

Original article

Camphor–Thujone Chemotype and Bioactivities of *Artemisia herba-alba* Asso Essential Oil from Zintan, Libya: Chemical Composition, Antibacterial, and Antiproliferative Effects

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Abstract

Artemisia herba-alba Asso is a medicinal plant renowned for its therapeutic essential oil. This study characterized the chemical profile and bioactivities of the essential oil from Libyan *A. herba-alba*. The essential oil, obtained via hydrodistillation with a 2% yield, was analyzed by GC-MS/FID and evaluated for antibacterial (disc diffusion) and anticancer (MTT assay) properties. The oil was dominated by camphor (30.53%) and Thujone (22.47%), classifying it as a camphor-thujone chemotype. It exhibited remarkable antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA; 17.33 ± 1.52 mm), and demonstrated potent, dose-dependent cytotoxicity against MCF-7 breast cancer cells with an IC₅₀ of 0.59 mg/mL. The results validate the traditional use of this plant and highlight its potential as a source of antimicrobial and anticancer agents.

Keywords: *Artemisia herba-alba*, Essential Oil, GC-MS, Antimicrobial Activity, Cytotoxicity.

Introduction

Artemisia herba-alba Asso (white wormwood) is a medicinal plant widely used in North Africa and the Middle East to treat gastrointestinal disorders, diabetes, hypertension, and infections [1,2]. Its therapeutic properties are mainly due to its essential oil (EO), which exhibits notable chemotypic diversity influenced by geography, climate, harvest time, and genetics [3,4]. Predominant chemotypes include camphor/thujone, 1,8-cineole, and α/β-thujone, each with distinct biological activities.

The urgency of discovering new antimicrobial and anticancer agents has renewed interest in plant EOs, especially against multidrug-resistant bacteria like MRSA and ESBL-producing *Klebsiella* species [5]. The MCF-7 breast cancer cell line is commonly used to screen the antiproliferative potential of new compounds [6]. Although EOs from Algeria, Morocco, and Jordan have been studied [7,8], data on Libyan specimens are limited. This study aimed to characterize the chemical composition of Zintan *A. herba-alba* EO using GC-MS/FID. Assess its antibacterial activity against clinically relevant resistant bacteria using the disc diffusion method. Evaluate its direct, solvent-free cytotoxicity and antiproliferative effects on MCF-7 cells, including IC₅₀ determination. This work provides data on the chemotype and bioactivity of Libyan *A. herba-alba*, supporting its traditional use and potential as a source of novel therapeutic agents.

Methods

Plant Collection

Aerial parts of *Artemisia herba-alba* were collected from the awled Belhol area in Zintan, Libya (coordinates: 31°56'45.8"N, 12°14'37.7"E) during October 2024. The plant was identified botanically by Dr. Ali Afilaly, Department of Botany, Faculty of Science, University of Zintan. The collected plant material was washed, shade-dried at room temperature for two weeks, and the leaves were separated and stored in airtight containers until extraction.

Essential Oil Extraction

The essential oil was extracted from the dried leaves using hydro-distillation for 3 hours in a Clevenger apparatus, with 50 g of plant material in 500 ml of distilled water at 60°C [9]. The obtained oil was separated, and its volume was measured to calculate the percentage yield. The oil was stored in sterile, opaque glass vials at 4°C until use.

Chemical Profiling of Essential Oils via GC-MS/FID

The chemical composition of the essential oils (EOs) was analyzed using gas chromatography coupled with mass spectrometry and flame ionization detection (GC-MS/FID), following well-documented analytical procedures [10], [11]. Each EO sample was diluted in absolute ethanol at a ratio of 1:99 (v/v), and 1 µL of the solution was introduced in splitless mode into an Agilent 8890/5977BMSD/FID system (Agilent Technologies, Santa Clara, CA, USA). Compound separation was carried out on an HP-INNOWax

polyethylene glycol capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). The injector was maintained at 250 °C, and high-purity helium (99.9995%) served as the carrier gas with a constant flow rate of 0.8 mL/min. The oven temperature began at 65 °C (held for 2 minutes), then increased to 220 °C at 3 °C/min, and was held at the final temperature for 15 minutes. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV, with the ion source and transfer line temperatures set to 150 °C and 250 °C, respectively.

