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Chemical profiling and biological assessment of essential oil from *Artemisia herba-alba*

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Recently, essential oils (EOs) have garnered attention for their biological properties as a source of natural compounds with anticancer and antioxidant effects. *Artemisia herba alba* grows commonly on the dry steppes of the Mediterranean regions in Northern Africa, Western Asia, and Southwestern Europe. This species is native to Palestine and the western region of Jordan, known as Sheeh. It is employed by the Bedouins for the remedy of many diseases. Recently, essential oils (EOs) have been booming with a growing interest in their use as palliative treatments to conventional medicine due to their biological qualities. The objective of this study is to identify the chemical composition of EO extracted from the dry leaves of *A. herba alba* and to evaluate their in vitro antioxidant, anticancer, and α-amylase and lipase inhibitory activity. The EO was extracted *via* hydrodistillation and examined using gas chromatography-mass (GC-MS) spectrometry to determine its chemical composition. The primary components included 1,8-cineole (28.67%), followed by trans-thujone (24.00%), cis-thujone (17.69%), camphor (12.76%), and concluding with terpinen-4-ol (8.34%). Biologically, the EO performed high antioxidant activity against 1,1-diphenyl-2-picrylhydrzyl (DPPH) with 22.17 ± 1.11 µg/mL and modest lipase and α-amylase activity. The EO displayed significant anticancer properties against B16F10 and MCF-7 with IC_{50} values of 12.39 and 13.60 µg/mL, respectively. A 1:1 combination of *Artemisia herba alba* and *Teucrium polium* EOs improved the anticancer activities against B16F10 and MCF-7 cell lines, with IC_{50} values of 6.184 and 9.427, respectively. Adding 25–50 µg/mL to Taxol (10 ng) caused a remarkable increase in the inhibition of cancer cells. This enhancement in cancer cell inhibition suggests a potential synergistic effect between the essential oil and Taxol, warranting further investigation into their combinatory use in therapeutic applications. Future studies may explore the underlying mechanisms of this synergy to optimize treatment strategies for cancer patients.

Keywords *Artemisia herba alba*, Essential oil, Chemical composition, Antioxidant, Anticancer, MCF-7, B16F10, α-amylase, Lipase

Medicinal plants are nature's gift to humanity, facilitating disease prevention and a healthy existence. Aromatic and medicinal plants are vital sources of secondary metabolites, which have extensive applications in the management of plant and human illnesses, as well as in the pharmaceutical, culinary, and cosmetic sectors[1]. Essential oils (EOs) represent a diverse and enduring category of medicinal plant preparations, derived from more than 17,500 aromatic species and contained within various plant structures, including fruits, flowers, leaves, stems, and roots[2]. Essential oils consist of a variety of molecular types across different structural categories, including hydrocarbons—specifically mono- and sesquiterpene hydrocarbons—as well as oxygenated mono- and sesquiterpenes featuring various functional groups, such as aldehydes, alcohols, ketones, esters, epoxides, and volatile phenolics[3]. Numerous studies have demonstrated that medicinal plants are a major source of bioactive phytochemicals with antibacterial capabilities. These molecules have the ability to protect the human body from free radical-induced stress, which can cause cardiac and neurological problems, joint inflammation, cancer, and other dysfunctions[4]. *Artemisia* is a diverse and significant genus of plants in the Asteraceae family, consisting of approximately 500 species that flourish in temperate climates across both hemispheres, primarily in arid or semiarid regions[5]. Palestine is home to just four species: *Artemisia jordanica*, *Artemisia*

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dracunculus, *Artemisia arborescens*, and *Artemisia herba alba*. *Artemisia herba alba*, commonly referred to as desert wormwood, is a medicinal and aromatic dwarf shrub found in arid regions of the Mediterranean, Middle East, northwestern Himalayas, and India. This plant can grow up to 2.4 m tall and features a tubular stem. Its leaves are arranged alternately and exhibit a dark green or brownish-green color. The aroma is distinctive and pleasant, while the taste is harsh[6]. *A. herba alba* thrives in the dry plains and steppes of Palestine. The oil derived from this plant, known as armoise oil, has a long-standing history of use in traditional and herbal medicine. Numerous scientists have investigated the biological and pharmacological properties of this oil, which include antibacterial, antioxidant, anti-inflammatory, anticancer, antispasmodic, and neuroprotective effects[7–9]. Researchers are currently exploring the potential therapeutic applications of armoise oil, particularly for the treatment of chronic diseases and infections. As the interest in natural remedies continues to grow, *A. herba-alba* is gaining recognition not only for its traditional uses but also for its potential contributions to modern medicine[8].

