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Assessing *Salvia dominica* L.: from chemical profiling to antioxidant, antimicrobial, anticancer, α-amylase, and α-glycosidase activities of the plant essential oil

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Abstract

Salvia dominica L. is a fragrant perennial shrub densely adorned with trichomes, found throughout the eastern Mediterranean, especially Palestine, Jordan, Lebanon and Syria. It is commonly used by the Bedouins for the remedy of many diseases. In recent years, essential oils (EOs) have attracted interest due to their biological qualities. This study sought to examine the chemical composition of EOs extracted from the dry and fresh leaves of *Salvia dominica* L. and to evaluate their in vitro antioxidant, anticancer, antibacterial and α-amylase and lipase inhibitory activity. The chemical compositions of EOs obtained by steam distillation were determined using gas chromatography–mass spectrometry. The principal constituents of the oil derived from fresh *Salvia dominica* L. leaves comprised linalyl acetate (43.69%), α-terpinyl acetate (12.35%), germacrene D (10.22%), linalool (9.40%), 1,8-cineole (7.07%), and α-terpineol (4.97%), with the predominant category being oxygenated monoterpenes (OM) at 74.60%. The principal constituents of the EO obtained from air-dried leaves included linalyl acetate (70.17%), germacrene D (10.20%), terpinyl acetate (7.49%), and 1,8-cineole (4.08%), with oxygenated monoterpenes (OM) representing the predominant class at 80.87%. The air-dried flowers of *Salvia dominica* L. were extracted with CO_2–CH_2Cl_2, yielding a dark brown sticky oil that was fractionated into five fractions via silica gel chromatography. Interestingly, fractions (F3 and F4) showed significant anticancer activity against MCF-7 and HeLa cell lines, with IC_{50} values ranging from 25.41 ± 1.27 to 40.94 ± 2.05 µg/mL, while both EOs showed reduced anticancer properties and poor α-amylase and lipase activities. Both EOs displayed outstanding antioxidant activity, and modest antibacterial activity against *K. pneumonia* and *S. aureus* with MIC values between 0.39 and 3.125 µL/mL. The fractions 4 and 5 of the CO_2 extract showed enhanced antibacterial efficacy relative to the commonly employed antibiotic gentamicin (31.25–125 µg/mL) against all tested microorganisms, with MIC values between 6.25 and 25 µg/mL.

Keywords *Salvia dominica* L., Essential oil, CO_2 extract, Phytochemicals, Antioxidant, Antimicrobial, Anticancer, α-amylase, α-glycosidase

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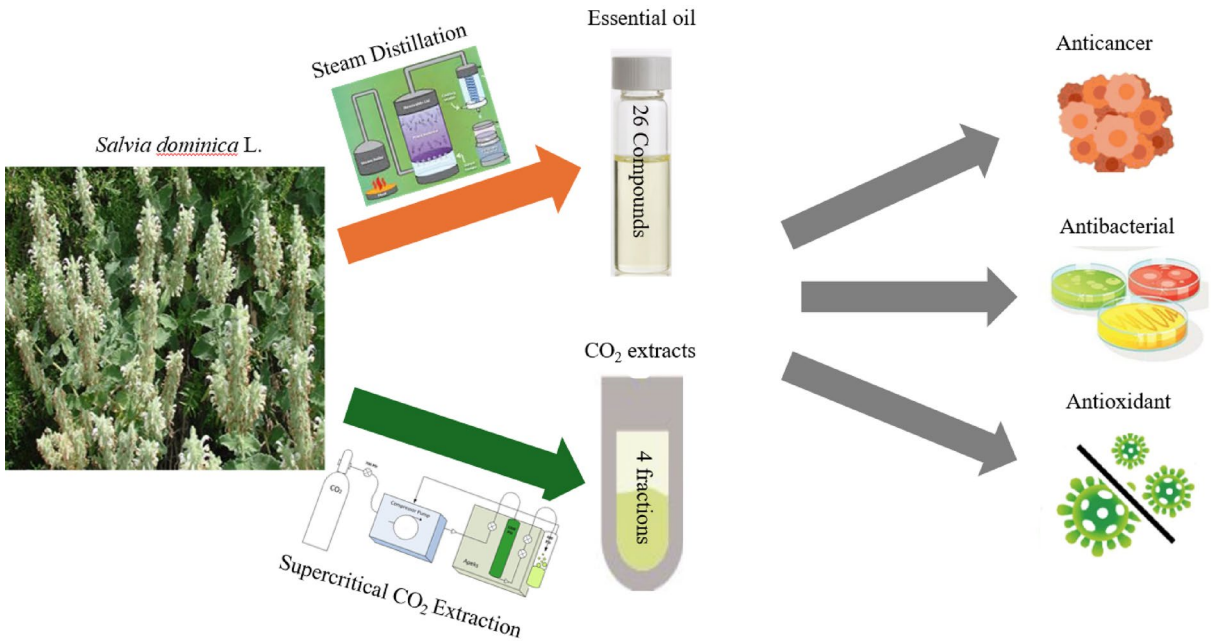
Graphical Abstract



Introduction

Since ancient times, medicinal plants have been recognized as a crucial source of novel chemicals with potential therapeutic properties. Recently, plant-derived natural products, especially essential oils, have garnered increased public interest owing to their distinctive biological features and purported absence of adverse effects [1]. Essential oils (EOs), also known as volatile oils, are lipophilic and highly volatile fragrant secondary metabolites obtained from plants. The mixtures of volatile compounds mostly comprise mono- and sesquiterpenoids and phenylpropanoids, with terpenoids being the most abundant and characteristic constituents. Mono-, bi-, or tricyclic mono- and sesquiterpenoids from several chemical families provide the primary constituents of essential oils, encompassing hydrocarbons, ketones, alcohols, oxides, aldehydes, phenols, and esters [2]. Owing to their structural compatibility within the same chemical group, EO components are easily converted into one another through oxidation, isomerization, cyclization, or dehydrogenation processes. These processes may be initiated chemically or enzymatically [3]. Essential oils are extensively utilized as flavorings; nevertheless, due to their established antibacterial, antiviral, antifungal, insecticidal, and antioxidant capabilities, they provide an eco-friendly option in the pharmaceutical, nutritional, and agricultural sectors [2–5].