To determine the Kovats Retention Index (RI) for each compound, a homologous series of n-alkanes (C₁₀–C₄₀; Sigma-Aldrich, St. Louis, MO, USA) was analyzed under identical conditions. Identification of constituents was based on comparing both mass spectral data and calculated RIs with reference standards, the Adams essential oil database [10], the NIST MS Search 2.4 library, and other relevant scientific sources. Relative abundance of each component was determined from FID peak areas, using MSD ChemStation software (F.01.03.2357, Agilent Technologies), with the FID detector maintained at 300 °C. Results are presented as relative percentages of the total chromatographic area.

Isolation and Identification of Bacteria

Clinical bacterial isolates were obtained from patient samples (using cotton swabs) at the National Research Center for Tropical and Transboundary Diseases (NRCTTD) in Zintan. Samples were collected from November 2024 to January 2025 from patients with tonsillitis, urinary tract infections, and dermatitis. The samples were cultured on Nutrient Agar, Mannitol Salt Agar (MSA), MacConkey Agar, and Blood Agar, and incubated at 37°C for 24 hours [12]. Initial identification was based on colony morphology, hemolytic patterns on Blood Agar, and reactions on selective media, such as MSA. This was followed by Gram staining [13] and biochemical tests, including catalase [14] and coagulase tests [15]. Final, conclusive identification of the bacterial species was performed using the automated MA 120 identification system (Render, China), following the manufacturer's operational protocol [16]. The isolates used in this study were confirmed as *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca*.

Antimicrobial Activity Assay

The antibacterial activity of the *A. herba-alba* essential oil was evaluated using the standard disc diffusion method [17]. Bacterial suspensions were prepared from fresh 24-hour cultures and adjusted to a 0.5 McFarland standard. A 100 µL aliquot of each suspension was spread uniformly onto Mueller-Hinton Agar (MHA) plates. Sterile filter paper discs (6 mm diameter) were impregnated with 10 µL of the pure essential oil and placed on the inoculated agar. A disc moistened with sterile distilled water served as a negative control. The plates were allowed to diffuse at room temperature for 15 minutes and then incubated at 37°C for 24 hours. The antibacterial activity was measured as the diameter of the zones of inhibition (IZD) in millimeters.

Cell Culture and Antiproliferative Assay

The MCF-7 human breast cancer cell line was acquired from the American Type Culture Collection (ATCC, USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco) enriched with 10% fetal bovine serum (FBS, Gibco) and 1% penicillin/streptomycin (Gibco) and incubated at 37 °C in a humidified 5% CO₂ atmosphere. Subculturing was performed every 2–3 days upon reaching approximately 80% of confluence.

Antiproliferative activity was assessed using a slightly modified version of the MTT assay originally developed by Mosmann [18]. At 80% confluence, cells were rinsed twice with phosphate-buffered saline (PBS) and detached using trypsin-EDTA (Gibco). Viable cells were counted with an automated cell counter (Invitrogen Countess) and plated in 96-well plates at a density of 2 × 10⁵ cells/mL in complete medium. After a 24-hour incubation to allow cell attachment, the medium was replaced with fresh medium containing varying concentrations of the essential oil. The oil was diluted directly into the culture medium to final concentrations of 1, 2, 4, and 10 µL/mL, corresponding to 0.853, 1.706, 3.412, and 8.53 mg/mL, assuming an oil density of 0.853 g/mL. This concentration range was selected based on preliminary experiments to capture the full dose-response curve, from minimal to near-complete inhibition. A negative control well contained only medium without oil. Cells were then incubated for an additional 48 hours.

Following treatment, 10 µL of MTT solution (5 mg/mL in PBS; Dojindo) was added to each well, and plates were incubated overnight at 37 °C. The formazan crystals formed were dissolved by adding 100 µL of 10% sodium dodecyl sulfate (SDS; Sigma-Aldrich) to each well, followed by a 24-hour incubation at 37 °C. Absorbance was measured at 570 nm using a microplate reader (Varioskan Flash, Thermo Scientific).

The percentage of growth inhibition was calculated as follows:

$$\% \text{ Inhibition} = [(OD \text{ control} - OD \text{ sample}) / OD \text{ control}] \times 100$$

Where OD control represents the optical density of untreated cells, and OD sample corresponds to the optical density of cells exposed to the essential oil.