Despite multiple studies on *Artemisia herba alba*, there remains a need to investigate the chemical composition and biological effects of the EO derived from this plant, which grows at the world's lowest point in Jericho, Palestine, near the Dead Sea. This research aims to clarify the chemical composition of the EO extracted from *A. herba alba* in Jericho, Palestine, utilizing GC/MS analysis, and to assess its antioxidant, anticancer, α-amylase, and α-glycosidase activities, particularly in relation to the treatment of chronic diseases and infections. As interest in natural remedies increases, *A. herba-alba* is gaining recognition not only for its traditional uses but also for its potential contributions to modern medicine.

Although numerous studies have been conducted on *A. herba-alba*, there remains a need to investigate the chemical composition and biological effects of the essential oil derived from *A. herba-alba* at the world's lowest point, Jericho in Palestine, near the Dead Sea. The EO of this plant has not been investigated before. This study seeks to elucidate the chemical composition of the essential oil derived from *A. herba-alba* sourced from Jericho, Palestine, employing GC/MS analysis, while also assessing its antioxidant, anticancer, α-amylase, and α-glycosidase activities.

Materials and methods

Species collection and identification

A. herba-alba leaves were harvested from the lowest elevation on the planet, Jericho-Palestine (latitude 31°52'0.01"N and longitude 35°27'0"E), in early June 2023. The collection of plant material is in accordance with institutional guidelines and legislation. The formal identification of the plant was done by Dr. Nidal Jaradat, and voucher specimens were deposited in An-Najah National University's Herbal Products Laboratory with the code Pharm-PCT-2815. Plant specimens kept in the herbarium of An-Najah National University to be available as scientific reference material for publicity. The leaves were cleaned and desiccated in the shade at ambient temperature (25 ± 3 °C) and humidity (55 ± 4 RH). The desiccated leaves were preserved in the refrigerator at 4 °C until utilized. Prior to the collection of *Artemisia herba-alba*, all required permissions and licenses were obtained from the relevant authorities, and our study is complied with relevant institutional, national, and international guidelines and legislation.

Extraction of essential oil

The stored *A. herba-alba* dried leaves were mechanically ground into tiny pieces to assist in the extraction of the EO and divided into three portions to repeat the study. A mass of 100 g of powdered leaves was subjected to hydro-distillation using a Clevenger-type device for 3 h. The resulting oil was extracted with diethyl ether (Et_2O, 50 mL x 2), the combined organic layers were dried over $MgSO_4$, followed by removing Et_2O under reduced pressure to yield 0.25% of pale-yellow oil. The oil was stored in a dark container and kept in a refrigerator at 4 °C until use. The stored dried leaves of *A. herba-alba* were mechanically ground into small pieces to facilitate the extraction of the EO and were divided into three portions to replicate the study. A mass of 100 g of the powdered leaves was subjected to hydrodistillation using a Clevenger-type apparatus for 3 h. The resulting oil was extracted with diethyl ether (Et_2O, 50 mL x 2); and the combined organic layers were dried over magnesium sulfate ($MgSO_4$). Following this, the diethyl ether was removed under reduced pressure, yielding 0.75% of a pale-yellow oil. The oil was stored in a dark container and kept in a refrigerator at 4 °C until needed.

Qualitative and quantitative analysis of the extracted EOs

The essential oil of *A. herba-alba* was analyzed using gas chromatography-mass spectrometry (GC-MS) with a nonpolar Perkin Elmer-5-MS capillary column (0.25 mm, 30 m, 0.25 µm). A 1 µL sample of EO, prepared at a concentration of 1000 ppm, was injected into the system. Helium served as the carrier gas, operating at a flow rate of 1 mL/min and maintaining a pressure of 20.41 psi in split mode, with a split ratio of 1:50. The injector temperature was set at 250 °C, and the transfer line was also held at 250 °C. The oven temperature was initially set to 50 °C for 5 min, followed by a gradual increase to 280 °C at a rate of 4 °C per minute. It was then maintained at a constant temperature of 280 °C for an additional 10 min. The total run time was 62.5 min, which included a 10-minute conditioning period after the run. Detection was performed using a Perkin Elmer Clarus 560 mass spectrometer. The data was collected using electron ionization (EI) mode at a voltage of 70 eV in standard scanning mode, looking at a mass range from 40 to 500 m/z. The identification of individual metabolites was achieved by comparing their relative retention indices (RRI) and mass spectral data with those from the MS library, NIST webbook, and relevant literature, along with an analysis of their fragmentation patterns. Detected compounds were expressed as percentages of the peak area of each component relative 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 (C7–C30) under identical experimental conditions as the EO samples.