Salvia plants are cultivated globally and are used to produce unique fragrant EOs that have significant commercial value in the cosmetics, fragrance, and pharmaceutical sectors [4]. The *Salvia* genus is the largest group of plants in the *Lamiaceae* family, encompassing around 1000 species of shrubs, herbaceous perennials, and annuals, of which 22 species are growing in Palestine, including *Salvia palaestina*, *S. microstegia*, *S. viridis*, *S. indica*, *S. syriaca*, and *S. dominica* [6]. Salvia species are abundant in several secondary metabolites, including polyphenols, flavonoids, and terpenoids, and have been associated with traditional medicinal practices since antiquity [7, 8]. They are widely consumed, particularly when combined with tea and seasonings [9]. Traditional medicine has recorded the effectiveness of many *Salvia* species in treating a range of ailments such as bronchitis, bleeding, and menstrual abnormalities [10]. Moreover, scientists have discovered that these plants possess antioxidant, anti-inflammatory, anticancer, antibacterial, antifungal, and antidiabetic characteristics [11, 12].

Salvia dominica L. is a scented perennial plant prevalent in the eastern Mediterranean, particularly in Palestine, Jordan, Lebanon, and Syria [12–16]. The plant has pale yellow flowers and possesses leaves that are densely covered with hair. It is known as Khowekha. It attains a vertical measurement of one meter with a strong and pleasant scent [17]. A literature review indicated that

only two studies focused on *S. dominica* L.; one examined the chemical composition of its EOs from two distinct regions in Jordan, while the other analyzed the phytochemicals in the $CHCl_3$–MeOH extract of *Salvia dominica* L. aerial parts, leading to the identification and characterization of 24 novel sesterterpenoids with notable properties in inhibiting tubulin tyrosine ligase [12, 18].

In light of our interest in the phytochemical composition of essential oils (EOs) of aromatic plants from the flora of Palestine, this study examined the phytochemical composition of EOs derived from dry and fresh *Salvia dominica* L. leaves, utilizing GC/MS analysis. In addition, we investigated their antioxidant, antimicrobial, antiviral, α-amylase, and lipase activities. We aim to investigate the potential anticancer properties of the CO_2 extract derived from the plant's dry flowers.

Materials and methods

Species collection and identification

The leaves and blossoms of *Salvia dominica* L. were gathered from the Albadhan mountains along the route connecting Jericho and Nablus (latitude 3°53′07.5″ N and longitude 59°40′10.9″ 312 W) in early May 2024. Dr. Nidal Jaradat identified the plant, and voucher specimens were deposited in An-Najah National University's Herbal Products Laboratory with the code Pharm-PCT-2115.

Distillation of essential oils

Half of the collected leaves were dried in a shaded location at room temperature (25 ± 3 °C) and humidity (55 ± 4 RH), while the rest was kept in the freezer for 2 days. The dried and frozen leaves were mechanically crushed into tiny pieces to assist in the distillation of the EO. A quantity of 200 g of powdered leaves were subjected to steam distillation for 2 h. The dispersed oil was extracted with diethyl ether (Et_2O, 50 mL ×2), the combined organic layers were dried over $MgSO_4$, and ether was carefully removed under reduced pressure at 35 ℃, yielding 2.46 g of pale-yellow oil. The same procedure has been applied to the distillation of the EO from the frozen leaves; offering 2.82 g of EO. The oils were stored in a sealed container in the dark at room temperature.

Supercritical carbon dioxide extraction

The extraction of *Salvia dominica* L. flowers was conducted using Careddi supercritical CO_2 extraction machine (HA120-50-005), manufactured in China with a 500 mL extraction vessel (extractors) [extraction kettle (0.5 L/50 MPa), separation kettle I (separators I) (0.3 L/30 MPa), separation kettle II (separators II) (0.3 L/30 MPa)]. The experimental procedure employed in this study was identical to that outlined in the previous study [19]. For the supercritical extraction, the mixture of CO_2 + CH_2Cl_2 was used as a solvent. 20 g of the ground flowers were immersed in 10 mL dichloromethane (CH_2Cl_2) at a mass ratio of 2:1 (mass of CH_2Cl_2: mass of material). Subsequently, the mixture was introduced into the extractor, which was then purged with carbon dioxide (CO_2). After charging the extractor with the sample, CH_2Cl_2, and CO_2 at the specified pressure and temperature, the static extraction phase began. At the end of the static period (which was set to 60 min), the dynamic extraction began with a compressed CO_2 flow rate of approximately 26 L/h with both pressure and temperature constantly controlled. The extraction was carried out under varying settings to identify the best parameters. Extraction Kettle (0.5 L/50 MPa) operated at 65 °C (338.15 K) and 25 MPa pressure; Separation Kettle I (0.3 L/30 MPa) at 50 °C (323.15 K) and 8 MPa pressure; and Separation Kettle II (0.3 L/30 MPa) at 50 °C (323.15 K) and 4 MPa pressure. The DCM residue was removed from the resulting dark brown oil by flushing with cold CO_2 gas at temperatures less than 4 °C, yielding 0.4 g of dark brown sticky oil.