Determination of Half-Maximal Inhibitory Concentration (IC₅₀)

The antiproliferative activity of *A. herba-alba* essential oil against the MCF-7 cell line was quantified by determining the half-maximal inhibitory concentration (IC₅₀). The dose-response data, presented as the mean percentage of inhibition at various essential oil concentrations, were subjected to log-linear regression analysis to calculate the IC₅₀ value. Briefly, the mean inhibition values (%) were plotted against the natural logarithm of the corresponding concentrations (mg/mL). A linear regression model was fitted to the data, resulting in an equation of the form:

$$\text{Inhibition (\%)} = a \times \ln(\text{Concentration}) + b$$

Where:

a is the slope of the regression line, representing the change in inhibition per unit change in $\ln(\text{concentration})$.

b is the y-intercept, representing the theoretical inhibition at $\ln(\text{concentration}) = 0$.

The IC₅₀ value, defined as the concentration that results in 50% inhibition of cell viability, was calculated by solving the regression equation for an inhibition value of 50%. This method of IC₅₀ determination, based on regression analysis of concentration–response data, is consistent with established pharmacological practices [19].

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS version 20). A one-way analysis of variance (ANOVA) was performed, followed by the Least Significant Difference (LSD) post-hoc test. Results are expressed as mean $\pm$ standard deviation (SD), and a p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

Results and discussion

This study provides a comprehensive analysis of the chemical composition, antibacterial efficacy, and cytotoxic potential of *Artemisia herba-alba* essential oil (EO) from Zintan, Libya. The hydrodistillation process yielded 2% essential oil, a value consistent with known ranges for this species, although influenced by geographical and environmental factors [20,21]. The most significant findings were the identification of a high-potency camphor–thujone chemotype, the oil's notable antibacterial activity against clinically relevant resistant strains, and its direct, solvent-free cytotoxic effects on MCF-7 breast cancer cells.

Extraction Yield and Essential Oil Properties

The hydrodistillation of the dried leaves of *A. herba-alba* yielded 2% (v/w) of essential oil. (Table 1). The obtained essential oil was characterized as a light-yellow liquid with a pH of 5.00.

Table 1. Extract yield from *A. herba-alba* leaves

Plant Material	Essential Oil Yield (%)
<i>A. herba-alba</i>	2.00

Chemical Composition of the Essential Oil

The chemical profile of the *A. herba-alba* essential oil, as determined by GC-MS analysis, is presented in (Table 2). A total of 28 compounds were identified, representing 98.72% of the total oil composition. The oil was overwhelmingly dominated by oxygenated monoterpenes (67.01%), with camphor (30.53%) and Thujone (22.47%) as the two most abundant constituents. This profile firmly classifies the oil as a camphor–Thujone chemotype. Other significant compounds included sabinene (10.29%), camphene (7.32%), and β -pinene (4.79%). Notably, several species-specific markers were identified, such as *Artemisia* ketone (1.06%) and *Santolina* triene (2.25%), reinforcing the botanical origin and chemotaxonomic identity of the sample. Monoterpene hydrocarbons constituted the second major class (27.83%), while sesquiterpenes and diterpenes were present in minor amounts.

The GC–MS analysis revealed a chemoprofile dominated by camphor (30.53%) and thujone (22.47%), classifying our Libyan *A. herba-alba* sample firmly within the camphor–thujone chemotype. This finding exhibits both strong consistencies and significant quantitative distinctions when compared to other regional studies. The high camphor content is a common feature in North African *A. herba-alba*, as seen in samples from Bejaia, Algeria [22]. However, the specific ratio and high combined concentration of camphor and thujone in our sample are distinctive.

This chemotype aligns with the well-established profile for the species in North Africa but presents a notable contrast to the only other chemical analysis of *A. herba-alba* from Zintan, Libya. Janackovic et al. [23] reported a chrysanthenone/chrysanthenyl acetate chemotype from the same region, with thujone isomers totaling 17.6% and a much lower camphor content (1.8%). The stark difference between our findings and this prior study—from the same geographical location—strongly underscores the profound intraspecific

chemical diversity in *A. herba-alba*, which can be driven by microclimatic conditions, soil composition, plant phenology, and genetic variation [3], [24], [25].

