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Chemical Composition and Multi-Target Bioactivity of *Salvia sclarea* L. Essential Oil From the Algerian Edough Massif: Antimicrobial, Enzyme-Inhibitory, and Molecular Docking Studies

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ABSTRACT

Salvia sclarea L. (clary sage) is widely used in Algerian traditional medicine, yet its essential oil from the Edough Mountains (northeastern Algeria) remains poorly characterized. An ethnobotanical survey among 85 informants revealed high recognition (RFC = 0.918) and versatility (UV = 1.671), with predominant uses against digestive, nervous, and gynecological disorders (ICF up to 0.88). Hydrodistillation of the aerial parts yielded 1.90% (w/w) of a pale-yellow essential oil dominated by oxygenated monoterpenes (68.95%), mainly linalyl anthranilate (47.75%), and linalool (16.24%). The oil showed potent antimicrobial activity (MIC 15.32–1000 µg/mL), strong antibiofilm effects (up to 76.02% inhibition), remarkable antioxidant capacity (DPPH EC₅₀ = 40.38 µg/mL), anti-inflammatory activity (EC₅₀ = 291.10 µg/mL), and interesting inhibitory effects on acetylcholinesterase (EC₅₀ = 64.40 µg/mL) and α-amylase (EC₅₀ = 69.49 µg/mL). These findings suggest that the Algerian chemotype of *S. sclarea* may represent a promising source of bioactive natural compounds. Molecular docking and 100 ns molecular dynamics simulations elucidated the binding modes and stability of the major constituents in the enzyme active sites. This study validates traditional knowledge and positions the oil as a promising natural resource for managing oxidative stress-related and microbial disorders.

1 | Introduction

Medicinal and aromatic plants constitute a precious source of bioactive natural products, generating increasing interest in drug discovery and the development of phytotherapy [1, 2]. Among plant-derived metabolites, essential oils represent complex mixtures of volatile compounds, mainly composed of monoterpenes and sesquiterpenes, which are known for their wide range

of biological properties, including antimicrobial, antioxidant, anti-inflammatory, and enzyme-inhibitory activities [3, 4].

Essential oils exhibit a wide variability in their chemical composition, influenced by genetic, ecological, geographical, and environmental factors. This variability leads to the existence of different chemotypes within the same species [5]. Studying plant populations growing in regions with high biodiversity therefore

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represents a significant approach for identifying new chemical profiles and exploring their biological relevance [6]. In this context, North African ecosystems constitute an important, yet still insufficiently explored, reservoir of botanical and phytochemical diversity [7].

Clary sage (*Salvia sclarea* L., *Lamiaceae*) is a widespread aromatic species cultivated throughout the Mediterranean region. Traditionally used in medicine to treat digestive, inflammatory, and neurological disorders, this plant has also gained economic importance through its applications in the essential oil industry [8, 9]. *S. sclarea* essential oil is generally characterized by a high proportion of monoterpenes and oxygenated esters, notably linalool and its linalyl derivatives, which are associated with antimicrobial and neuroprotective effects [10, 11]. Previous studies have demonstrated that geographical variations can significantly influence the chemical composition of this species, thus affecting its biological properties [12, 13].

In recent years, there has been growing interest in natural compounds capable of modulating biological targets involved in metabolic and neurodegenerative disorders. Enzymes such as acetylcholinesterase (AChE) and α -amylase play key roles in cognitive processes and carbohydrate metabolism, respectively, and their inhibition represents a promising therapeutic strategy [14, 15]. Furthermore, the ability of plant-derived compounds to interfere with the formation of microbial biofilms has become a key aspect of developing alternative approaches to combat antimicrobial resistance [16].

Although *Salvia sclarea* essential oil has been investigated in different geographical regions, data concerning Algerian populations [17], particularly those growing in the Edough Massif, remain scarce [18].

The novelty of the present study lies in the integrated approach combining ethnobotanical documentation, GC–MS-based chemical profiling, antimicrobial and antibiofilm evaluation, antioxidant and anti-inflammatory screening, enzyme inhibition assays against acetylcholinesterase and α -amylase, and in silico analyses including molecular docking and 100 ns molecular dynamics simulations. To our knowledge, this is one of the first studies linking traditional uses of *S. sclarea* from the Edough region with multi-target biological evaluation and molecular-level interpretation of selected bioactive constituents.

2 | Results and Discussion

2.1 | Study Area

For sampling and ethnobotanical research, this study was conducted in the Edough Mountains, located in the far northeast of Algeria, covering 35 423 hectares of forest and 4880 hectares of coastal area [19].

The Bouzizi Forest (36°53'59.7" N–7°39'10.2" E) is located in Algeria (Figure 1), southeast of the Edough Massif, at an elevation between 800 and 850 m. This site is characterized by cork oak forests (*Quercus suber* L.) and a rich associated flora [18].

2.2 | Ethnobotanical Survey

Of the 85 informants, 78 (91.8%) mentioned *Salvia sclarea* (local name: “Toulbouna” or “Toute-bonne”), resulting in 142 reported uses. The main reported indications are digestive, gynecological, and nervous disorders as well as wound healing, which is consistent with the traditional uses described [7, 20] (Table 1). The predominant dosage form remains a decoction of dried leaves and flowers (steeped for 10–15 min), often combined with honey. The calculated ethnobotanical indices were as follows:

- RFC = $78/85 = 0.918$ (very high local recognition)
- UV = $142/85 = 1.671$ (high versatility)

The informant consensus factor (ICF) values ranged from 0.50 to 0.88, indicating a relatively high agreement among informants regarding the medicinal use of plants for specific ailment categories. The highest consensus was observed for gynecological disorders (ICF = 0.88) and digestive disorders (ICF = 0.86), suggesting that local communities rely on a relatively small number of plant species for treating these conditions.

The indices obtained reflect an exceptional consensus of traditional knowledge in Edough, exceeding the national average for *Lamiaceae* reported by Miara et al. [6]. This perfect convergence indicates that the plant is perceived as highly effective and versatile in the region, confirming previous observations by Boudjelal et al. [7] and Benarba et al. [12].

These results fully justify the phytochemical and pharmacological investigations conducted in this study on its essential oil, which is rich in linalool and linalyl acetate. They also underscore the need to preserve the Edough communities and to promote this ancestral knowledge with a view to sustainable development and modern pharmacopoeia.

2.3 | Extraction Yield and Chemical Composition of *Salvia sclarea* (L.) Essential Oil

Hydrodistillation of 100 g of dried aerial parts of *Salvia sclarea* (L.) using a Clevenger-type apparatus yielded a very pale-yellow essential oil with a yield of 1.90% (w/w, dry weight basis). This value falls within the range previously reported by Mahboubi [1], who described extraction yields varying from 0.11% to 2.5% depending on the geographical origin and experimental conditions [21].

Chemical characterization by gas chromatography–mass spectrometry (GC–MS) enabled the identification of 64 constituents.

Compound identification was achieved by comparing the obtained mass spectra with those available in commercial and literature databases and by comparing experimentally calculated linear retention indices with published retention indices obtained on columns of similar polarity. Whenever available, authentic standards were co-injected to confirm compound identity. Therefore, compound identification was not based

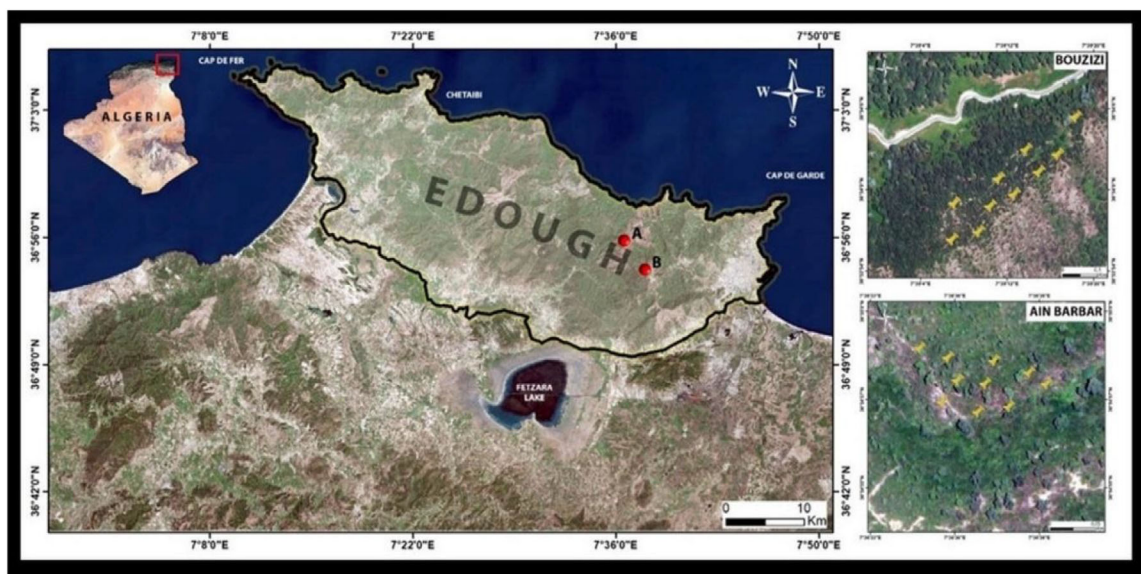


FIGURE 1 | Map of the study area showing the sampling sites of *Salvia sclarea* (L.) in the Edough Massif, northeastern Algeria: (A) Ain Barbar and (B) Bouzizi Forest (36°53'59.7" N, 7°39'10.2" E; elevation 800–850 m a.s.l.). The Edough Massif covers 35 423 ha of forest and 4880 ha of coastal area. Source: Google Earth.

TABLE 1 | Ethnobotanical uses of *Salvia sclarea* (L.) in the Edough region (Annaba, Algeria).

Ailment Category	Nur	Nt	ICF	Plant part used	Preparation method	Route of administration	FC	RFC
Digestive disorders	38	6	0.86	Leaves	Decoction	Oral	32	0.918
Nervous system disorders	27	5	0.85	Leaves + flowers	Infusion	Oral	21	0.918
Gynecological disorders	25	4	0.88	Leaves	Decoction with honey	Oral	18	0.918
Skin infections/wound healing	18	5	0.76	Leaves	Poultice	Topical	12	0.918
Respiratory disorders	10	4	0.67	Flowers	Infusion	Oral	7	0.918
Rheumatism/inflammation	8	3	0.71	Leaves	Decoction	Oral	6	0.918
Urinary disorders	6	3	0.60	Leaves	Decoction	Oral	2	0.918
General tonic	5	3	0.50	Leaves	Decoction	Oral	4	0.918
Headache	4	1	—	Leaves	Infusion	Oral	3	0.918
Mild infections	3	1	—	Leaves	Infusion	Oral	1	0.918

Note: Total informants (N) = 85; total use reports (UR) = 142; overall relative frequency of citation (RFC) = 78/85 = 0.918; mean ICF = 0.78 (very high consensus). Abbreviations: FC, frequency of citation; ICF, informant consensus factor; Nt: number of taxa cited for the category; Nur: number of use reports for the ailment category; RFC, relative frequency of citation.

solely on mass spectral library matching, but also on retention index comparison, which increases the reliability of the GC–MS assignments.

The oil was predominantly composed of oxygenated monoterpenes (68.95%), followed by sesquiterpene hydrocarbons (16.30%), monoterpene hydrocarbons (5.68%), oxygenated sesquiterpenes (2.60%), and diterpenes (1.88%) (Figure S1, Table 2).

The results presented in Table 2 indicate that oxygenated monoterpenes constitute the predominant chemical class, mainly represented by linalyl anthranilate (47.75%), followed by linalool (16.24%) and geranyl formate (1.00%).