Qualitative and quantitative analysis of the extracted EOs

Gas chromatography–mass spectrometry analysis was used to determine the chemical composition of the extracted EOs in a qualitative and quantitative manner. This analysis utilized a nonpolar Perkin Elmer-5-MS capillary column with specific dimensions: an inner diameter of 0.25 mm, a length of 30 m, and a width of 0.25 µm. 1 µL of EO samples, produced at a concentration of 1000 parts per million (ppm), were injected. Helium was employed as the carrier gas with a flow rate of 1 mL/min, maintained at a pressure of 20.41 psi in split mode, utilizing a split ratio of 1:50. The injector was maintained at a temperature of 250 °C, while the transfer line was also held at a temperature of 250 °C. The oven was initially set to a temperature of 50 °C for 5 min, after which it was gradually increased to 280 °C at a rate of 4 °C/min. It was then kept at a constant temperature of 280 °C for a period of 10 min. The duration of the run was 62.5 min, including a 10-min period after the run for conditioning. The detection was performed utilizing a Perkin Elmer Clarus 560 mass spectrometer. The acquisition was performed via electron ionization (EI) mode, employing an ionization voltage of 70 eV in a standard scanning mode covering a mass range of 40–500 *m*/*z*. The identification of individual metabolites was achieved by comparing their relative retention indices (RRI) and mass spectral data with the MS library, NIST webbook, and relevant literature sources, as well as analyzing their fragmentation patterns. The compounds that were detected were represented as the percentages of the peak area of each individual component in relation to the total peak area of the

EO. The RRI for each phytoconstituent was determined by comparing their retention times to those of a standard solution of *n*-alkanes (C_7–C_{30}) under the same experimental circumstances as the EO samples. The relative proportions of distinct substances were calculated from the peak regions obtained using gas chromatography using a normalization method, without the application of correction factors.

DPPH free radical scavenging assay

The antioxidant activity of extracted EOs was evaluated through 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging capacity. The DPPH assay was carried out following methods reported in the literature [20]. Freshly prepared methanolic-0.1 mM DPPH free radical solution was added to and mixed thoroughly with a methanolic solution of EOs and synthetic antioxidants (BHA and BHT, taken as standards) of varying concentrations in the ratio 3:1, respectively. The mixtures were incubated for 30 min in the dark at room temperature. The resultant absorbance was recorded at 517 nm using a UV–visible spectrophotometer (LABINDIA®, India). The DPPH radical scavenging capacity was calculated using the following formula:

$$\text{Scavenging capacity} = A_{\text{control}} - A_{\text{sample}}/A_{\text{control}} \times 100, \quad (1)$$

where A_{control} is the absorbance of DPPH radical without any additive and A_{sample} is the absorbance of DPPH radical with oil samples and control solutions of various concentrations. A graph of scavenging activity as a percent inhibition was plotted against the concentrations of essential oils and standards to determine their IC_{50} value.

α-Amylase inhibition assay

The inhibitory activity of the EOs against α-amylase was determined by the Worthington enzyme method [21]. The EO was dissolved in 10% DMSO to prepare dilutions: 10, 50, 70, 100, 500, and 1000 µg/mL. A total of 0.2 mL of EO solution and 0.2 mL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing α-amylase solution (1.0 U/mL) were incubated at 30 °C for 10 min. After preincubation, 0.2 mL of 1% starch solution was added to 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl). The reaction mixture was then incubated at 25 °C for 10 min. Then, 0.2 mL of dinitrosalicylic acid (DNS) colour reagent was added to stop the reaction. Followed by a dilution of each solution by adding 5 mL of distilled water and incubation in a boiling water bath for 5 min, then letting it cool to room temperature. The absorbance was measured at 540 nm. The α-amylase inhibitory activity was calculated according to the equation: α-amylase inhibition (%) $=(A_B - A_E)/A_B \times 100$, where A_B is the absorbance of the blank solution and A_E is the absorbance of the EO. A graph of α-amylase inhibition (%) was plotted against the concentrations of oils and standards to determine their IC_{50} value.

Porcine pancreatic lipase inhibition assay

The lipase inhibition activity was assessed using the methodology outlined in the literature [22]. The EOs were initially dissolved in 10% DMSO to produce a stock solution, which was subsequently diluted to prepare concentrations of 50, 100, 200, 300, 500, and 1000 µg/mL. Lipase was diluted in a 75 mM buffer solution at pH 8.5. A *p*-nitrophenyl butyrate (PNPB) solution was prepared by dissolving 20.9 mg in 2 mL of acetonitrile. Subsequently, 100 µL of test solutions were combined with 200 µL of lipase solution (0.8 µg/mL), and a Tri–HCl solution was added to achieve a total volume of 1 mL. The mixture was maintained in darkness at 37 °C for 15 min; subsequently, 20 µL of *p*-nitrophenyl palmitate (4 µg/mL) was added and incubated for 30 min at 37 °C. The analyses were conducted at a wavelength of 450 nm. The lipase inhibitory activity was calculated using the following equation: lipase inhibition (%) =(AB-AE)/AB ×100, where AB represents the absorbance of the blank solution and AE denotes the absorbance of the EO. A graph depicting lipase inhibition (%) was constructed in relation to the concentrations of oils and standards to ascertain their IC_{50} value.