When compared to other regional variants, our Libyan oil also differs from the EO from Jericho, Palestine [26], which was dominated by 1,8-cineole and cis-thujone, and from some Algerian oils characterized by an even higher thujone content [27]. The presence of other characteristic compounds, such as Artemisia ketone and Santolina triene in our oil, further confirms its botanical authenticity and aligns with the typical profile of the genus [4].

Table 2. Chemical composition of *A. herba-alba* essential oil from Zintan, Libya, as determined by GC-MS

Compound	Molecular formula	Retention time (RT)	Composition (%)
α -Thujene	C10H16	5.597	0.31
Sabinene	C10H16	6.809	10.29
α -Pinene	C10H16	7.460	2.93
Camphene	C10H16	7.836	7.32
β -Pinene	C10H16	8.418	4.79
α -Terpinene	C10H16	9.452	0.40
p-Cymene	C10H14	9.641	0.95
Limonene	C10H16	9.698	0.23
β -Phellandrene	C10H16	9.755	0.46
1,8-Cineole (Eucalyptol)	C10H18O	9.828	3.05
Artemisia ketone	C10H16O	10.475	1.06
Santolina triene	C10H16	10.694	2.25
β -Thujone	C10H16O	11.194	0.22
Chrysanthenone	C10H14O	11.485	1.43
Camphor	C10H16O	11.631	0.27
Camphor	C10H16O	11.964	30.53
Borneol	C10H18O	12.053	0.27
Thujone	C10H14O	12.644	22.47
trans-Pinocarveol	C10H16O	12.835	1.03
Verbenone	C10H14O	12.998	0.40
trans-Carveol	C10H16O	13.070	0.63
Carvone	C10H14O	13.329	4.24
Piperitone	C10H16O	13.452	0.17
Myrtenyl acetate	C12H18O2	13.593	0.69
Carvacrol	C10H14O	14.418	0.95
α -Terpinyl acetate	C12H20O2	15.311	0.49
α -Copaene	C15H24	15.692	0.45
Germacrene D	C15H24	18.012	0.50
δ -Cadinene	C15H24	19.055	0.15
Phytol	C20H40O	28.280	0.48
Total identified compounds			98.72
Main class %			
Monoterpene hydrocarbons			27.83%
Oxygenated monoterpenes			67.01%
Sesquiterpene hydrocarbons			1.10%
Oxygenated sesquiterpenes			0.48%
Diterpenes			0.48%
Number of compounds			28

Antibacterial Activity

The essential oil of *A. herba-alba* exhibited notable in vitro antibacterial activity against all tested clinical isolates, as measured by the disc diffusion assay (Table 3). The oil was most effective against *Staphylococcus aureus* (MRSA), with an inhibition zone diameter of 17.33 ± 1.52 mm. This was followed by activity against *Staphylococcus epidermidis* (16.00 ± 1.73 mm). The Gram-negative bacteria, *Klebsiella pneumoniae* and *Klebsiella oxytoca*, showed lower susceptibility, with inhibition zones of 14.66 ± 0.57 mm and 14.00 ± 0.00 mm, respectively. The observed antibacterial activity can be attributed to the synergistic action of the major oxygenated monoterpenes, primarily camphor and thujone, both of which are known for their ability to disrupt microbial cell membranes and increase permeability [28]. Thujone, in particular, is a well-documented antimicrobial agent, and its high concentration likely plays a critical role in the observed

bioactivity. The oil showed the highest activity against methicillin-resistant *Staphylococcus aureus* (MRSA) with an inhibition zone diameter of 17.33 ± 1.52 mm. This result is comparable to the findings of Bouhouia et al. [7], who also reported significant activity against *S. aureus*. The oil also inhibited *Staphylococcus epidermidis* (16.00 ± 1.73 mm), confirming the general susceptibility of Gram-positive bacteria to this monoterpene-rich essential oil.

For Gram-negative strains, the oil produced more moderate inhibition against *Klebsiella pneumoniae* (14.66 ± 0.57 mm) and *Klebsiella oxytoca* (14.00 ± 0.00 mm). This aligns with the known greater intrinsic resistance of Gram-negative bacteria, which possess a complex outer membrane that acts as a barrier to hydrophobic compounds [29]. The potent activity against MRSA is of particular therapeutic interest and is likely a function of the combined effect of camphor, thujone, and other minor constituents like carvacrol, which can fluidize the bacterial membrane and lead to cell death.