Sesquiterpene hydrocarbons represented the second major fraction, with β -cubebene (5.62%) and caryophyllene (5.29%) as the principal constituents, together with γ -elemene (1.28%). Among monoterpene hydrocarbons, α -ocimene (2.51%) was the dominant compound, followed by limonene (0.84%) and limonene oxide (0.64%).

Regarding oxygenated sesquiterpenes, caryophyllene oxide (1.79%) was the dominant compound, followed by β -eudesmol (0.38%) and α -eudesmol (0.27%). Diterpenes were detected in minor proportions, mainly represented by sclareol (1.55%), manool (0.25%), and manoyl oxide (0.08%). These findings are consistent with previous reports indicating that *Salvia sclarea*

TABLE 2 | Chemical composition of *Salvia sclarea* (L.) essential oil determined by GC and GC–MS analyses.

N°	RT (min)	Compounds	IUPAC name	[%]
1	4.18	3-Hexen-1-ol, (Z)-	(Z)-Hex-3-en-1-ol	0.04
2	6.19	α -Pinene	(1S,5S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	0.04
3	7.51	Sabinene	(1S,5S)-4-Methylidene-1-methylbicyclo[3.1.0]hexane	0.02
4	7.63	β -Pinene	(1R,5R)-6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane	0.02
5	7.72	Camphene	(1R,4R)-2,2-Dimethyl-3-methylenebicyclo[2.2.1]heptane	0.02
6	8.22	Limonene	(4R)-1-Methyl-4-(prop-1-en-2-yl)cyclohex-1-ene	0.84
7	8.84	β -Myrcene	7-Methyl-3-methyleneocta-1,6-diene	0.15
8	9.55	p-Cymene	1-Methyl-4-(propan-2-yl)benzene	0.04
9	9.76	D-Limonene	(4R)-1-Methyl-4-(prop-1-en-2-yl)cyclohex-1-ene	0.26
10	9.85	Camphor	(1R,4R)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one	0.13
11	10.20	β -Ocimene	(3E)-3,7-Dimethylocta-1,3,6-triene	0.33
12	10.71	Alloocimene	(2E,4E)-2,6-Dimethylocta-2,4,6-triene	0.57
13	12.61	δ -4-Carene	(1S,6R)-3,7,7-Trimethylbicyclo[4.1.0]hept-3-ene	0.17
14	14.51	Linalool	(3R)-3,7-Dimethylocta-1,6-dien-3-ol	16.24
15	19.27	α -Terpineol	(2R)-2-(4-Methylcyclohex-3-en-1-yl)propan-2-ol	0.89
16	24.76	Linalyl anthranilate	(3R)-3,7-Dimethylocta-1,6-dien-3-yl 2-aminobenzoate	47.75
17	25.08	Linalyl formate	(3R)-3,7-Dimethylocta-1,6-dien-3-yl methanoate	0.09
18	25.25	Anethole	(E)-1-Methoxy-4-(prop-1-en-1-yl)benzene	0.11
19	25.94	Geranyl formate	(2E)-3,7-Dimethylocta-2,6-dien-1-yl methanoate	0.19
20	28.06	Unidentified compound	Unidentified compound	0.18
21	28.33	α -Cubebene	(1R,4S,4aS,8aR)-1,4-Dimethyl-7-(propan-2-ylidene)decahydronaphthalene	0.20
22	28.66	L-Borneol	(1S,2R,4S)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol	0.06
23	29.61	Geranyl formate	(2E)-3,7-Dimethylocta-2,6-dien-1-yl methanoate	1.00
24	29.96	Copaene	(1R,2S,6S,7S,8S)-8-Isopropyl-1,3,3-trimethyltricyclo[4.4.0.0 ^{2,7}]dec-3-ene	0.69
25	30.39	β -Bourbonene	(1S,4S,4aS,8aR)-1,4-Dimethyl-7-methylidene-2,3,4,4a,5,6,7,8-octahydronaphthalene	0.69
26	30.74	Sclareol	(4aS,6R,8aS)-2,2,4a,8-Tetramethyl-6-(2-hydroxypropan-2-yl)decahydronaphthalene-1-methanol	1.55
27	30.80	Geranyl acetate	(2E)-3,7-Dimethylocta-2,6-dien-1-yl ethanoate	0.94
28	31.59	Carvacrol	2-Methyl-5-(propan-2-yl)phenol	0.13
29	32.69	Caryophyllene	(1R,4E,9S)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	5.29
30	32.96	(+)-Epi-bicyclosquiphellandrene	(1R,4aR,8aS)-1,4-Dimethyl-7-(propan-2-ylidene)decahydronaphthalene	0.19
31	33.74	Bicycloelemene	(1S,2R,6R,7R)-2,6-Dimethyl-7-(prop-1-en-2-yl)tricyclo[5.3.0.0 ^{2,6}]decane	0.08
32	34.27	Germacrene D	(1E,5E,9S)-1,5-Dimethyl-8-(propan-2-ylidene)cyclodeca-1,5-diene	30
33	34.94	Unidentified compound	Unidentified compound	0.16

(Continues)

TABLE 2 | (Continued)

N°	RT (min)	Compounds	IUPAC name	[%]
34	36.30	β -Cubebene	(1R,4S,4aR,8aS)-1,4-Dimethyl-7-(propan-2-yl)decahydronaphthalene	5.62
35	36.44	Eremophilene	(4aS,8aR)-1,1,4a-Trimethyl-7-(propan-2-ylidene)decahydronaphthalene	0.30
36	37.02	γ -Elemene	(1S,2S,6R)-1-Methyl-2-(prop-1-en-2-yl)-6-(propan-2-yl)cyclohex-1-ene	1.28
37	37.48	Unidentified compound	Unidentified compound	0.21
38	37.83	α -Farnesene	(3E,6E)-3,7,11-trimethyldodeca-1,3,6,10-tetraene	0.22
39	37.91	Unidentified compound	Unidentified compound	0.15
40	38.43	δ -Cadinene	(1S,4aR,8aS)-1,6-Dimethyl-4-(propan-2-ylidene)decahydronaphthalene	0.46
41	39.60	Linalyl acetate	(3R)-3,7-Dimethylocta-1,6-dien-3-yl ethanoate	1.00
42	40.17	Unidentified compound	Unidentified compound	0.12
43	40.93	Caryophyllene oxide	(1R,4R,6R,7S,9S)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene-9,10-epoxide	1.79
44	41.59	Caryophyllene	(1R,4E,9S)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	0.47
45	41.88	Unidentified compound	Unidentified compound	0.18
46	42.01	β -Humulene	(1E,4E,8E)-2,6,6,9-Tetramethylcycloundeca-1,4,8-triene	0.05
47	42.78	Geranyl-p-cymene	Geranyl-p-cymene	0.35
48	43.06	β -Eudesmol	(4aS,8aR)-2,5,5,8a-Tetramethyldecahydronaphthalen-2-ol	0.38
49	43.15	α -Eudesmol	(4aR,8aS)-4a,8-Dimethyl-2-(propan-2-yl)decahydronaphthalen-1-ol	0.27
50	43.60	Unidentified compound	Unidentified compound	0.15
51	44.61	Trans-sabinene hydrate	trans-(1S,2R,4S)-4-Methyl-1-(propan-2-yl)bicyclo[3.1.0]hexan-2-ol	0.07
52	46.09	β -Caryophyllene	(1R,4E,9S)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	0.04
53	45.70	Octadecane	Octadecane	0.09
54	47.27	Trans- β -ocimene	(3E)-3,7-Dimethylocta-1,3,6-triene	0.04
55	47.46	α -Cadinol	(1R,4aS,8aR)-4-Isopropyl-1,6-dimethyldecahydronaphthalen-1-ol	0.07
56	47.65	Hexahydrofarnesyl acetone	6,10,14-Trimethylpentadecan-2-one	0.09
57	49.13	α -Copaene	(1R,2S,6S,7S,8S)-8-Isopropyl-1,3,3-trimethyltricyclo[4.4.0.0 ^{2,7}]dec-3-ene	0.33
58	49.28	Bicyclogermacrene	(1S,4aS,8aR)-1,4-dimethyl-7-methylidenedeca-hydronaphthalene	0.09
59	49.73	Unidentified compound	Unidentified compound	0.18
60	49.85	Limonene oxide	2-(4-Methylcyclohex-3-en-1-yl)oxirane	0.67
61	50.43	Manoyl oxide	(4aR,8aS)-13-Epoxyabdo-14-ene	0.08
62	51.53	Manool	(13R)-Labda-8(17),14-dien-13-ol	0.25
63	51.90	Pyrethrin	(5-Benzyl-3-furyl)methyl (1R,3R)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane-1-carboxylate	0.23

(Continues)

TABLE 2 | (Continued)

N°	RT (min)	Compounds	IUPAC name	[%]
64	54.25	α -Ocimene	(3Z)-3,7-dimethylocta-1,3,7-triene	2.51
Oxygenated monoterpenes				68.95
Sesquiterpene hydrocarbons				16.30
Oxygenated sesquiterpenes				2.60
Monoterpene hydrocarbons				5.68
Diterpenes				1.88

Abbreviation: RT, retention time.

TABLE 3 | Antimicrobial activity of *Salvia sclarea* (L.) essential oil.

Microorganisms	<i>Salvia sclarea</i> (L.) essential oil			Gentamicin	Amphotericin B
	MIC	MBC	MBC/MIC	MIC	MIC
<i>C. albicans</i>	15.32	31.25	2	NA	125
<i>C. albicans</i> ATCC 10231	NE	NE	NE	NA	125
<i>E. coli</i>	500	1500	3	NE	NA
<i>E. coli</i> ATCC 25922	250	250	1	250	NA
<i>E. faecalis</i>	NE	NE	NE	NE	NA
<i>E. faecalis</i> ATCC 29212	NE	NE	NE	500	NA
<i>K. pneumoniae</i>	1000	2000	2	NE	NA
<i>K. pneumoniae</i> ATCC 13883	500	1000	2	500	NA
<i>P. aeruginosa</i>	125	125	1	500	NA
<i>P. aeruginosa</i> ATCC 27853	250	250	1	250	NA
<i>S. aureus</i>	62.5	125	2	250	NA
<i>S. aureus</i> ATCC 25923	250	1000	4	125	NA

Abbreviations: MBC, minimum bactericidal concentration($\mu\text{g/mL}$); MIC, minimum inhibitory concentrations ($\mu\text{g/mL}$); NA, not applicable; NE, no effect.

essential oils are typically characterized by high levels of linalyl derivatives, caryophyllene, linalool, α -terpineol, sclareol, and germacrene D, with comparable qualitative profiles to those identified in the present study [1, 3, 22–25].

2.4 | Antimicrobial Activity

Antimicrobial efficacy was assessed by determining the minimum inhibitory concentration (MIC, $\mu\text{g/mL}$) as a quantitative indicator for each susceptible strain. The data presented in Table 3 demonstrate a pronounced antimicrobial activity of the *Salvia sclarea* essential oil against tested strains.

The antimicrobial activity of the essential oil was evaluated by determining the minimum inhibitory concentrations (MICs) and compared with gentamicin and amphotericin B, used as reference antibacterial and antifungal agents, respectively (Table 3). The results revealed a notable inhibitory effect of the *Salvia sclarea* essential oil against the tested microorganisms, with MIC values ranging from 15.32 to 1000 $\mu\text{g/mL}$. The highest sensitivity was observed for *C. albicans* (15.32 $\mu\text{g/mL}$) and *S. aureus* (62.5 $\mu\text{g/mL}$). In contrast, the two *E. faecalis* strains and *C. albicans* ATCC 10231 exhibited marked resistance to this essential oil. Determination

of the MBC/MIC ratio indicated a bactericidal effect against all susceptible bacterial strains, with MBC/MIC values ≤ 4 .