Antibacterial tests

The bacterial isolates being investigated were acquired from the American Type Culture Collection (ATCC). The collection comprised the following bacterial strains: *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Proteus vulgaris* (ATCC 8427), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6538P), and *Staphylococcus epidermidis* (ATCC 12228). Along with a single clinical strain of vancomycin-resistant *Staphylococcus aureus*. The broth turbidity of the tested bacterial strains was adjusted to 0.5 McFarland (equal to 1.5×10^8 CFU/mL), which was then combined with saline solution to achieve a concentration of 1×10^7 CFU/mL.

Determination of antibacterial susceptibility

The disk diffusion susceptibility method was used to determine the antibacterial susceptibility [23]. In brief, Muller–Hinton agar was cross-contaminated with a standardized bacterial isolate (1.5 ×108 CFU/mL). Next, standardized concentrations of each substance (0.1 mg/mL) were applied to 6 mm diameter filter paper disks, which were then placed on the surface. After an overnight

incubation at 37 °C, the size of the zone of inhibition (in mm) surrounding the disk was measured.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The antibacterial effectiveness of the two EOs against the preceding four isolates was assessed by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values using the method described by Wayne PA [23]. A stock solution with a concentration of 50 µL/mL was prepared by mixing each EO (50 µL) with 900 µL of 20% DMSO and 100 µL of 60% Muller–Hinton broth. The prepared EO solutions were serially diluted by a factor of two in Muller–Hinton broth medium. Duplicates of each dilution (1000, 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91, and 1.95 µg/mL) were inoculated with 1 µL from 1×10^7 CFU/mL standardized bacterial isolated suspension. The last two duplicate wells were not inoculated (negative controls). Then, the inoculated microplates were incubated at 37 °C overnight. The lowest concentration of each compound that inhibited the growth of the tested isolate was considered the MIC. After that, the contents of the wells resulting from MIC were streaked using sterile cotton swabs on nutrient agar plates free of antibacterial agents and incubated at 37 °C overnight. The lowest concentration of each compound that showed no bacterial growth was considered MBC.

Data analysis

All conducted tests on *S. dominica* EO and extracts were performed in triplicate. The results were expressed as means ± standard deviation (SD), while the outcomes were considered significant when p values were <0.05.

Results and discussion

Phytochemistry

The chemical composition of EOs derived from dry and fresh *Salvia dominica* L. leaves was examined. The plant material was harvested in early May 2023. Steam distillation of the dried and fresh leaves yielded light yellow oil at 1.23% and 1.41%, respectively. The chemical content of both oils was analyzed by GC–MS. Table 1 presents the names, retention times (RT), retention indices (RI), and percentages of identified constituents for the EOs. The GC chromatograms for both EOs (Figures S1 and S2) and the mass spectra of the principal components (Figures S4–S14) are included in the supplementary material. In the EO obtained from fresh leaves (FLEO), 26 compounds were identified, with linalyl acetate **1** (43.69%), α-terpinyl acetate **2** (12.35%), germacrene D **3** (10.22%), linalool **4** (9.40%), 1,8-cineole **5** (7.07%), and α-terpineol **6** (4.97%) being the predominant components. The EO obtained from dried leaves (DLEO) comprises the same constituents, albeit in varying amounts, with the principal components being linalyl acetate **1** (70.17%), germacrene D **3** (10.20%), α-terpinyl acetate **2** (7.49%), and 1,8-cineole **5** (4.08%). In comparison to the EO derived from fresh leaves (FLEO), the EO from dried leaves (DLEO) included linalool **4** and α-terpineol **6** at low concentrations of 1.17% and 0.45%, respectively.

Our results significantly diverge from previous studies regarding the chemical composition of *Salvia dominica* L. leaves (EO). Abdallah et al. found 35 and 24 compounds from fresh and dried leaves of *Salvia dominica* L. plants gathered in the Jordan Valley in April 2009, which constituted 99.1% and 82.4% of the total essential oils extracted, with 1.9% and 1.3% obtained via hydrodistillation, respectively [18]. The major components of the EO from fresh aerial parts were reported as dehydrolinalool (31.4%), α-terpineol **6** (24.4%), α-terpinyl acetate **2** (4.7%), and germacrene D **3** (4.3%), while the principal components identified in the dry aerial parts were dehydrolinalool (18.3%), α-terpineol **6** (15.0%), neo-isodihydrocarveol (10.7%), thymol (7.5%), and *cis*-carveol (7.5%) [25]. In 2020, Al-Jaber et al. investigated the chemical composition of the EOs of *Salvia dominica* L., extracted via hydrodistillation from the leaves, pre-flowering buds, flowers, sepals, and petals gathered in Jordan from the Mediterranean and Irano-Turanian regions [26]. The primary constituents EO sourced from the Mediterranean region were identified as α-terpinyl acetate **2** (54.87%), *trans*-sabinene hydrate acetate (24.44%), 1,8-cineole **5**, linalool acetate **1** (2.2%), and sabinene (1.50%). In contrast, the predominant components of EO obtained from the Irano-Turanian region included *trans*-sabinene hydrate acetate (38.54%), *Z*-β-ocimene (24.33%), α-thujene (4.90%), sabinene (3.84%), and *E*-β-ocimene (2.43%). Furthermore, Jaber et al. study indicated that the chemical compositions of plant parts, as well as their growing locations, exhibit significant quantitative and qualitative differences. The supercritical CO_2 extraction using solely CO_2 as a solvent was unsuccessful. The use of dichloromethane under identical extraction conditions (25 MPa and 50 °C) markedly enhanced the extraction yield to 4.84 wt%. The GC–MS analysis of the extract allowed the identification of 12 chemicals constituting 100% of the volatile components in the crude extract (Figure S3), with diterpene sclareol **7** (80.98%) (Figure S15), linalyl acetate **1** (17.40%), and neryl acetate (1.26%) being the major components. It was feasible to utilize silica gel flash chromatography with gradient elution (Hexane:DCM:EtOAc) to partition the viscous extract (300 mg) into five fractions (F1–F5). F1 (36 mg) was eluted using hexane:CH_2Cl_2 (8:2), whereas F2 (31 mg) was eluted using a 7:3 mixture of hexane:CH_2Cl_2. F3 (73 mg) was eluted using a solvent