Table 3. Antibacterial activity of *A. herba-alba* essential oil expressed as inhibition zone diameter (IZD, Mean $\pm$ SD, mm)

Bacterial Isolate	IZD (mm) Mean $\pm$ SD
<i>K. pneumoniae</i>	14.66 ± 0.57
<i>K. oxytoca</i>	14.00 ± 0.00
<i>S. aureus</i> (MRSA)	17.33 ± 1.52
<i>S. epidermidis</i>	16.00 ± 1.73

Antiproliferative Activity against MCF-7 Cells

The essential oil demonstrated a potent and dose-dependent inhibitory effect on the viability and proliferation of human breast cancer cells (MCF-7) (Table 4 and Figure 1). At the lowest concentration tested (0.853 mg/mL), the oil inhibited cell growth by 57.37%, corresponding to a reduction in viability to 42.63% of the control. This antiproliferative effect increased with concentration, reaching a maximum inhibition of 84.78% (viability of 15.22%) at the highest concentration of 8.53 mg/mL. The calculated IC_{50} value shown in (Figure 1), derived from the log-linear regression analysis of the dose-response data, was 0.59 mg/mL, indicating strong cytotoxic potential. The regression analysis yielded the equation:

$$\text{Inhibition (\%)} = 12.91 \times \ln(\text{Concentration}) + 56.81$$

Accordingly, the IC_{50} was obtained from the following expression:

$50 = 12.91 \times \ln(IC_{50}) + 56.81$, which simplifies to $\ln(IC_{50}) = -0.5276$ and $IC_{50} = e^{-0.5276} = 0.59$ mg/mL. This result confirms the potent dose-dependent cytotoxic activity of *A. herba-alba* essential oil against the MCF-7 cell line.

A key finding of this study is the potent, dose-dependent cytotoxicity of the Libyan *A. herba-alba* essential oil (EO) against MCF-7 breast cancer cells, with an IC_{50} value of 0.59 mg/mL. It is important to note that in our anticancer assays, the essential oil was applied directly to the culture medium without the use of any solvent such as DMSO, representing a *direct, solvent-free cytotoxicity assay*. This approach eliminates solvent-induced enhancement of solubility and cellular uptake, thereby providing a more physiologically relevant assessment of the oil's intrinsic bioactivity [30,31]. Although solvent-free conditions may yield slightly higher IC_{50} values, they more accurately reflect the oil's native behavior under biological conditions. For instance, Bsharat et al. [26] reported a substantially lower IC_{50} for their Jericho EO, which was dissolved in DMSO, underscoring how the use of organic solvents can artificially increase apparent potency.

Despite this methodological difference, the direct cytotoxic activity of our solvent-free EO remains significant. The observed bioactivity can be strongly linked to the high concentrations of both camphor and thujone. Camphor is well known for its cytotoxic and pro-apoptotic properties [32,33], while thujone has also been implicated in inducing apoptosis and disrupting mitochondrial integrity in various biological models. Furthermore, the multi-targeted mechanistic insights provided by Bou Malhab et al. [31] are relevant; they demonstrated that *A. herba-alba* extracts induce apoptosis, cause cell cycle arrest, and inhibit the PI3K/AKT/mTOR pathway in cancer cells. This provides a plausible framework for the bioactivity of our camphor-thujone chemotype, where these two major components may work synergistically to exert cytotoxic effects, even in the absence of a solubilizing agent.

The cytotoxic and antibacterial activities observed in this study can thus be mechanistically attributed to the combined action of camphor and thujone, the two dominant oxygenated monoterpenes in the oil. Both compounds are known to disrupt microbial and cancer cell membranes through their lipophilic nature, which allows them to penetrate lipid bilayers, increasing membrane permeability, causing leakage of intracellular contents, and loss of membrane potential [28]. In cancer cells, camphor induces mitochondria-mediated apoptosis via reactive oxygen species (ROS) generation, leading to oxidative stress, mitochondrial membrane depolarization, and activation of the caspase cascade [34]. Similarly, thujone exhibits pro-apoptotic and anti-proliferative effects by interfering with cell cycle progression and promoting DNA fragmentation through ROS-mediated pathways [34]. The coexistence of these two bioactive molecules likely enhances cytotoxic potency via synergistic interactions, where thujone's strong oxidative stress-inducing capacity complements camphor's mitochondrial targeting. This combined mechanism may underlie the

potent, dose-dependent inhibition of MCF-7 cell proliferation and the broad antibacterial spectrum observed in this study.