The richness of *Salvia* species in oxygenated monoterpenes and sesquiterpene hydrocarbons, the major classes of compounds commonly found in their essential oils, is well documented, and these constituents are widely recognized for their antimicrobial properties against various pathogenic microorganisms [26–28]. In the present study, the essential oil exhibited a pronounced inhibitory effect against most of the tested strains.

The differences in susceptibility observed among gram-positive and gram-negative bacteria may be partly explained by differences in cell envelope architecture. Gram-negative bacteria possess an outer membrane rich in lipopolysaccharides, which can act as an additional permeability barrier and limit the diffusion of hydrophobic terpenoid compounds [29, 30]. In contrast, gram-positive bacteria lack this outer membrane, and their thick peptidoglycan layer is generally more accessible to lipophilic essential oil constituents [31]. This may explain the relatively high sensitivity of *S. aureus* compared with some gram-negative strains. However, the strong activity observed against *P. aeruginosa* suggests that the essential oil may also affect membrane integrity, permeability, and intracellular targets in gram-negative

bacteria. The antimicrobial effect is probably related to the high proportion of oxygenated monoterpenes, particularly linalool and linalyl acetate, as well as sesquiterpene hydrocarbons such as caryophyllene and β -cubebene. These compounds are known to disrupt microbial membranes, alter ion gradients, increase leakage of intracellular constituents, and interfere with cellular metabolism.

The clinical *C. albicans* isolate was markedly more sensitive (MIC = 15.32 μ g/mL) than the reference strain ATCC 10231 (no effect). This discrepancy is frequently observed with essential oils and is attributed to phenotypic adaptations acquired by laboratory reference strains during prolonged subculturing, resulting in altered membrane permeability and reduced susceptibility to terpenoid compounds [32, 33]. This pattern has been consistently reported with various essential oils against *Candida* spp., where clinical isolates show significantly lower MIC values than reference strains [34, 35].

These findings are consistent with several previous studies reporting strong antimicrobial activity of *Salvia sclarea* essential oil against pathogens such as *S. aureus*, *S. epidermidis*, *E. coli*, and *C. albicans*, often with relatively low MIC values [28, 36–39].

The antimicrobial activity of *Salvia sclarea* essential oil is largely attributed to its major constituents, including linalool, linalyl derivatives, caryophyllene, γ -elemene, and cubebene, along with related terpenoid compounds. These constituents have been reported to exhibit significant antimicrobial activity against a wide range of pathogenic microorganisms, including *E. coli*, *S. aureus*, *P. aeruginosa*, and *C. albicans* [3, 40–42].

2.5 | Biofilm Inhibition and Metabolic Activity (MTT Reduction Assay)

The ability of the essential oil to inhibit biofilm formation was evaluated using the crystal violet staining assay, while the metabolic activity of the biofilm was assessed using the MTT reduction method. The corresponding results are presented in Figures 2 and 3.

The inhibitory effect of the essential oil on biofilm formation was evaluated using the crystal violet staining assay. The results showed inhibition rates ranging from 55.01% $\pm$ 1.21% to 76.02% $\pm$ 0.56%, with *C. albicans* exhibiting the highest inhibition (76.02% $\pm$ 0.56%). In comparison, gentamicin, used as the reference antibiotic, showed inhibition rates between 45.31% $\pm$ 0.56% and 57.65% $\pm$ 1.49%, while amphotericin B exhibited an inhibition rate of 54.43% $\pm$ 1.17%. These findings indicate that the tested essential oil exerted a stronger inhibitory effect on biofilm formation than the reference agents.

Regarding biofilm metabolic activity, assessed by the MTT reduction assay, inhibition rates ranged from 44.89% $\pm$ 0.89% to 66.87% $\pm$ 0.47%, again exceeding those obtained with gentamicin (39.31% $\pm$ 1.10%–51.08% $\pm$ 0.58%) and amphotericin B (39.51% $\pm$ 1.03%). These results suggest that the *Salvia sclarea* essential oil significantly impaired the metabolic activity of established biofilms.

The antibiofilm activity of the essential oil was further evaluated by determining the metabolic activity of biofilm-forming cells using the MTT reduction assay. This method is based on the ability of mitochondrial dehydrogenases in viable cells to reduce the tetrazolium salt MTT into soluble formazan, thereby allowing discrimination between metabolically active and nonviable cells. The intensity of the resulting coloration is proportional to cellular metabolic activity and can be quantified by measuring absorbance at 540 nm using a microplate reader (BioRad—USA) [43].

The obtained results revealed a marked reduction in the metabolic activity of the biofilm after treatment with the essential oil, indicating a significant decrease in the viability of microbial cells embedded within the biofilm matrix. Similar observations have been reported for *Salvia sclarea* essential oil, which has been shown to inhibit biofilm formation and reduce performed biofilms. According to Đurić et al. [44], this antibiofilm activity is mainly associated with the disruption of cellular viability within the biofilm structure.

Furthermore, previous studies have demonstrated that *Salvia sclarea* essential oil can interfere with bacterial metabolic activity by inducing membrane damage and altering cellular permeability. These alterations promote the leakage of essential intracellular components, including ATP and DNA, thereby significantly reducing the metabolic activity of pathogenic microorganisms such as *E. coli* and *S. aureus* [36]. This mechanism may account for the reduction in biofilm metabolic activity observed in the present study.

The antibiofilm activity may also be attributed to several major constituents of the essential oil, particularly linalool and linalyl derivatives, as well as caryophyllene and γ -elemene. These compounds have been reported to inhibit biofilm formation and reduce microbial metabolic activity by disrupting cell membrane integrity and destabilizing the biofilm matrix [45, 46]. The potential synergistic interactions among these constituents may therefore explain the observed reduction in metabolic activity and the disruption of biofilm architecture.

Although the crystal violet and MTT assays provide complementary information on biofilm biomass and metabolic activity, respectively, they do not allow direct visualization of biofilm architecture. Therefore, future studies using scanning electron microscopy or confocal laser scanning microscopy would be valuable to confirm the structural disruption of the biofilm matrix and to better characterize the spatial distribution of viable and non-viable cells after essential oil treatment.

2.6 | Antioxidant and Anti-Inflammatory Activities

The antioxidant and anti-inflammatory activities were expressed as maximal inhibition (%) and EC₅₀ values (μ g/mL), calculated from dose–response curves obtained by plotting the tested concentrations against the corresponding percentages of antioxidant and anti-inflammatory activity. Values are summarized in Table 4.

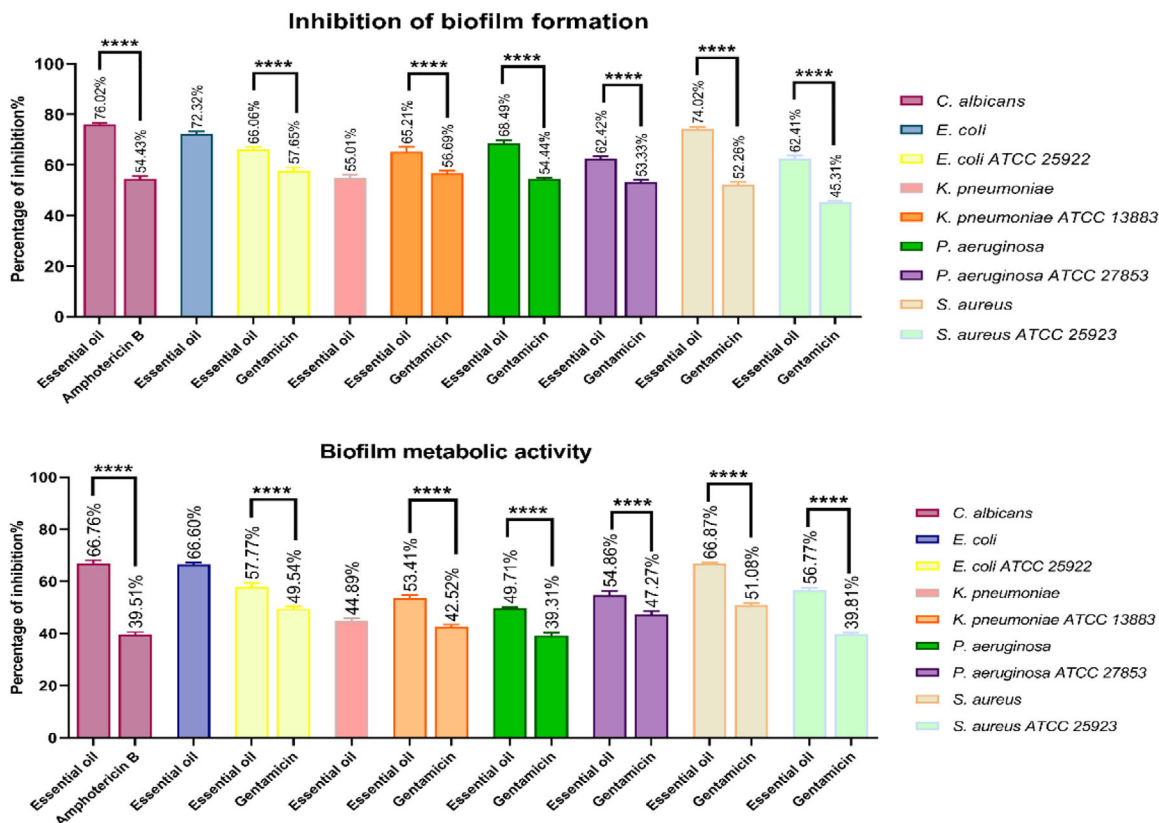


FIGURE 2 | Inhibitory effects of *Salvia sclarea* (L.) essential oil on (A) biofilm formation assessed by crystal violet (CV) staining (absorbance at 570 nm) and (B) biofilm metabolic activity assessed by the MTT reduction assay (absorbance at 550 nm), against clinical and reference microbial strains. The essential oil was tested at its minimum inhibitory concentration (MIC); gentamicin (GM) and amphotericin B (AM-B) served as antibacterial and antifungal reference agents, respectively. Negative control: untreated biofilm (100% formation). Data are expressed as mean percentage inhibition $\pm$ standard deviation (SD); $n = 3$ independent experiments. * $p < 0.05$ compared to reference agents (two-way ANOVA, Tukey's post hoc test).

The antioxidant potential of the essential oil was evaluated using the DPPH radical scavenging assay (Table 4). The oil exhibited significant antioxidant activity compared with ascorbic acid as a reference standard. Specifically, the essential oil showed an EC_{50} of 40.38 $\mu\text{g/mL}$ and a maximal inhibition of $87.84\% \pm 0.27\%$, whereas ascorbic acid displayed an EC_{50} of 23.79 $\mu\text{g/mL}$ with a maximal inhibition of $94.48\% \pm 0.26\%$. These results are consistent with previous reports, where *Salvia sclarea* essential oil showed an EC_{50} of $27.67 \pm 0.98 \mu\text{g/mL}$ [3]. The observed antioxidant activity is largely attributed to the chemical composition of the oil, which is rich in linalool, α -pinene, and α -terpineol compounds known for their antioxidant properties [47, 48].

The antioxidant activity was evaluated using the DPPH assay, which provides useful preliminary information on radical-scavenging capacity. However, antioxidant activity is a multi-mechanistic process, and the use of additional assays such as ABTS, FRAP, or CUPRAC would provide a more comprehensive evaluation of the redox properties of the essential oil. Therefore, the antioxidant results should be interpreted as preliminary and should be confirmed by complementary assays in future studies.

The anti-inflammatory activity was assessed in vitro by the inhibition of protein denaturation using the BSA assay (Table 4).