Table 1 The GC–MS area percentage, retention time (RT), retention index (RI), and phytochemical composition of *Salvia dominica* L. essential oils and CO_2 extract

No	Name	RT	RI[a]	RI[b]	CO_2 extract	CO_2 F1	CO_2 F2	CO_2 F3	CO_2 F4	EO from dry leaves	EO from fresh leaves
					% Content						
1	Sabinene	11.79	975	975						0.11	0.10
2	Myrcene	12.61	993	992		2.27				0.39	0.12
3	Limonene	14.3	1032	1029		1.16				–	–
4	1,8-Cineole	14.42	1035	1035	0.16					4.08	7.07
5	*Z*-β-Ocimene	14.63	1040	1039		0.86				0.40	0.70
6	*E*-β-Ocimene	15.07	1050	1050		1.48				0.09	0.12
7	*cis*-Linalool oxide	16.06	1074	1075						1.18	0.37
8	*trans*-Linalool oxide	16.74	1090	1090		0.20				0.26	0.32
9	Linalool	17.28	1103	1102						1.17	9.40
10	Camphor	19.12	1150	1149						0.05	0.57
11	Ocimenol	20.06	1174	1174						0.04	0.24
12	Borneol	20.13	1176	1177						0.02	0.20
13	4-Terpineol	20.44	1184	1184						0.04	1.29
14	α-Terpineol	21.03	1199	1193						0.45	4.97
15	Linalool acetate	23.02	1254	1257	17.42	81.49				70.17	43.69
16	α-Terpinyl acetate	26.37	1352	1352	1.26	11.05				7.49	12.35
17	Neryl acetate	26.74	1362	1361		0.45				0.04	0.46
18	Geranyl acetate	27.38	1382	1381		1.05				0.22	1.06
19	β-Bourbonene	27.64	1389	1388	0.01					0.28	0.31
20	β-Elemene	27.78	1393	1390	0.02					0.18	0.21
21	α-Gurjunene	28.77	1424	1420	0.03		5.82			0.20	0.44
22	*α-trans*-Bergamotene	29.13	1437	1434	0.01		55.68			0.05	0.11
23	Germacrene D	30.75	1488	1482	0.07		1.08			10.20	10.22
24	δ-Selinene	30.94	1493	1492	0.00		9.10			0.02	0.12
25	Bicyclogermacrene	31.21	1503	1500	0.01		27.97			1.23	1.52
26	α-Springene	43.46	1969.1	–	0.03					1.55	2.23
27	Sclareol	49.87	2298	2346	80.98			100	100	0.09	1.80
	Total				99.99	100.0	99.65	100	100	100	99.99
Monoterpene hydrocarbons (MH)					0	5.76	5.76	0.00	0.00	0.98	1.04
Oxygenated monoterpenes (OM)					18.69	94.04		0.00	0.00	80.87	74.60
Sesquiterpene hydrocarbons (SH)					0.15	0.00	99.65	0.00	0.00	12.16	12.93
Oxygenated diterpenes (OD)					81.17	0.00	0.20	100.00	100.00	5.99	11.43
Others						0.20	0.00	0.00	0.00	5.99	11.43

[a] RI represents the retention index calculated in relation to a homologous series of *n*-alkanes on a DB-5MS column

[b] RI Retention indices in NIST webpage [24]

mixture of CH_2Cl_2 and EtOAc at an 8:1 ratio. F4 (68 mg) was eluted using a solvent mixture of CH_2Cl_2 and EtOAc in a ratio of 7:3, whereas F5 (17 mg) utilized a ratio of 6:4 for the same solvents. GC–MS analysis of F1 identified nine chemicals, with linalyl acetate **1** (81.49%) and α-terpinyl acetate **2** (11.05%) being the predominant constituents. Germacrene D 3 (55.68%), α-springene (27.17%), bicyclogermacrene (9.10%), and aromadendrene (5.82%) were discovered as the predominant products among the nine chemicals identified in F2. GC–MS analysis showed F3 and F4 only sclareol as the only compound; HPLC revealed that F3 contains more than one compound.

Evaluation of the cytotoxicity effect

The cytotoxicity of the EOs from fresh and dried leaves of *Salvia dominica* L., along with the CO_2:CH_2Cl_2 extract and its four fractions (F1–F4), was assessed on HeLa and MCF7 cell lines. All evaluated materials exhibited a concentration-dependent anticancer effect on both cell lines (Table 2), with fractions F3 and F4 demonstrating the most significant cytotoxic activity against the MCF7 prostate cancer and the HeLa cervical cancer cell lines, presenting IC_{50} values between 25.41 ± 1.27 and 40.94 ± 2.05 µg/mL. Fraction F4 exhibited the most effective anticancer activity against MCF7 and Hela cell lines, with IC_{50} values of 25.41 ± 1.27 and 33.60 ± 1.68 µg/mL, respectively. The dark CO_2–CH_2Cl_2 viscous oil showed significant anticancer efficacy against MCF-7 and HeLa cancer cell lines, with IC_{50} values of 45.14 ± 2.26 and 73.00 ± 3.66 µg/mL, respectively. The significant cytotoxicity of CO_2 extract and fractions F3 and F4 could be attributed to the elevated levels of sclareol, a diterpene alcohol derived from *Salvia sclarea*. Literature review revealed that sclareol possesses significant cytotoxicity against MCF-7 and Hela cell lines, with IC_{50} values of 31.11 µM and 20 µM, respectively [27].