The relative proportions of distinct substances were calculated based on the peak areas obtained through gas chromatography, employing a normalization method without applying correction factors.

DPPH free radical scavenging assay

The antioxidant activity of the *A. herba-alba* EO was evaluated using a spectrophotometric assay that employed the stable radical 2,2′-diphenylpicrylhydrazyl (DPPH•) as a reagent, in accordance with established methods in the literature[10,11]. An EO methanolic solution was prepared at a concentration of 1 mg/mL and subsequently diluted with methanol to achieve concentrations of 1, 2, 3, 5, 7, 10, 20, 30, 40, 50, 80, and 100 µg/mL. Each essential oil solution was mixed with 1.0 mL of 0.1 mM DPPH• in methanol, and the total volume was adjusted to 3.0 mL with methanol. The mixture was incubated for 30 min at room temperature in the absence of light. The absorbance was recorded at 517 nm using a UV-visible spectrophotometer (LABINDIA®, India). The absorbance of methanol and DPPH• radical without oil served as a control. Trolox solution (10–160 µg/mL) acted as the reference compound for comparison. The DPPH radical scavenging capacity was calculated using the following formula: Scavenging capacity = $(A_{control}-A_{sample})/A_{control} \times 100\%$, where $A_{control}$ refers to the absorbance of the DPPH radical without any additive, and A_{sample} represents the absorbance of the DPPH radical with oil samples and control solutions of varying concentrations. A plot of percentage inhibition versus the concentrations of essential oils and standards was utilized to determine their IC_{50} value.

Cytotoxicity of EO

Cell lines and cell culture

The B16F10 melanoma cell lines (ATCC CRL-6475) and mouse Embryonic Fibroblast-1 (MEF-1; ATCC CRL-2214) cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Waltham, Massachusetts, USA) enriched with high glucose, L-glutamine, phenol red (Wako, Japan), 10% fetal bovine serum (FBS; HyClone, Logan, UT, USA), and 1% penicillin/streptomycin (P/S) (Sigma, USA). MCF-7 breast cell lines (ATCC HTB-22) were cultured in ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. The basal medium was supplemented with 0.01 mg/mL of human recombinant insulin, fetal bovine serum to a final concentration of 10%, and 1% penicillin-streptomycin. The cells were incubated at 37 °C in a 5% CO_2 atmosphere.

Chemicals and EOs

The EOs stock solutions were prepared by dissolving them in DMSO to a concentration of 500 µg/mL. In our study, all experimental treatments were compared against vehicle control groups containing the same concentration of DMSO (0.1%) to ensure that any observed cytotoxic effects were attributable solely to the essential oil and not to the solvent.

Cell cultures with EOs

B16F10 and MCF-7 cells (2×10^5 cells/well) were cultured in 6-well plates (TPP, Switzerland) and incubated overnight prior to the introduction of DMSO (EO control), EO, and Mix at doses between 5 and 100 µg/mL EO. At 24 h, viable cells were enumerated utilizing trypan blue dead-cell exclusion dye (Sigma, USA). In several tests, B16F10 or MCF-7 cells were grown with or without Taxol (10 ng/mL) with essential oils[12,13].

α-amylase inhibition assay

The assessment of *A. herba-alba* essential oil's capacity to inhibit α-amylase enzyme activity was conducted in accordance with the Worthington enzyme protocol[14]. A stock solution of 10% *A. herba-alba* EO in DMSO was prepared to create dilutions of 10, 50, 70, 100, 500, and 1000 µg/mL. 200 µL of pancreatic α-amylase enzyme and 200 µL of PBS (pH = 6.9) were mixed with 200 µL of each prepared concentration of EO and incubated at 30 °C for 10 min. Subsequent to preincubation, 200 µL of a 1% starch solution was introduced to 0.02 M sodium phosphate buffer (pH 6.9 containing 0.006 M NaCl). The reaction mixture was subsequently incubated at 25 °C for 10 min. Subsequently, 200 µL of dinitrosalicylic acid (DNS) reagent was introduced to terminate the reaction. Next, each solution was diluted by the addition of 5 mL of distilled water and incubated in a boiling water bath for 8 min, followed by immediate cooling in an ice bath, and the absorbance was measured at 540 nm. The α-amylase inhibitory activity was determined using the formula: α-amylase inhibition (%) = (AB-AE)/AB×100, where AB represents the absorbance of the blank solution and AE denotes the absorbance of EO. A graph depicting α-amylase inhibition (%) was constructed against the concentrations of oil and standard to ascertain their IC_{50} value.