A concentration-dependent inhibitory effect on heat-induced protein denaturation was observed. At 1600 $\mu\text{g/mL}$, the essential oil exhibited maximal inhibition of $76.27\% \pm 0.74\%$ with an EC_{50} of 291.10 $\mu\text{g/mL}$. In comparison, the reference anti-inflammatory drug diclofenac showed stronger inhibition ($92.52\% \pm 1.18\%$) with an EC_{50} of 89.24 $\mu\text{g/mL}$. These findings align with previous in vivo studies on wound healing, where *Salvia sclarea* essential oil reduced pro-inflammatory cytokines (TNF- α , IL-6), enhanced granulation tissue formation, increased collagen synthesis, and promoted angiogenesis [22].

The anti-inflammatory effects of *Salvia sclarea* oil are attributed to its complex mixture of bioactive compounds. Linalool, α -terpineol, and linalyl derivatives contribute significantly to its efficacy, with the whole essential oil showing greater activity than individual constituents, highlighting the importance of synergistic interactions [49, 50]. These compounds were also identified in the analyzed essential oil in this study.

The protein denaturation assay is useful as a preliminary screening method for anti-inflammatory potential. However, it does not provide direct mechanistic information on inflammatory pathways. Further investigations targeting COX-2, LOX, nitric oxide production, and pro-inflammatory cytokines such as TNF- α and IL-6 are required to better define the anti-inflammatory mechanisms of *S. sclarea* essential oil.

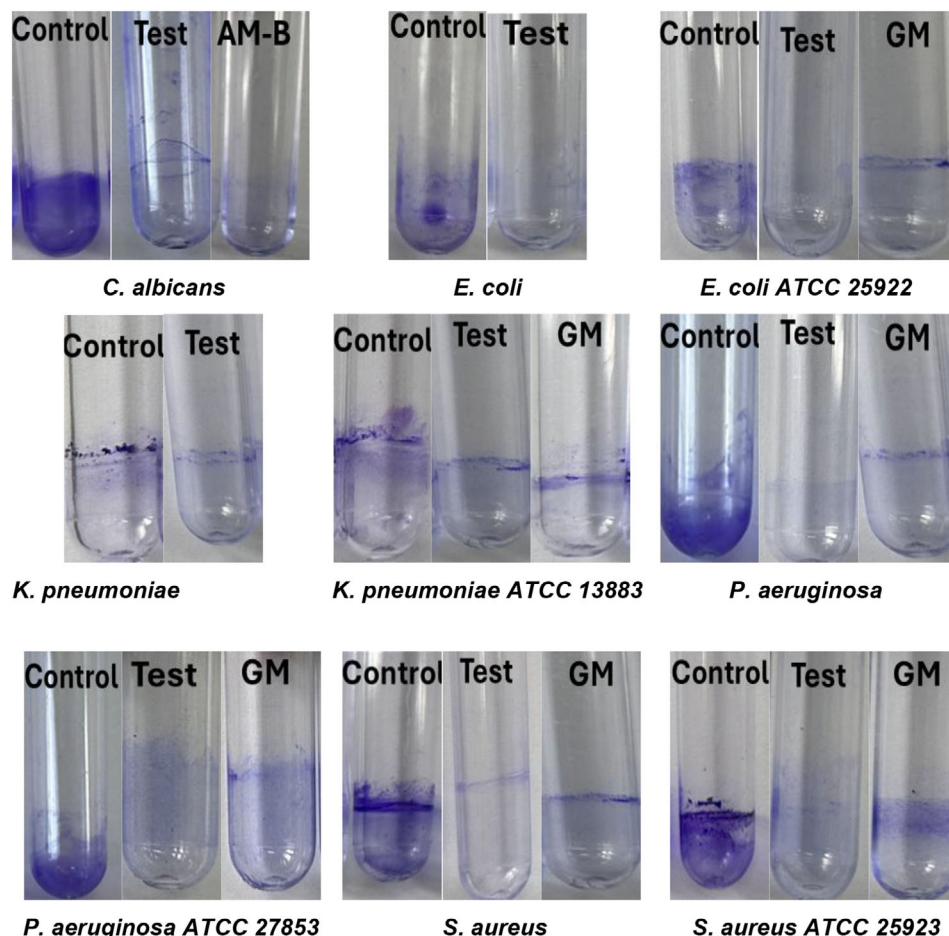


FIGURE 3 | Representative photographs of crystal violet-stained polystyrene tubes illustrating the inhibitory effect of *Salvia sclarea* (L.) essential oil on biofilm formation. For each microbial strain (arranged in rows from top-left to bottom-right: *C. albicans*, *E. coli* clinical, *E. coli* ATCC 25922, *K. pneumoniae* clinical, *K. pneumoniae* ATCC 13883, *P. aeruginosa* clinical, *P. aeruginosa* ATCC 27853, *S. aureus* clinical, *S. aureus* ATCC 25923), three tubes are shown from left to right: untreated control, essential oil at MIC, and gentamicin positive control (GM). Reduced intensity of crystal violet staining in treated tubes indicates successful disruption of biofilm architecture. Each condition was performed in triplicate ($n = 3$).

2.7 | Acetylcholinesterase and α -Amylase Inhibitory Activities

Alzheimer's disease is the most prevalent form of dementia, accounting for approximately 60%–80% of cases, and represents a major cause of functional decline in the elderly population. Increasing evidence indicates that impaired glycemic regulation may contribute to the risk of developing Alzheimer's disease, as disturbances in carbohydrate metabolism play an important role in the onset and progression of this neurodegenerative disorder [51, 52].

In this context, the inhibitory activity of *Salvia sclarea* essential oil against acetylcholinesterase and α -amylase was evaluated. The results were expressed as percentage inhibition (Figure 4 and Table 5), together with the corresponding EC_{50} values calculated from the dose–response curves (Figure 4).

The results presented in Figure 4 and Table 5 indicate that the essential oil exhibited a moderate inhibitory activity against acetylcholinesterase, with an EC_{50} value of 64.40 $\mu\text{g/mL}$ and a maximal inhibition of $86.47\% \pm 1.99\%$. In comparison, the

reference inhibitor galantamine showed a maximal inhibition of $99.12\% \pm 0.50\%$ with a markedly lower EC_{50} value of 15.94 $\mu\text{g/mL}$.

Similarly, for α -amylase inhibition, the essential oil displayed an EC_{50} value of 69.49 $\mu\text{g/mL}$ and a maximal inhibition of $82.50\% \pm 2.80\%$, whereas acarbose, used as the reference inhibitor, exhibited an EC_{50} of 36.15 $\mu\text{g/mL}$ with a maximal inhibition of $94.61\% \pm 1.70\%$.

These results are consistent with previous reports indicating that essential oils from the genus *Salvia* possess inhibitory potential against both enzymes. Species of this genus have long been used in traditional medicine for their memory-enhancing properties, and several species have demonstrated beneficial effects in disorders of the central nervous system. In addition, antidiabetic potential has also been documented for some members of this genus [5, 53]. Essential oils from certain *Salvia* species, particularly *Salvia officinalis*, are increasingly recognized for their neuroprotective properties and cholinesterase-inhibitory potential, making them promising candidates for the prevention or delay of neurodegenerative disorders such as Alzheimer's disease [54, 55].

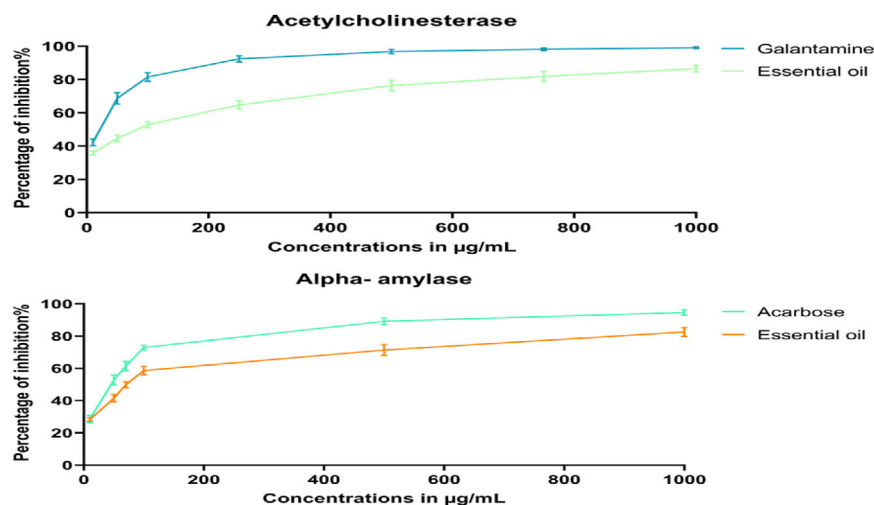


FIGURE 4 | Dose-response inhibition curves of *Salvia sclarea* (L.) essential oil and reference inhibitors against acetylcholinesterase (AChE) and α -amylase. (A) AChE inhibitory activity (Ellman's colorimetric assay): essential oil (filled circles, •) versus galantamine (filled triangles, ▲). (B) α -Amylase inhibitory activity (DNSA colorimetric assay): essential oil (filled circles, •) versus acarbose (filled triangles, ▲). Curves were fitted by non-linear regression using a sigmoidal dose-response model. EC₅₀ values and 95% confidence intervals are reported in Table 5. Data are expressed as mean percentage inhibition $\pm$ SD ($n = 3$).

The antioxidant capacity of *Salvia officinalis* essential oil also plays an important role in counteracting oxidative stress, a major contributing factor in neurodegenerative disorders such as Alzheimer's disease. Previous studies have shown that this essential oil can effectively reduce oxidative stress markers, thereby potentially slowing the progression of Alzheimer's disease [56].

The inhibitory activity observed for the essential oil may be attributed to its richness in oxygenated monoterpenes and sesquiterpenes, particularly linalool, α -pinene, α -terpineol, caryophyllene, and cubebene. These terpenoid compounds can interact with the catalytic and anionic sites of acetylcholinesterase through hydrogen bonding and hydrophobic interactions, which are crucial for enzymatic inhibition. Moreover, synergistic interactions among the different constituents of the oil may enhance the overall inhibitory effect [4, 15, 57, 58].

Furthermore, the *Lamiaceae* family, to which *Salvia sclarea* belongs, includes several species whose α -amylase inhibitory activity has been well documented. Essential oils from *Salvia tomentosa* and related species have demonstrated significant enzymatic inhibition, suggesting a comparable potential for *Salvia sclarea* [59]. In addition, the essential oil of *Salvia officinalis* has shown significant inhibitory effects on α -amylase and α -glucosidase, enzymes involved in carbohydrate digestion. Such inhibition contributes to the reduction of postprandial hyperglycemia, a key factor in diabetes management, with reported EC₅₀ values of approximately 69.23 μ g/mL for α -amylase.

Similarly, the essential oil of *Salvia fruticosa* contains bioactive compounds such as 1,8-cineole that have been associated with antiglycation properties. These compounds may inhibit the formation of advanced glycation end products, a major complication associated with diabetes [60].

The inhibition of α -amylase by oxygenated terpenoids such as linalool, α -pinene, α -terpineol, and linalyl anthranilate may

result from their ability to interact with the enzyme's active site through hydrogen bonding and hydrophobic interactions. Such interactions can hinder substrate access to the catalytic site, thereby reducing enzymatic activity, a mechanism similar to that reported for other natural α -amylase inhibitors [5, 61, 62]. The inhibitory activity observed in the present study may therefore arise from synergistic or additive effects among the oil constituents, in agreement with previously reported findings.