The anticancer efficacy of EOs derived from fresh and dried *Salvia dominica* L. leaves against HeLa cell lines was inferior to that of the CO_2 extract, exhibiting IC_{50} values of 92.03 ± 4.60 and 94.97 ± 4.75 µg/mL, respectively. However, against MCF-7 cell lines, both fresh and dried *Salvia dominica* L. exhibited restricted anticancer efficacy, with IC_{50} values of 100.94 ± 5.05 and 100.88 ± 5.04 µg/mL, respectively.

Numerous *Salvia* species have been investigated for their anticancer properties; however, to the best of our knowledge, there are limited reports available in the literature regarding *Salvia dominica* L. In Jordan, Abu-Dahab and Afifi investigated the anticancer efficacy of chloroform, aqueous, and ethanolic extracts of *Salvia dominica* L. against breast cancer cell lines (MCF-7) over a 72-h exposure. The ethanolic extract exhibited the highest potency, with a calculated IC_{50} value of 7.28 ± 1.150 (µg/mL), followed by the chloroform and aqueous extracts, which had IC_{50} values of 47.43 ± 4.57 and 100.96 ± 8.30 (µg/mL), respectively [18].

The literature reviews on *Salvia* species indicate that extracts from these plants may serve as natural anticancer agents, attributed to their flavonoid and terpenoid content. Durgha et al. [28] demonstrated that *S. sclarea* EO inhibited the proliferation of Hela cells through apoptosis, exhibiting an IC_{50} value of 80.69 µg/mL. This finding aligns with our results for both EOs and CO_2 extract. Russo et al. from Lebanon investigated the EOs of three *Salvia* species: *S. aurea*, *S. judaica*, and *S. viscosa*, for their potential inhibitory effects on the growth of M14, A2058, and A375 human melanoma cells [28]. The IC_{50} values ranged from 11.6 to 23.6 µg/mL across the three species and three cell types, demonstrating significant inhibitory activity. The research indicated that the anticancer properties of *Salvia* species are linked to the presence of terpenoids and diterpenoids with diverse structures [29]. The research on *S. lavandulifolia* EO, which is rich in camphor, demonstrated limited anticancer efficacy against human cervix carcinoma (HeLa) and lung adenocarcinoma (A549) cells, with an IC_{50} value of 131.50 µg/mL observed for human fetal lung fibroblast cells (MRC-5) [30]. According to a different study, after 48 h of incubation, EO extracted from *S. officinalis* L. grown in Sicily, Italy, had in vitro antiproliferative activities at concentrations between 100 and 200 µg/mL, dramatically lowering cell viability in MCF7, LNCaP, and HeLa cell lines [30].

Referring to the literature: Eucalyptol, linalool, α-gurjunene, germacrene D, β-elemene, α-terpineol, and sclareol are all documented to have anticancer activity, and all the mentioned compounds are present in both EOs in a notable percent, which could explain the strong anticancer activity of all extracts. The variation in results could be due to the variation in percent content and the synergetic effect of other components [30–37].

Evaluation of the antioxidant, anti-lipase, and anti-α-amylase activities

The antioxidant activity was assessed using the DPPH assay, a rapid, simple, sensitive, and widely used method [37]. Figure S16 demonstrates that the FLEO exhibited significant antioxidant capacity, indicated by an IC_{50} value of 45.52 ± 2.28 µg/mL. The DLEO exhibited marginally reduced antioxidant activity, with an IC_{50} value of 58.19 ± 2.91 µg/mL. Multiple studies demonstrate that *Salvia* EOs possess antioxidant properties; however, the IC_{50} value for *Salvia dominica* L. has yet to be established. A study conducted in Tunisia evaluated the antioxidant activity of *S. officinalis* EO, determining its

IC_{50} to be 8.31 ± 0.55 mg/L [38]. The significant activity observed can be ascribed to the predominance of 1,8-cineole **5**, along with the presence of terpinen-4-ol and linalool **4**, which are acknowledged as potent free radical scavengers [39]. Iravani et al. showed that the EOs of *S. ceratophylla* and *S. limbate*, which were collected in Iran, have strong antioxidant effects, with IC_{50} values of 30.08 µg/mL for *S. ceratophylla* and 80.08 µg/mL for *S. limbate* [40]. The notable antioxidant activity of the oil extracts may be attributed to the identification of eucalyptol 5 (1,8-cineole) and linalool **4** [41, 42]. The recent studies demonstrated a correlation between the antioxidant activity of EOs and their chemical composition. It is crucial to examine the synergistic effects of both major and minor constituents in the oils to comprehensively understand their biological activity [43].

