyama et al. 2012; Park et al. 2012; Wu et al. 2014; Chang et al. 2015; Seol et al. 2016; Dias et al. 2017; Villamizar et al. 2017; Liu et al. 2020). Linalool exhibits strong preclinical evidence for mitigating amyloid-induced neurodegeneration and inflammation in animal models (Sabogal-Guáqueta et al. 2016; Yuan et al. 2021). Due to the weaker binding of linalool to COX-2, its anti-inflammatory effects are most likely not due to enzyme inhibition. However, due to its terpenic origin, linalool has poor water solubility, which results in poor bioavailability when administered orally. Studies show that complexing linalool with β-cyclodextrin or in nanostructured lipid carriers significantly improves its bioavailability and peak plasma concentration (Shi et al. 2016; Pedreira et al. 2024). Beneficial health effects have also been established for phytol, whose concentration in the infusion is lower than that in the essential oil but deserves attention due to its effects (Santos et al. 2013; de Moraes et al. 2014; Silva et al. 2014; Ghaneian et al. 2015; Bobe et al. 2020; Kim et al. 2022). No articles discussing the bioavailability of β-bisabolene, hexahydrofarnesyl acetone, and phytol were identified. Maulydia et al. (2023) provided a pharmacokinetic prediction indicating that phytol is moderately absorbed in the gastrointestinal tract. The incorporation of β-bisabolene into polymeric nanocapsules may enhance its bioavailability (Rodrigues et al. 2023). Consequently, a definitive assessment of the poten-

tial pharmacological effects of the oil and infusion cannot be made, despite the fact that some of their constituents are known to possess bioactivity. In addition, the limited information available on most compounds underscores the necessity for further research.

Conclusion

The analysis confirmed the presence of previously reported compounds, supplemented existing data with the identification of new components in the essential oil of *A. eupatoria*, and examined the volatile profile of the herb infusion for the first time. The results showed that the extraction method had a significant effect on the profile of the oil and the infusion.

Molecular docking analyses were performed to evaluate interactions between the major phytochemicals and COX-2, MAO-B, AChE, and BChE. Isophytol and hexahydrofarnesyl acetone exhibited the highest predicted affinities, suggesting beneficial potential against inflammation, oxidative stress, and neurodegenerative diseases.

Despite the identification of numerous constituents, data on their pharmacokinetics, bioavailability, and biological activities remain limited, restricting the assessment of the therapeutic potential of *A. eupatoria* extracts. Further *in vitro* and *in vivo* studies, including mechanistic investigations and evaluation of compound interactions, are needed to clarify their physiological effects and applications in herbal medicine and functional foods.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

The authors declared that no experiments on animals were performed for the present study.

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

Artificial Intelligence (AI) use

The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI.
No AI tools were used in the preparation of this manuscript.

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Author contributions

Conceptualization, O.T. and G.Y.; methodology, I.D., I.K. and H.Ş.; validation, O.T. and M.B.; formal analysis, S.S. and M.B.; investigation, I.D., I.K. and H.Ş.; data curation, P.O., S.S. and M.B.; writing—original draft preparation, S.S. and M.B.; writing – review and editing, P.O., G.Y. and O.T.; visualization, S.S.

Author ORCIDs

Stoyan Stoyanov https://orcid.org/0009-0000-7441-2057
Ivayla Dincheva https://orcid.org/0000-0003-0276-4170
Iliyan Kolev https://orcid.org/0000-0001-5523-3555
Halil Şenol https://orcid.org/0000-0002-8333-035X
Momchil Barbolov https://orcid.org/0009-0003-7595-1636
Pawel Olczyk https://orcid.org/0000-0001-7387-9587
Galina Yaneva https://orcid.org/0000-0002-6194-2902
Oskan Tasinov https://orcid.org/0000-0001-7268-3937

Data availability

All of the data that support the findings of this study are available in the main text.

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Supplementary material 1

Supplementary figures S1–S7

Authors: Stoyan Stoyanov, Ivayla Dincheva, Iliyan Kolev, Halil Şenol, Momchil Barbolov, Pawel Olczyk, Galina Yaneva, Oskan Tasinov

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