Although the essential oil showed inhibitory activity against AChE and α -amylase, kinetic analyses were not performed in the present study. Therefore, the inhibition mode, whether competitive, non-competitive, or mixed, remains to be determined. Future studies using different substrate concentrations and Lineweaver–Burk or non-linear kinetic modeling will be necessary to clarify the mechanism of enzyme inhibition.

2.8 | Molecular Docking

Acetylcholine (ACh) is a key neurotransmitter involved in the cognitive processes of memory and learning. In Alzheimer's disease, the cholinergic hypothesis suggests that a drastic decrease in ACh levels, resulting from neuronal degeneration, is responsible for the decline in brain functions [63]. The selection of acetylcholinesterase (AChE) as a target for molecular docking is based on the necessity of blocking this enzyme, which is responsible for ACh degradation. In this study, we performed molecular docking simulations against this target using four molecules identified by GC–MS from our plant essential oil (*Salvia sclarea* L.). This was conducted to confirm the enzymatic activity observed against ACh and to identify which specific molecules are responsible for the biological activity. The docking protocol was successfully validated by redocking the co-crystallized ligand (Tacrine), achieving a root-mean-square deviation (RMSD) of less than 1.0 Å, as shown in Figure 5.

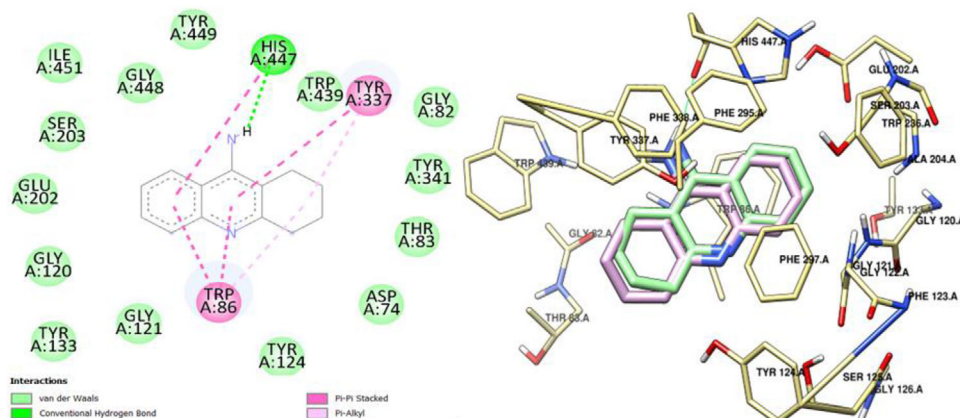


FIGURE 5 | Validation of the molecular docking protocol for acetylcholinesterase (AChE; PDB ID: 7XN1, resolution 2.85 Å). Superimposition of the redocked pose of the co-crystallized ligand tacrine (green sticks) onto its experimentally determined native conformation (yellow sticks) within the AChE binding site. The root-mean-square deviation (RMSD) between both poses was < 1.0 Å, confirming the reliability and accuracy of the Glide SP docking protocol. Key catalytic residues (TRP86, TYR337, HIS447) forming the active site gorge are shown as gray sticks.

The molecular docking study of the four major compounds within the active site of acetylcholinesterase (AChE) revealed significant binding affinities, suggesting a strong inhibitory potential. Linalyl anthranilate was identified as the most stable ligand, recording the lowest binding free energy (docking score) of −7.640 kcal/mol (Table 6). This high stability is primarily attributed to a dense network of interactions with key catalytic site residues (Figure 6A). Notably, the amine (NH) group of this compound forms a critical hydrogen bond with the HIS447 residue, mimicking the binding mode of the co-crystallized ligand. The complex is further stabilized by two π - π stacked interactions between its aromatic ring and the key residues TYR337 and TRP86, along with several hydrophobic π -alkyl interactions involving TRP86, HIS447, TYR124, PHE338, PHE295, and PHE297. Caryophyllene ranked second in terms of stability with a docking score of −6.749 kcal/mol (Table 6). Its affinity is driven by a dense cluster of hydrophobic π -alkyl interactions with the residues TRP86, HIS447, and TYR337 (Figure 6B). This was followed by beta-cubebene, which recorded a docking score of −6.653 kcal/mol (Table 6). This binding affinity is due to numerous π -sigma and π -alkyl hydrophobic interactions with residues TRP86, HIS447, and TYR124 (Figure 6C). Finally, linalool showed a docking score of −6.093 kcal/mol (Table 6). Despite the higher score, the molecule maintains its position by forming two hydrogen bonds between its hydroxyl (OH) group and residues HIS447 and TYR337. Additionally, it establishes several hydrophobic π -alkyl interactions with TRP86, HIS447, TRP439, TYR449, and TYR337 (Figure 6D).

The biological contribution of each constituent cannot be inferred solely from docking scores. For example, Linalyl anthranilate and linalool were the dominant constituents of the essential oil, representing 47.75% and 16.24%, respectively, and are therefore likely to contribute substantially to the global bioactivity through concentration-dependent effects. Conversely, linalyl anthranilate showed favorable predicted interactions with AChE but represented only 1.00% of the oil. Its contribution may therefore be limited quantitatively, although minor compounds may still participate in synergistic interactions. Thus, the observed AChE inhibition is more plausibly explained by the combined

action of abundant oxygenated monoterpenes and selected minor constituents with favorable binding properties.

2.9 | Molecular Dynamics Simulations

To gain comprehensive insights into the time-dependent behavior of intermolecular interactions, 100 ns molecular dynamics (MD) simulations were performed on the two top-ranked complexes identified from the initial molecular docking study: Complex A (ACh-linalyl acetate) and Complex B (ACh-caryophyllene). The structural integrity and stability of these systems were rigorously monitored through root mean square deviation (RMSD) and root mean square fluctuation (RMSF) analyses. Furthermore, a detailed examination of the resulting trajectories allowed for a deeper understanding of the key stabilization mechanisms governing these ligands within the enzymatic active sites.

2.9.1 | Protein and Ligand RMSD Analysis

The molecular dynamics simulation analysis over a 100 ns trajectory reveals distinct stability profiles for the two complexes based on their root mean square deviation (RMSD) values (Figure 7). For Complex A (ACh-Linalyl anthranilate), the system exhibits remarkable structural equilibrium; the protein C-alpha atoms stabilize rapidly at approximately 1.3 Å, while the ligand (fit on protein) maintains a steady plateau around 3.0 Å. This minimal fluctuation suggests that Linalyl anthranilate achieves an optimal fit within the binding pocket, preserving the enzyme's structural integrity without inducing significant conformational changes. In contrast, Complex B (ACh-Caryophyllene) demonstrates a more dynamic behavior, with the protein RMSD reaching higher values between 1.5 and 1.9 Å. The Caryophyllene ligand shows a more pronounced deviation, stabilizing only after 60 ns at a level of 4.8 Å, which indicates a period of structural readjustment and greater mobility within the active site. Ultimately, while both systems reach a state of convergence, Complex A displays superior structural stability and binding complementarity compared to the more flexible Complex B.

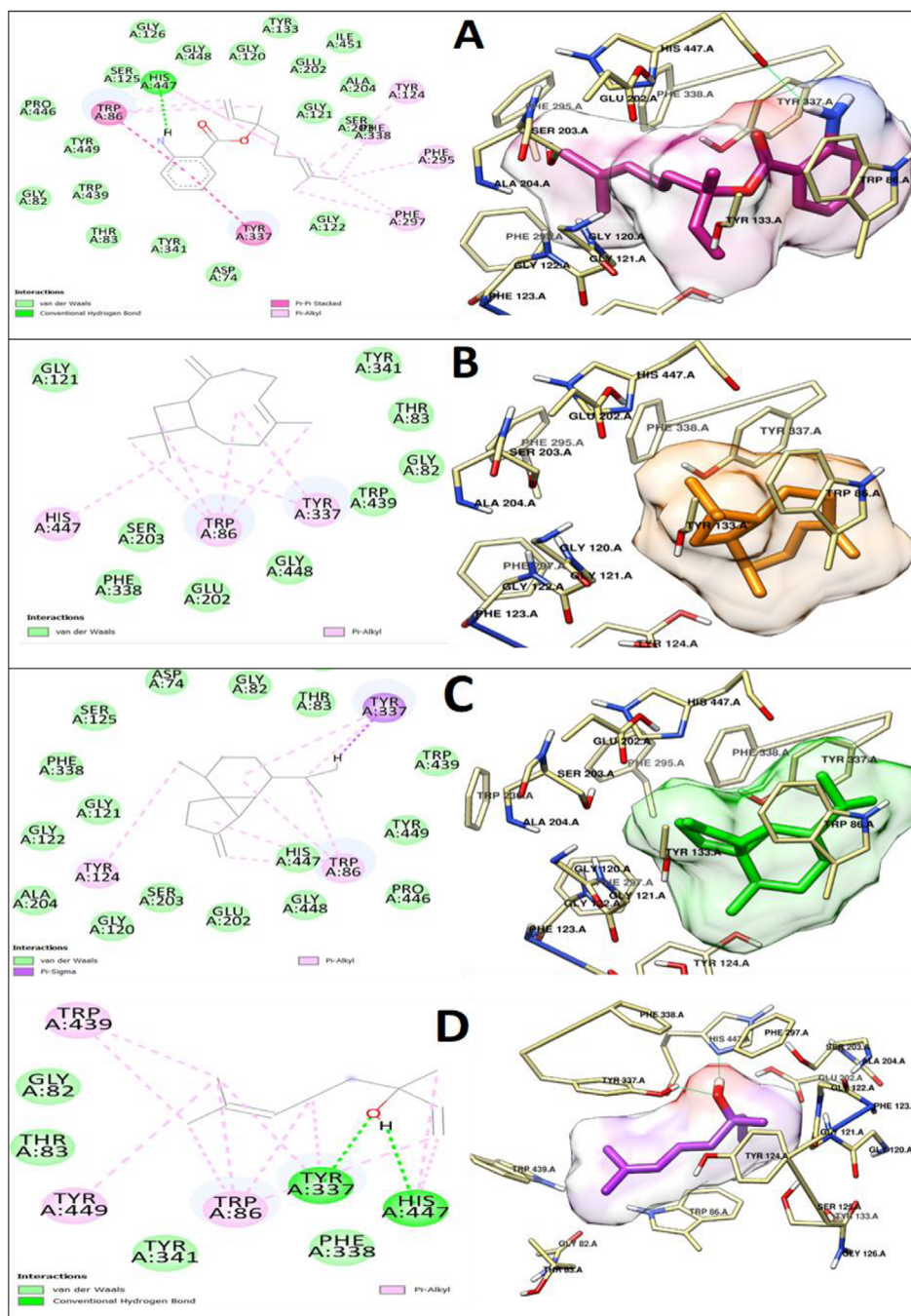


FIGURE 6 | Two-dimensional (2D, left panels) and three-dimensional (3D, right panels) binding orientations of the four major *Salvia sclarea* essential oil constituents docked within the active site gorge of acetylcholinesterase (AChE; PDB ID: 7XN1), as determined by Glide SP molecular docking. (A) Linalyl anthranilate (docking score: -7.640 kcal/mol): H-bond with HIS447 (NH group), π - π stacking with TYR337 and TRP86, hydrophobic π -alkyl contacts with PHE338, PHE295, PHE297. (B) Caryophyllene (-6.749 kcal/mol): hydrophobic π -alkyl interactions with TRP86, HIS447, TYR337. (C) β -Cubebene (-6.653 kcal/mol): π -sigma and π -alkyl hydrophobic interactions with TRP86, HIS447, TYR124. (D) Linalool (-6.093 kcal/mol): two H-bonds (OH group) with HIS447 and TYR337; hydrophobic contacts with TRP86, TRP439, TYR449. The co-crystallized ligand tacrine (docking score: -8.235 kcal/mol) served as a reference. Hydrogen bonds: green dashed lines; hydrophobic contacts: pink arcs; π - π interactions: green lines.