The inhibitory effects of both EOs on lipase and α-amylase were assessed, as shown in Figure S17. Fresh and dry EOs demonstrated limited lipase inhibition activity, with IC_{50} values surpassing 2000 µg/mL for the FLEO and 891 µg/mL for the DLEO. Literature on the lipase inhibition activity of EOs from *Salvia* species is scarce. A study conducted by an Iranian group examined the anti-obesity effects of *S. Officinalis* L extracts utilizing various solvents. The MeOH extract exhibited the most significant inhibition of lipase activity, with an IC_{50} of 0.32 mg/mL. This was followed by the 70% MeOH extract (IC_{50} = 0.36 mg/mL), the 70% EtOH extract (IC_{50} = 0.39 mg/mL), and finally the EtOH extract (IC_{50} = 0.42 mg/mL). The study demonstrated that the inhibition of lipase activity increased with higher concentrations of crude extracts [44].

Both EOs demonstrated slightly greater inhibition of α-amylase as compared to lipase inhibition; however, both oils are considered weak inhibitors. DLEO exhibited greater α-amylase inhibition, with an IC_{50} of 561 ± 28.05 µg/mL, as compared to FLEO, which showed an IC_{50} value of 875.75 ± 43.79 µg/mL (Figure S18). Our findings are in agreement with the results of the study of the α-amylase inhibitory activities of six *Salvia* species [45]. Nikavar reported that IC_{50} values were 18.34 mg/mL for *S. verticillate* and 19.73 mg/mL for *S. virgata* and 1.19 ± 0.45–25.01 ± 1.68% for *S. reuterana* extract, while the inhibitory activity of *S. reuterana* on α-amylase was very weak. Surprisingly, two negative inhibition values were observed for *S. hydrangea* (−13.42 ± 1.20%) and *S. officinalis* (3.91 ± 1.29%) which could indicate the activation of α-amylase rather than being inhibited [45]. α-Terpinyl acetate is recognized as an inhibitor of α-amylase and lipase [46]. Both EOs constitute 12.35% of the essential oil extracted from the fresh species and 7.49% from the dried species. The observed values prompted an investigation into the inhibitory activity against α-amylase. Both EO extracts, derived from dry and fresh species, exhibited minimal inhibitory activity.

Evaluation of the antibacterial activity

The in vitro antibacterial efficacy of the EOs and the CO_2–CH_2Cl_2 extract, together with its fractions F1–F4, was assessed utilizing the disk diffusion method [47]. The EOs examined exhibited potential antibacterial action against some bacterial isolates (Table 3). The results indicated that both EOs were more effective against Gram-positive bacteria (*S. aureus* and *S. epidermidis*) than against Gram-negative bacteria (*E. coli* and *K. pneumoniae*). *S. epidermidis* was evidently the most susceptible isolate. Both EOs showed lower efficacy than the commonly used antibiotic gentamicin. Furthermore, FLEO showed greater efficacy against the Gram-positive bacteria *S. aureus* (14 mm) and *S. epidermidis* (16 mm). The CO_2 extract was the most effective against *S. aureus* (18 mm), while the studied fractions were more efficient against Gram-positive bacteria, specifically fractions F3 (28 mm) and F4 (24 mm), as both fractions were more powerful than gentamicin (22 mm) in their action against *S. aureus*.

The two EOs, as well as the CO_2 extract and its fractions (F1–F4) analyzed, were subsequently evaluated for their minimum inhibitory concentration (MIC) (Tables 3, 4). The results demonstrated that the inhibitory effects of FLEO and DLEO ranged from 3.125 to 12.5 µL/mL and 0.39 to 12.5 µL/mL, respectively. *S. aureus* and *K. pneumoniae* had the highest sensitivity to FLEO, being inhibited by 3.125 µL/mL of it. Furthermore, DLEO suppressed *K. pneumoniae* at a concentration of 0.39 µL/mL. The minimum bactericidal concentrations (MBC) indicated that the bactericidal efficacy of FLEO and DLEO ranged from 3.125 to 12.5 µL/mL and 3.125 to 25 µL/mL, respectively. *S. aureus* was eradicated by 3.125 µL/mL of FLEO, while *K. pneumoniae* was eliminated by 3.125 µL/mL of DLEO.

CO_2 extract (3.125–12.5 µg/mL) as well as fractions 3 (MIC = 12.5–25 µg/mL) and 4 (MIC = 6.25–25 µg/mL) exhibited superior bacteriostatic activity as compared to the positive control (gentamicin) against all tested bacteria, particularly showing substantial inhibition of Gram-positive pathogens. Moreover, CO_2 extract (MIC = 3.125–12.5 µg/mL) as well as fraction F3 (MIC = 12.5–50 µg/mL) showed the highest bactericidal efficacy against the tested pathogens, demonstrating particularly notable effectiveness against *S. aureus*.

The literature review indicated that the antibacterial activity of *Salvia dominica* L. EO has been assessed solely against *L. monocytogenes* [48]. Bozzini et al. reported that the EO of *Salvia dominica* L., extracted via hydrodistillation from leaves cultivated in the

Table 2 IC_{50} values (µg/mL) for cytotoxicity of *Salvia dominica* L. CO_2–CH_2Cl_2 extract, fraction 1–4 essential oils, and 5-fluorouracil against HeLa and MCF-7 cells

Name of sample	IC_{50} value (µg/mL) MCF-7	IC_{50} value (µg/mL) Hela
CO_2–CH_2Cl_2 extract of dried flowers	73 ± 3.65	45.14 ± 2.26
Fraction F1 of the CO_2–CH_2Cl_2 extract	389.19 ± 19.45	453.705 ± 22.69
Fraction F2 of the CO_2–CH_2Cl_2 extract	377.42 ± 18.87	338.92 ± 16.95
Fraction F3 of the CO_2–CH_2Cl_2 extract	40.94 ± 2.05	36.65 ± 1.83
Fraction F4 of the CO_2–CH_2Cl_2 extract	25.41 ± 1.27	33.6 ± 1.68
EO of fresh leaves (FLEO)	100.94 ± 5.05	92.03 ± 4.60
EO of dried leaves (DLEO)	100.88 ± 5.04	94.97 ± 4.75
5-Fluorouracil (positive control)	1.29 ± 0.45	2.16 ± 1.01