Porcine pancreatic lipase Inhibition assay

The inhibitory effect on pancreatic lipase was assessed using the methodology described in the literature[14]. A solution of p-nitrophenyl butyrate (PNPB) was formulated by dissolving 20.0 mg in 2 mL of acetonitrile for use as the substrate. The EO was dissolved in 10% DMSO to create a stock solution with a concentration of 1000 µg/mL, which was subsequently serially diluted to concentrations of 50, 100, 200, 300, 500, and 1000 µg/mL. A solution of porcine pancreatic lipase was prepared by dissolving 10 mg of the enzyme in 10 mL of Tris HCl buffer at pH 8 immediately before use. A mixture was prepared to conduct the inhibition activity test by combining 200 µL of lipase at 1 mg/mL, 200 µL of the plant extract solution at different concentrations, and 0.60 mL of Tris HCl buffer at pH 8 to achieve a total volume of 1 mL. The resulting mixture was incubated at 37 °C for 15 min, followed by adding 20 µL of the substrate solution (PNPB) and further incubation for 30 min at 37 °C. Using a UV–visible spectrophotometer, the hydrolysis of (PNPB) at 405 nm was measured to evaluate the inhibitory activity of pancreatic lipase. Orlistat was used as a positive control, and the same protocol was followed with the previously specified concentrations. Every test was run in triplicate. The porcine pancreatic

lipase inhibitory activity was calculated using the following formula: lipase inhibition (%) = (AB-AE)/AB×100, where AB represents the absorbance of the blank solution and AE is the absorbance of the EO. A graph of lipase inhibition (%) was plotted in relation to the concentrations of oils and standards to calculate their IC_{50} value.

Statistical analysis

All experiments were performed in biological triplicates ($n = 3$), unless otherwise stated. Data are presented as mean ± standard deviation (SD). One-way ANOVA was used to compare means among more than two groups, followed by the post hoc Tukey's test where appropriate, to identify specific group differences. Student's t-test was employed to compare means between two groups. Statistical significance was defined as $p < 0.05$.

Results and discussion

Phytochemistry

Hydrodistillation of dried *A. herba alba* leaves for three hours produced a pale-yellow oil with a yield of 0.75%. The GC-MS analysis of *A. herba alba* essential oil found 50 different compounds, making up 98.97% of the total oil, as shown in Fig. 1; Table 1. Table 1 displays the names, retention times (RT), retention indices (RI), and percentages of detected constituents. The main group of compounds found were oxygenated monoterpenes, making up 90.32% of the total, with 1,8-cineole being the most common at 28.67%, followed by cis-thujone at 17.69%, camphor at 12.76%, chrysanthenone at 8.91%, terpinen-4-ol at 8.34%, borneol at 4.66%, and α-terpineol at 2.29%, along with other compounds in smaller amounts. Monoterpene hydrocarbons (4.12%) were the second biggest group, with amounts of p-cymene (1.91%) and γ-terpinene (1.25%), along with other chemicals in smaller quantities. The scarcest categories are oxygenated sesquiterpenes (1.32%) and sesquiterpene hydrocarbons (0.13%). These compounds, though less abundant, play crucial roles in the aroma and flavor profiles of various plants, contributing to their unique characteristics and potential therapeutic benefits. Understanding their distribution and concentrations can provide insights into the ecological functions and uses of these plant species. A notable quantity of dimethylphenol (1.75%) is present in the EO alongside smaller quantities of other molecules. The literature reveals notable variability in the composition of the essential oil of *A. herba-alba*, influenced by factors such as geographical location, harvest timing, and the specific plant organ utilized for extraction[15–22]. In accordance with current literature, our findings demonstrate that oxygenated monoterpenes constitute the predominant class of molecules, as noted in all previously described *A. herba-alba* essential oils[15–19] but they exhibit unique chemotypes relative to those already reported. The literature study revealed considerable quantitative and qualitative heterogeneity in the composition of *A. herba alba* EOs from wild populations across different geographical locations, highlighting distinct chemotypes. This diversity indicates that environmental factors, including soil composition and climate, significantly influence the chemical profiles of these oils. Additional research is required to investigate how these variations influence the medicinal characteristics and prospective applications of *A. herba alba* across different industries. Our results align with the research conducted by Feuerstein et al., which examined four populations of *A. herba alba* from distinct geographical regions in Palestine. This study identified two chemotypes: 1,8-cineole (4.8–50%), thujone (4.2–22%), camphor (3.0–25%), and the pinane chemotype[15]. The GC-MS analysis of essential oil derived from the aerial parts of *A. herba-*