2.9.2 | Protein RMSF Analysis

The RMSF analysis was conducted to evaluate the local flexibility of the protein residues upon ligand binding (Figure 8). For both complexes, the majority of the amino acids exhibited RMSF values below $2.0 \text{ \AA}$, indicating that the enzyme's core structural integrity is well-maintained during the 100 ns trajectory. The

observed peaks correspond to the flexible loop regions and the N- and C-terminal extremities.

In Complex A (ACh-Linalyl anthranilate), the RMSF profile is notably rigid, with most residues fluctuating below $1.6 \text{ \AA}$. Specifically, the active site residues (highlighted by green vertical bars) show minimal displacement (often $< 0.8 \text{ \AA}$),

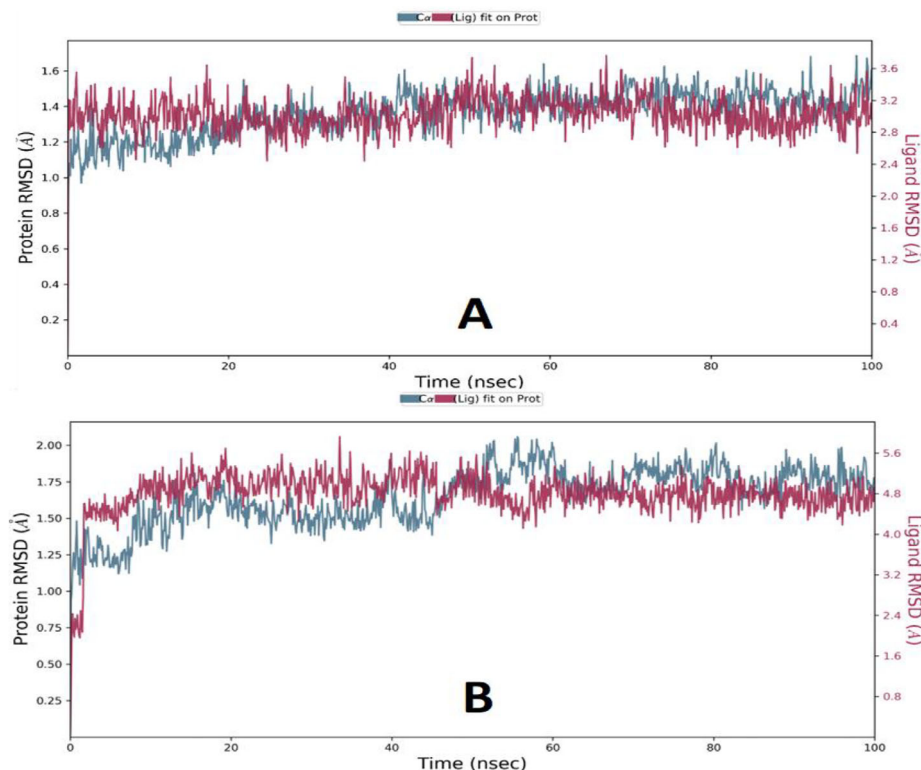


FIGURE 7 | Root-mean-square deviation (RMSD) of the protein α atoms and the bound ligand as a function of simulation time over 100 ns, for Complex A (AChE–linalyl anthranilate, upper panel) and Complex B (AChE–caryophyllene, lower panel). Protein RMSD (black line) and ligand RMSD fitted on the protein backbone (red line) are shown. In Complex A, the system reaches rapid equilibrium (protein RMSD $\approx$ 1.3 Å; ligand RMSD plateau $\approx$ 3.0 Å), indicating high structural stability of the binding complex. In Complex B, greater conformational dynamics are observed (protein RMSD 1.5–1.9 Å; ligand RMSD converging to $\approx$ 4.8 Å only after 60 ns). Simulations were conducted using Desmond (Schrödinger Suite), OPLS-2005 force field, TIP3P explicit solvent, 300 K, and 1 atm (NPT ensemble).

suggesting that Linalyl anthranilate effectively stabilizes the catalytic triad and surrounding pocket. Conversely, Complex B (ACh–Caryophyllene) displays slightly higher local flexibility, with several fluctuations exceeding 2.0 Å. This increased mobility in the protein backbone suggests that Caryophyllene binding allows for a more dynamic environment compared to the highly stabilized state observed with Linalyl acetate.

2.9.3 | Ligand RMSF Analysis

The ligand RMSF was analyzed to determine the positional stability of individual atoms within the binding pocket during the simulation (Figure 9).

In Complex A (linalyl anthranilate), the ligand atoms displayed high stability, with fluctuations ranging between 0.4 and 0.7 Å. A localized peak at atom index 9 indicates slight flexibility at the terminus of the terpene chain, but the overall low RMSF values suggest that the molecule is firmly anchored, likely due to specific interactions involving the ester and aromatic moieties. In contrast, Complex B (caryophyllene) exhibited higher overall fluctuations, with RMSF values ranging from 0.7 to 1.1 Å. The increased mobility, particularly at the macrocyclic ring extremities, reflects a more dynamic binding mode. This suggests that the hydrophobic nature of Caryophyllene results in less restrictive

interactions compared to the more stabilized binding observed with Linalyl anthranilate.

2.9.4 | Protein–Ligand Contacts Analysis

The protein–ligand contact analysis highlights the specific residues and types of interactions responsible for complex stabilization during the 100 ns trajectory (Figure 10). In Complex A (Linalyl anthranilate), the binding is characterized by a high density of hydrophobic contacts, particularly with TRP_86, TYR_124, and PHE_338. Most notably, the complex is further reinforced by highly persistent hydrogen bonds (H-bonds) with TYR_337 and TYR_341, which maintain an interaction fraction near 1.0. These strong, directional bonds explain the significantly lower RMSD and the enhanced structural stability of this complex compared to the other. Conversely, Complex B (caryophyllene) relies solely on hydrophobic interactions, with TRP_86 and TYR_341 serving as the primary anchors. The total absence of polar interactions or water bridges reflects the pure hydrocarbon nature of the Caryophyllene molecule. While these hydrophobic forces provide adequate binding, the lack of directional hydrogen bonding results in the increased flexibility and higher atomic fluctuations observed in the RMSF analysis.

RMSD and RMSF analyses indicated that the selected complexes reached stable conformational behavior during the 100 ns MD

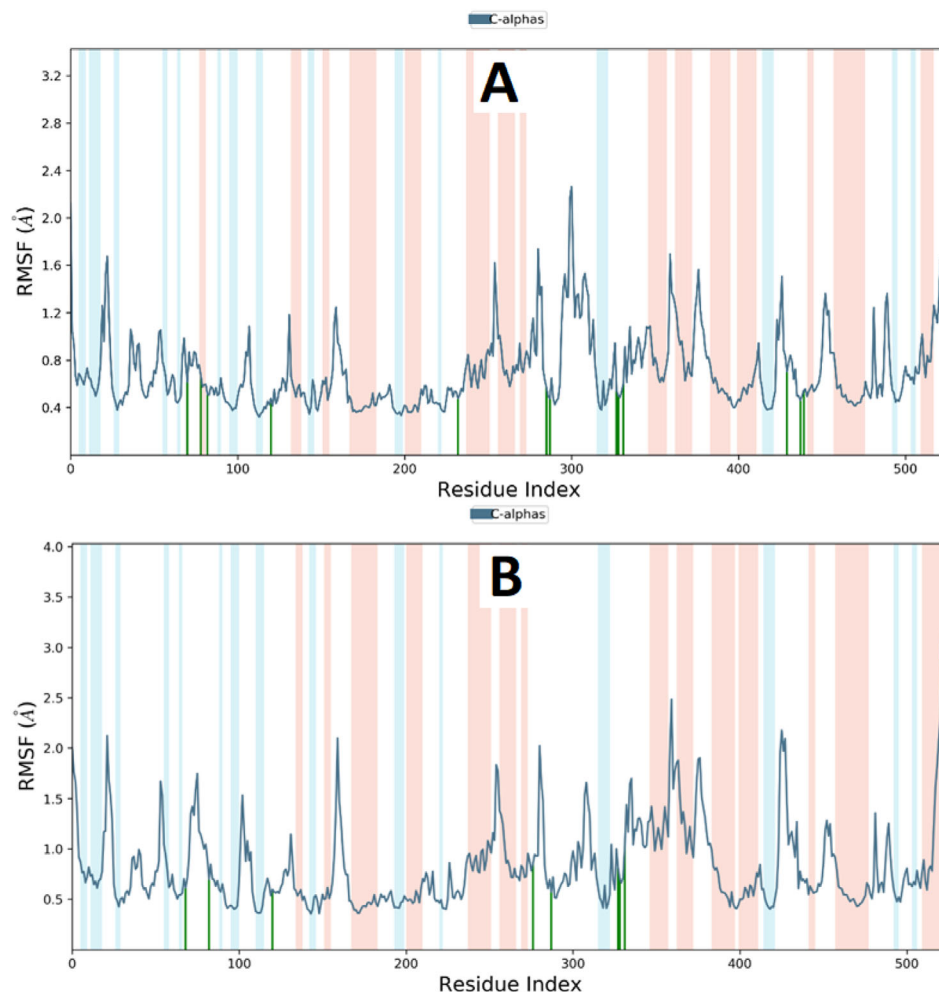


FIGURE 8 | Per-residue root-mean-square fluctuation (RMSF) of AChE C α atoms over the 100 ns molecular dynamics simulation trajectory for Complex A (AChE–linalyl anthranilate, upper panel) and Complex B (AChE–caryophyllene, lower panel). Green vertical bars indicate active site residues (TRP86, TYR124, TYR337, HIS447, TRP439, TYR449, PHE295, PHE297, PHE338). The majority of residues exhibit RMSF < 2.0 Å in both complexes, reflecting preserved enzyme core structural integrity. Complex A shows markedly reduced fluctuation at active site residues (RMSF < 0.8 Å), indicating effective stabilization of the catalytic triad. Complex B displays slightly higher local flexibility (several residues > 2.0 Å), consistent with the more dynamic binding mode of caryophyllene. High-RMSF peaks correspond to flexible loop regions and N-/C-terminal extremities of the enzyme.

simulations. The lower protein and ligand RMSD values observed for the linalyl anthranilate complex suggest a more stable binding mode compared with caryophyllene, which showed higher ligand mobility. RMSF analysis further indicated reduced fluctuations in active-site residues for the linalyl anthranilate complex, supporting better stabilization of the binding pocket. Protein–ligand contact analysis showed persistent hydrogen bonding with key residues, whereas caryophyllene was stabilized mainly by hydrophobic interactions. However, radius of gyration and MM/GBSA binding free-energy calculations were not performed, and this represents a limitation of the present MD analysis. Future studies including these parameters would provide a more quantitative estimation of complex compactness and binding free energy.

3 | Conclusions

This study provides ethnobotanical, phytochemical, and biological evidence supporting the potential interest of *Salvia sclarea*

essential oil from the Edough Massif. The essential oil was characterized by a high proportion of oxygenated monoterpenes, mainly Linalyl anthranilate and linalool, and showed antimicrobial, antibiofilm, antioxidant, anti-inflammatory and enzyme-inhibitory activities under in vitro conditions.

Molecular docking and 100 ns molecular dynamics simulations supported the stable binding of selected constituents, particularly linalyl anthranilate and caryophyllene, within the acetylcholinesterase active-site gorge. However, because linalyl anthranilate was present only as a minor constituent, the overall AChE inhibitory activity of the essential oil is likely to result from additive or synergistic effects involving both major and minor terpenoid constituents.