Botanic Garden and Museum of the University of Pisa, exhibited moderate inhibitory activity, with MIC and MBC values of 12.31 and 24.61 mg/mL, respectively [49]. The antimicrobial properties of EOs from various *Salvia* species have been extensively evaluated. EO of *Salvia officinalis*, with α-thujone, 1,8-cineole, camphor, borneol, and β-pinene being the predominant compounds, displayed moderate antibacterial activity against *E. coli*, *S. aureus*, and *S. epidermidis*, with MIC values ranging from 5 to 10 mg/mL [50]. Delamare indicated in the same investigation that the EO of *S. triloba*, comprising α-thujone, 1,8-cineole, camphor, and β-caryophyllene as predominant constituents, had significant antibacterial efficacy against *S. aureus* and *S. epidermidis*, with MIC values of 0.2 and 1 mg/mL, respectively, and modest activity against *E. coli*, with MIC values of 5–10 mg/mL [13]. Maache et al. discovered that EOs derived from *S. officinalis* leaves, predominantly comprising camphor (26%), β-thujone (17.14%), and 1,8-cineole (16.96%), exhibited antibacterial efficacy against *S. aureus*, with MIC and MBC values of 18.75 and 37.5 mg/mL, respectively [49]. Boutebouhart et al. showed that the EO of *S. officinalis* exhibits notable inhibitory effects, measuring 17 ± 0.55 mm against *S. aureus* and 9 ± 0.33 mm against *E. coli*, among others [13]. The EO of *S. lavandulifolia*, comprising camphor (39.24%), 1,8-cineole (22.01%), and camphene (9.71%) as primary constituents, exhibited notable antibacterial efficacy against *S. aureus*, with MIC and MBC values of 3.75 and 7.50 mg/mL, respectively [49]. The efficacy of these EOs differed among various species, presumably due to their distinct chemical makeup. Overall, higher concentrations of oxygenated sesquiterpenes appeared to correlate with enhanced antibacterial properties. Eucalyptol and linalool possess antibacterial properties [48, 51], which account for the significant activity of the essential oil in a study of Bozzini et al. [52]. The oil extract of other types of *Salvia*, such as *Salvia libanotica* (Lebanese sage), was shown to possess strong antimicrobial and antitumor effects due to the presence of α-terpineol and linalyl acetate [53].

Conclusion

Twenty-six compounds were identified in the essential oils extracted from the steam distillation of both fresh and air-dried *Salvia dominica* L. leaves. Twelve compounds were identified in the CO_2–CH_2Cl_2 extract, with sclareol constituting 80.98% of the total extract composition. The primary compounds identified in the EO of fresh *Salvia dominica* L. leaves included linalool acetate and α-terpinyl acetate, along with germacrene D, linalool, and 1,8-cineole. In contrast, the EO extracted from air-dried *Salvia dominica* L. leaves predominantly contained linalool acetate, germacrene D, α-terpinyl acetate, and 1,8-cineole. The antioxidant activity assays indicated that

Table 3 Antibacterial efficacy of FLEO and DLEO (µL/mL) alongside the antibiotic gentamicin (µg/mL) employing the micro-broth dilution technique

	Fresh		Dry		Gentamicin	
	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	12.5	12.5	12.5	12.5	62.5	125
K. pneumoniae	3.125	6.25	0.39	3.125	31.25	62.5
S. aureus	3.125	3.125	3.125	6.25	31.25	62.5
S. epidermidis	12.5	12.5	6.25	25	125	250

MIC minimum inhibitory concentration, *MBC* minimum bactericidal concentration

Table 4 Antibacterial activity of the CO_2 extract and its fractions (F1–F4) (μg/mL) utilizing the micro-broth dilution method

	CO_2 extract		F1		F2		F3		F4	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	12.5	12.5	50	100	25	50	25	50	50	100
K. pneumoniae	6.25	12.5	50	100	25	50	12.5	25	50	50
S. aureus	3.125	6.25	50	100	12.5	25	6.25	12.5	12.5	25
S. epidermidis	12.5	625	50	100	12.5	25	12.5	25	50	50

both EOs displayed inadequate antioxidant effects when compared with the Trolox standard, along with limited α-amylase and lipase inhibitory activity. Conversely, both essential oils demonstrated significant anticancer activity against Hela and MCF-7 cancer cell lines, whereas the CO_2–CH_2Cl_2 extract, and its polar fractions (F3 and F4) showed notable anticancer effects attributed to the presence of sclareol. In comparison to essential oils, CO_2 extract and its components F3 and F4 demonstrated significant efficacy in eradicating all tested strains, surpassing the effectiveness of gentamicin, particularly against *K. pneumoniae* and *S. aureus*, which exhibited the greatest vulnerability. The limited solubility of essential oils in water is a considerable constraint, leading to a diminished concentration of these hydrophobic substances in the aqueous phase. Our findings suggest that CO_2 extract and its polar fractions may behave as potential antibacterial and anticancer agents; therefore, further in vivo studies and analysis of the mechanism of action are necessary.

Supplementary Information

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Additional file 1.

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Declarations

Competing interests
The authors declare no competing interests.

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