Table 4. Dose-dependent effect of *A. herba-alba* essential oil on the viability and proliferation of MCF-7 cells after 48 hours of treatment

Concentration (mg/mL)	Inhibition (% of Control)	Viability (% of Control)
0.000	0.0 ± 0.0	100.0 ± 0.0
0.853	57.37 ± 0.52	42.63 ± 0.52
1.706	58.86 ± 0.80	41.14 ± 0.80
3.412	74.56 ± 3.57	25.44 ± 3.57
8.530	84.78 ± 1.54	15.22 ± 1.54

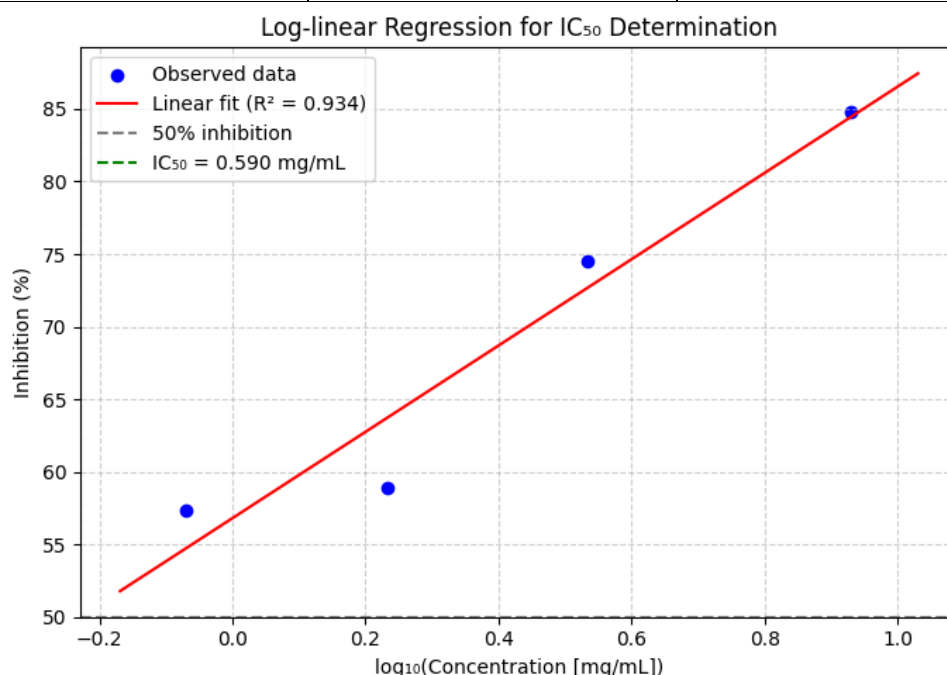


Figure 1. Dose-response curve showing the cytotoxic effect of *Artemisia herba-alba* essential oil on MCF-7 breast cancer cells after 48 hours of treatment.

The percentage of cell growth inhibition was plotted against the natural logarithm of the tested concentrations (mg/mL). The fitted log-linear regression model ($\text{Inhibition (\%)} = 12.91 \times \ln(\text{Concentration}) + 56.81$) yielded an IC₅₀ value of 0.59 mg/mL, indicating a strong, dose-dependent antiproliferative activity of the essential oil.

Conclusion

In conclusion, the essential oil of *Artemisia herba-alba* from Zintan, Libya, was characterized as a high-potency camphor-thujone chemotype, with these two compounds constituting over half of its total composition. This specific chemical profile underpins its notable antibacterial activity against multidrug-resistant pathogens like MRSA and its direct, solvent-free cytotoxic effects on MCF-7 breast cancer cells. The comparative analysis with EOs from other regions, including a contrasting chemotype from the same location, reveals a compelling narrative of extreme chemotypic diversity in this species, driven by local environmental and genetic factors. The results validate the traditional use of this plant and highlight this Libyan chemotype as a promising source of antimicrobial and anticancer agents. Future work should focus on elucidating the specific contributions and synergistic interactions of camphor and thujone to the observed bioactivities, optimizing delivery systems to enhance their bioavailability, and evaluating their safety and efficacy in in vivo models.

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Conflicts of Interest

The authors declare that they have no conflicts of interest

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