These findings suggest that Algerian *S. sclarea* essential oil could represent a promising natural source of bioactive compounds. However, further studies, including toxicity assessment, kinetic enzyme analyses, complementary antioxidant and anti-inflammatory assays, in vivo validation, and sustainable

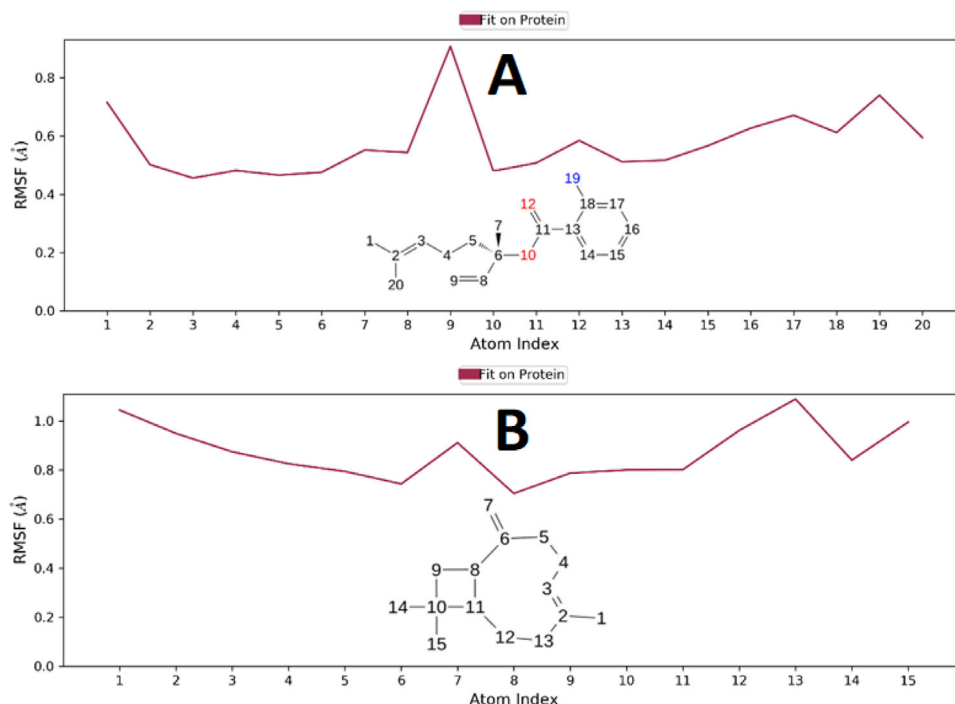


FIGURE 9 | Atomic root-mean-square fluctuation (RMSF) of individual ligand atoms within the AChE binding site over the 100 ns molecular dynamics simulation. (A) Linalyl anthranilate (Complex A): per-atom fluctuations range from 0.4 to 0.7 Å, with a localized peak at atom index 9 (terpene chain terminus), reflecting minor flexibility at the chain extremity while the ester and aromatic moieties remain firmly anchored through H-bond and hydrophobic contacts. (B) Caryophyllene (Complex B): per-atom fluctuations range from 0.7 to 1.1 Å, with increased mobility at the macrocyclic ring extremities, consistent with a more dynamic and exclusively hydrophobic binding mode. Atom indices follow the canonical numbering of each ligand as generated by LigPrep (Schrödinger).

harvesting evaluation, are required before any therapeutic application can be proposed.

4 | Experimental Section

4.1 | Ethnobotanical Survey

A semi-structured ethnobotanical survey was conducted between March and December 2025 in the Edough region (Annaba Forest Massif, Algeria), covering the municipalities of Berrahal, El Bouni, and Ain Berda. A total of 85 key informants (herbalists, midwives, traditional healers, and users aged 48 to 82, 62% of whom were women) were selected using the snowball sampling method [7]. The interviews, conducted in Arabic dialect and French with free and informed consent, allowed for the collection of local names, parts used, preparation methods, therapeutic indications, and frequency of use. The data were analyzed according to standard ethnobotanical indices: The data were analyzed according to classical ethnobotanical indices [6, 12]:

- Relative frequency of citation (RFC) = FC/N
- Use value (UV) = $\Sigma U/N$
- Informant consensus factor (ICF) = $(Nur - Nt)/(Nur - 1)$

Nur = Number of use reports

Nt = Number of species used in the category.

4.2 | Plant Material, Essential Oil Extraction, and Chemical Analysis

The aerial parts of *Salvia sclarea* (L.) were collected on May 25, 2025, in the Annaba region (eastern Algeria). Botanical identification was performed by Prof. Tarek Hamel, and a voucher specimen of *Salvia sclarea* L. was deposited under the number Sal_er18_45 in the herbarium of Prof. Gérard de Bélair, Badji-Mokhtar-Annaba University, Algeria.

The essential oil was obtained from 100 g of air-dried powdered aerial parts by hydrodistillation using a Clevenger-type apparatus, in accordance with the European Pharmacopoeia (3rd ed.) [21] and as described by Elyemni et al. [64], under optimized conditions. The yield was expressed as a percentage of dry plant material according to the equation:

$$\text{Yield (\%)} = (\text{mass of essential oil} / \text{mass of dry plant material}) \times 100.$$

Chemical characterization was performed by gas chromatography with flame ionization detection (GC-FID) and gas chromatography–mass spectrometry (GC–MS).

Compound identification was based on three complementary criteria: comparison of mass spectra with reference databases, comparison of experimentally calculated linear retention indices with literature values obtained on columns of similar polarity, and co-injection with authentic standards whenever available. Linear

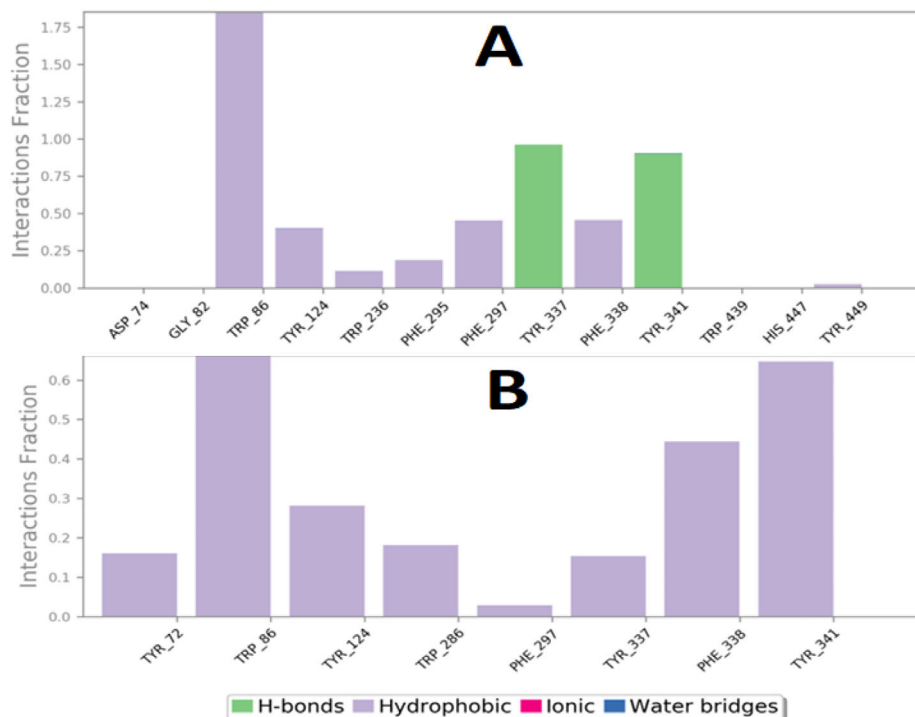


FIGURE 10 | Protein–ligand interaction frequency histograms for Complex A (AChE–linalyl anthranilate, upper panel) and Complex B (AChE–caryophyllene, lower panel) across the full 100 ns molecular dynamics simulation, generated with the Simulation Interaction Diagram module (Schrödinger Desmond). Bar height represents the fraction of simulation time (0.0–1.0) during which a specific contact between the ligand and an AChE residue was maintained. In Complex A, highly persistent H-bonds (fraction ≈ 1.0) with TYR_337 and TYR_341 reinforce the binding, with dominant hydrophobic contacts at TRP_86, TYR_124, and PHE_338. In Complex B, only hydrophobic interactions are present (TRP_86, TYR_341), with no polar or water-bridge contacts, reflecting the purely apolar nature of caryophyllene and explaining its greater flexibility (cf. panel B). Interaction types are color-coded: hydrogen bond (green), hydrophobic (gray), water bridge (blue).

retention indices were calculated relative to a homologous series of n-alkanes (C_8 – C_{28}) [65].

GC analyses were conducted on a MASTER GC DANI system equipped with a split/splitless injector, autosampler, and flame ionization detector. Separation was achieved on an HP-5 capillary column (30 m $\times$ 0.25 mm i.d., 0.25 μ m film thickness; 5% phenyl–95% dimethylpolysiloxane). The oven temperature program was 60°C (8 min), increased to 240°C at 2°C/min, followed by a 10 min isothermal hold. Injector and detector temperatures were set at 250°C and 260°C, respectively. Nitrogen was used as carrier gas at a flow rate of 0.5 mL/min. A 0.2 μ L aliquot was injected in split mode (50:1). Mass spectra were recorded under standard operating conditions.

4.3 | Evaluation of Antimicrobial Activity

The antimicrobial activity was assessed by broth microdilution in accordance with the standard protocol for the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) [66, 67]. The essential oil was tested against a panel of reference and clinical strains. Reference strains were obtained from the Pasteur Institute of Algeria, whereas clinical isolates were recovered from urinary catheter samples.

Bacterial suspensions were adjusted to 0.5 McFarland standard (1×10^8 CFU/mL) and further diluted in Mueller–

Hinton/Sabouraud broth (Sigma-Aldrich, France) to obtain a final inoculum of 5×10^5 CFU/mL per well. Twofold serial dilutions of the essential oil were prepared in 96-well microplates from a 4 mg/mL stock solution in dimethyl sulfoxide (DMSO). Each well contained 100 μ L of essential oil dilution and 100 μ L of bacterial inoculum. Growth control (medium plus inoculum), sterility control (medium only), solvent control, and positive controls (gentamicin for bacteria and amphotericin B for fungi) were included. Plates were incubated at 37°C for 24 h. Bacterial growth was evaluated by measuring optical density at 600 nm (OD_{600}). The MIC was defined as having the lowest concentration showing no visible growth.

For MBC determination, aliquots from wells showing no apparent growth were subcultured on Mueller–Hinton/Sabouraud dextrose agar and incubated for 24 h. The MBC was defined as the lowest concentration resulting in $\geq 99.9\%$ reduction of the initial inoculum.

4.4 | Inhibition of Biofilm Formation

Biofilm inhibition was evaluated in polystyrene tubes according to Hassan et al. [68]. Microbial suspensions in brain–heart infusion broth (Sigma-Aldrich, France) were adjusted to an optical density of 0.4 at 600 nm. Aliquots of 500 μ L of essential oil, tested at its minimum inhibitory concentration (MIC), were mixed with

TABLE 4 | Antioxidant and anti-inflammatory activities of *Salvia sclarea* (L.) essential oil.

	Antioxidant activity		Anti-inflammatory activity	
	Ascorbic acid	Essential oil	Diclofenac	Essential oil
EC ₅₀ in µg/mL	23.79 (19.98–28.06; R ² = 0.9751)	40.38 (34.89–46.45; R ² = 0.9796)	89.24 (81.36–97.15; R ² = 0.9970)	291.10 (258.9–326.2; R ² = 0.9840)
Inhibitory activity %	94.48 ± 0.26	87.84 ± 0.27	92.52 ± 1.18	76.27 ± 0.74

Note: EC₅₀: Effective concentration 50% (EC₅₀) is defined as the concentration of a compound required to produce 50% of its maximal biological effect under specified experimental conditions. The EC₅₀ values (µg/mL), expressed with their 95% confidence intervals and corresponding coefficients of determination (R²).

TABLE 5 | Percentage inhibition and EC₅₀ values (µg/mL, 95% CI) of essential oil against acetylcholinesterase and α-amylase activities.

	Acetylcholinesterase		α-Amylase	
	Galantamine	Essential oil	Acarbose	Essential oil
EC ₅₀ in µg/mL	15.94 (13.85–18.14; R ² = 0.9806)	64.40 (50.23–81.43; R ² = 0.9575)	36.15 (91.91–60.61; R ² = 0.9812)	69.49 (58.8–81.6; R ² = 0.9638)
Inhibitory activity %	99.12 ± 0.50	86.47 ± 1.99	94.61 ± 1.70 36.15 µg/mL, 95% CI = lower value – upper value	82.50 ± 2.80

Note: EC₅₀: The half-maximal effective concentration represents the concentration of a compound required to achieve 50% of its maximal biological response under defined experimental conditions. EC₅₀ values are expressed in µg/mL and reported together with their 95% confidence intervals and corresponding coefficients of determination (R²).

TABLE 6 | Docking scores and key interactions of the four major compounds within the AChE active site.

Compound	Docking score (kcal/mol)	Hydrogen bonds (H-bonds)	Key hydrophobic/ π -interactions
Linalyl anthranilate	−7.640	HIS447 (NH group)	TYR337, TRP86, PHE338, PHE295, PHE297
Caryophyllene	−6.749	/	TRP86, HIS447, TYR337
β -Cubebene	−6.653	/	TRP86, HIS447, TYR124
Linalool	−6.093	HIS447, TYR337 (OH group)	TRP86, TRP439, TYR449, TYR337
Co-crystallized ligand (tacrine)	−8.235	HIS447 (NH group)	TRP86, HIS447, TYR337

500 μ L of microbial suspension and incubated statically at 37°C to allow biofilm formation.

After incubation, tubes were stained with 0.1% (w/v) crystal violet for 45 min. Excess stain was removed, and the bound dye was solubilized with an ethanol–acetone mixture (1:3, v/v). Biofilm biomass was quantified by measuring absorbance at 570 nm using a SECOMAM spectrophotometer. The percentage of biofilm inhibition was calculated as follows:

$$\text{Percentage of Inhibition (\%)} = \frac{[(\text{Abs Control} - \text{Abs Test}) / \text{Abs Control}] \times 100}{}$$

4.5 | Evaluation of Biofilm Metabolic Activity (MTT Reduction Assay)

Biofilm metabolic activity was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay, which estimates the metabolic competence of viable cells through mitochondrial dehydrogenase activity.

The assay was performed according to Negreiros et al. [69] with minor modifications. A 5 mg/mL MTT stock solution (Sigma-Aldrich, France) was prepared in sterile phosphate-buffered saline (PBS) and filter-sterilized. After 24 h incubation under the conditions described for the biofilm inhibition assay, cells were collected by centrifugation (12 000 rpm, 10 min), and the pellet was resuspended in 10% MTT solution, followed by incubation for 4 h at 37°C in the dark. After centrifugation, the resulting pellet was resuspended in 1 mL dimethyl sulfoxide (DMSO) and incubated for 30 min at 37°C to ensure complete dissolution of formazan crystals.

Cellular debris was removed by centrifugation, and 200 μ L of the supernatant (four technical replicates per sample) were transferred to flat-bottom 96-well plates. Absorbance was recorded at 550 nm using a microplate reader (FilterMax F5, Molecular Devices, Austria). Percentage inhibition by essential oils was calculated from the mean optical density values of treated and control samples using the following equation:

$$\text{Percentage of Inhibition (\%)} = \frac{[(\text{Abs Control} - \text{Abs Test}) / \text{Abs Control}] \times 100}{}$$

4.6 | DPPH Radical-Scavenging Assay

The antioxidant activity of the essential oil of *Salvia sclarea* (L.) was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay, following the method described by Bouzina et al. [70] with minor modifications.

A 0.1 mM methanolic DPPH solution was prepared by dissolving 4 mg of DPPH in 100 mL of methanol. Briefly, 200 μ L of essential oil solution (dissolved in dimethyl sulfoxide, DMSO) was added to 800 μ L of DPPH solution. The mixtures were incubated for 30 min at room temperature in the dark to allow the reaction to reach steady state.

The assay was performed using a twofold serial dilution of the essential oil over the concentration range of 1000–15.62 μ g/mL. Ascorbic acid was used as a positive control, whereas the negative control consisted of 200 μ L DMSO mixed with 800 μ L DPPH solution.

Absorbance was measured at 517 nm using a SECOMAM spectrophotometer. The percentage of DPPH radical scavenging activity was calculated according to the following equation:

$$\text{Percentage of Inhibition (\%)} = \frac{[(\text{Abs Control} - \text{Abs Test}) / \text{Abs Control}] \times 100}{}$$

4.7 | Anti-Inflammatory Activity (Protein Denaturation Assay)

The anti-inflammatory activity of the essential oil was evaluated by assessing its ability to inhibit heat-induced protein denaturation, according to the method described by Gunathilake et al. [71].

Briefly, 0.2 mL bovine serum albumin (BSA, 0.1% w/v; Sigma-Aldrich) was mixed with 4.78 mL phosphate-buffered saline (PBS, pH 6.4) and 0.02 mL of the tested substances at various concentrations (100–1600 μ g/mL). The reaction mixtures were incubated at 37°C for 15 min and subsequently heated at 70°C for 5 min to induce protein denaturation. After cooling to room temperature, turbidity was measured spectrophotometrically at 660 nm.

The PBS solution without a test sample served as the negative control, while diclofenac sodium, prepared under identical conditions, was used as the positive control.

The percentage inhibition of protein denaturation (I%) was calculated using the following equation:

$$\text{Percentage of Inhibition (\%)} = \left[1 - \left(\text{Abs}_{\text{sample}} / \text{Abs}_{\text{negative control}} \right) \right] \times 100$$

4.8 | Acetylcholinesterase Inhibitory Activity

The acetylcholinesterase (AChE) inhibitory activity was assessed using a modified Ellman colorimetric assay in a 96-well microplate format, as described by Hung et al. [72].

Each well contained 140 μL phosphate buffer (0.1 M, pH 8.0), 20 μL essential oil (EO) at final concentrations of 10, 50, 100, 250, 500, 750, and 1000 $\mu\text{g/mL}$, and 20 μL AChE solution (0.25 U/mL). After pre-incubation for 15 min at 25°C, the reaction was initiated by adding 10 μL DTNB (2.5 mM) and 10 μL acetylthiocholine iodide (2.5 mM). Following 10 min incubation at 25°C, absorbance was measured at 405 nm using a microplate reader (FilterMax F5, Molecular Devices, Austria).

A reaction mixture containing buffer, enzyme, and substrate without EO served as the positive control (100% activity), whereas a mixture without enzyme was used as the negative control. A solvent control (1% DMSO) was included to assess potential solvent effects. Galantamine was used as the reference inhibitor under identical conditions.

The percentage of AChE inhibition was calculated according to the following equation:

$$\text{Acetylcholinesterase Inhibition (\%)} = \left[(\text{Abs Control} - \text{Abs Test}) / \text{Abs Control} \right] \times 100$$

4.9 | α -Amylase Inhibitory Activity

The α -amylase inhibitory activity was determined using the 3,5-dinitrosalicylic acid (DNSA) colorimetric method [73].

The essential oil (EO) was initially dissolved in a minimal volume of 10% DMSO and further diluted in phosphate buffer ($\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$, 0.02 M, containing 0.006 M NaCl, pH 6.9) to obtain final concentrations of 10, 50, 70, 100, 500, and 1000 $\mu\text{g/mL}$.

Briefly, 200 μL of α -amylase solution (2 U/mL) was mixed with 200 μL of EO solution and pre-incubated for 10 min at 30°C. Subsequently, 200 μL of 1% (w/v) soluble starch solution was added, and the mixture was incubated for 3 min. The reaction was terminated by adding 200 μL of DNSA reagent (containing sodium potassium tartrate tetrahydrate in 2 M NaOH and 3,5-dinitrosalicylic acid at 96 mM), followed by heating in a water bath at 85–90°C for 10 min.

After cooling to room temperature, the mixture was diluted with 5 mL of distilled water, and absorbance was measured at 540 nm using a UV–visible spectrophotometer (752N). All experiments were performed in triplicate.

The control representing 100% enzymatic activity was prepared by replacing the EO with buffer. For each EO concentration, a corresponding control without enzyme was performed to correct for non-enzymatic starch hydrolysis. Acarbose was used as the reference inhibitor under identical experimental conditions.

The α -amylase inhibitory activity was expressed as percentage inhibition and calculated using the following equation:

$$\alpha \text{ amylase inhibition (\%)} = \left[(\text{Abs control} - \text{Abs test}) / \text{Abs control} \right] \times 100$$

4.10 | Molecular Docking

The three-dimensional structure of the target enzyme was retrieved from the Protein Data Bank using its corresponding PDB identifier: acetylcholinesterase (PDB ID: 7XN1; resolution 2.85 Å) [74]. Protein preparation was carried out using the Protein Preparation Wizard [75] of the Schrödinger Suite, which included the addition of hydrogen atoms, assignment of bond orders and appropriate protonation states, and restrained energy minimization to optimize the protein structure. Ligand structures were prepared using the LigPrep module [76], ensuring the generation of correct protonation states, atom types, bond orders, and stereochemical configurations required for accurate docking simulations. Subsequently, receptor grids were generated with the Receptor Grid Generation module in Maestro to define the active-site binding region for docking calculations [77]. Flexible molecular docking was then performed using the Glide module in standard precision (SP) mode [78], allowing reliable prediction of protein–ligand binding interactions while considering ligand conformational flexibility.

4.11 | Molecular Dynamics Simulations

To evaluate the conformational stability and validate the docking results for Complex A (ACh-linalyl acetate) and Complex B (ACh-caryophyllene) [79], 100 ns molecular dynamics (MD) simulations were performed using the Desmond software package [80]. Each system underwent rigorous geometry optimization and energy minimization before being solvated in an orthorhombic box using the TIP3P water model and the OPLS-2005 force field [81]. To replicate physiological conditions and ensure system neutrality, a 0.15 M NaCl concentration was maintained [82]. Simulations were executed in the NPT ensemble at a constant temperature of 300 K and a pressure of 1 atm, with trajectories recorded every 100 ps. The overall stability and binding persistence were subsequently assessed via RMSD, RMSF, and detailed protein–ligand interaction profiling [83].

4.12 | Statistical Analysis

All experiments were performed in triplicate, and results are expressed as mean $\pm$ standard deviation (SD). Statistical

differences among groups were evaluated using two-way ANOVA followed by Tukey's post hoc test using GraphPad Prism 9 software. Differences were considered statistically significant at $p < 0.05$.

Author Contributions

Manel Lina Djendi conducted the in vitro tests and wrote the original draft. **Chahrazed Benzaid** (corresponding author) designed and supervised the study, validated the results, and revised the manuscript. **Ali Dekir** conducted the molecular docking. **Housseem Chenna** conducted the molecular dynamics simulations. **Fouzia Tread**, **Sarah Rahal**, and **Lina Raghad Klaiaia** contributed to the ethnobotanical survey and statistical analysis. **Saglam Kübra** performed the GC-MS analysis and compound identification. All authors read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable requests.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

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