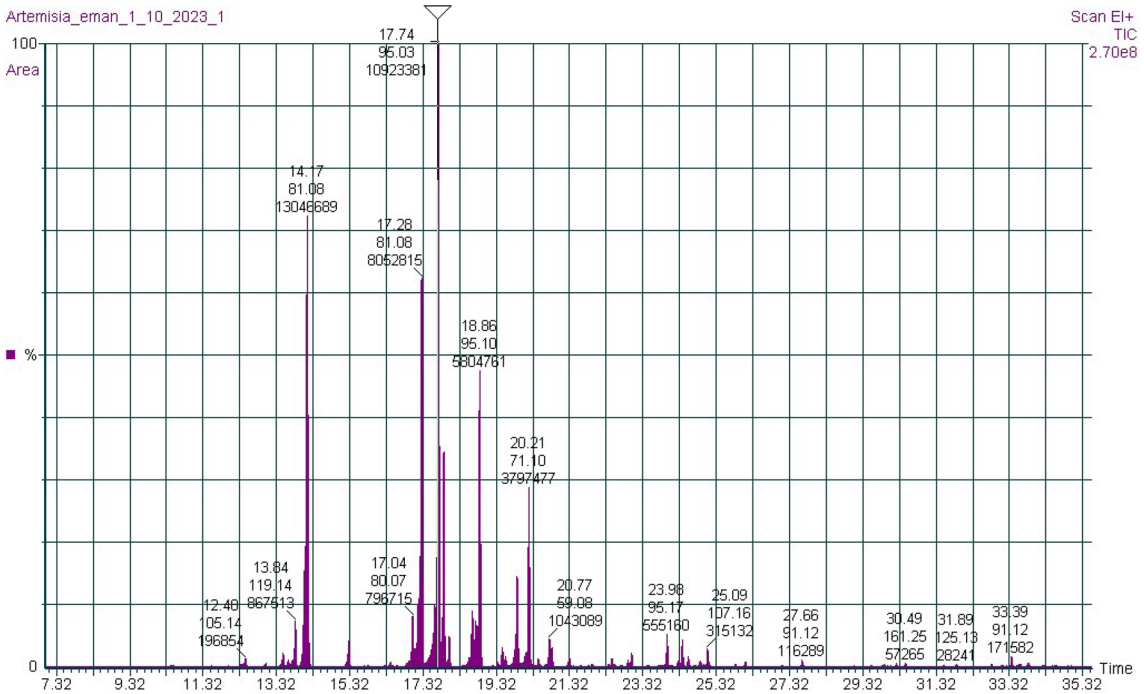

Fig. 1. Gas chromatography Chromatogram of *A. herba-alba* EO.

No	RT	RI	Name	%
1	11.57	970	Sabinene	0.03
2	12.01	980	Octen-3-ol	0.03
3	12.48	990	Mesitylene	0.43
4	13.04	1003	α -Phelandrene	0.14
5	13.51	1014	α -Terpinene	0.52
6	13.65	1017	1,2,4-Trimethyl benzene	0.22
7	13.84	1022	p-Cymene	1.91
8	14.17	1029	**1,8-Cineole**	**28.67**
9	14.42	1035	ND	0.01
10	14.9	1047	3-Methylcyclohex-2-en-1-one	0.01
11	15.29	1056	γ-Terpinene	1.25
12	15.7	1065	(3Z)-Hexenyl oxy-acetaldehyde	0.02
13	15.83	1068	p-Mentha-3,8-diene	0.04
14	16.26	1079	Artemisia alcohol	0.03
15	16.44	1083	Isoterpinolene	0.13
16	16.5	1084	ND	0.03
17	16.62	1087	p-Cymenene	0.04
18	16.86	1093	cis-4-Thujanol	0.04
19	16.96	1095	ND	0.03
20	17.04	1097	Dimethylphenol	1.75
21	17.28	1103	**cis-Thujone**	**17.69**
22	17.89	1118	**Chrysanthenone**	**8.91**
23	18.03	1122	trans-Pinene hydrate	1.13
24	18.59	1136	E-Epoxyocimene	0.10
25	18.67	1138	4(10)-Thujen-3-ol	1.94
26	18.86	1143	**Camphor**	**12.76**
27	19.22	1153	ND	0.03
28	19.35	1156	Sabina ketone	0.15
29	19.48	1159	Pinocarvone	0.73
30	19.88	1169	**Borneol**	4.66
31	20.21	1178	**Terpinen-4-ol**	8.34
32	20.47	1185	ND	0.31
33	20.77	1192	**α -Terpineol**	2.29
34	21.31	1207	trans-Piperitol	0.45
35	21.63	1216	ND	0.04
36	21.94	1224	m-Cuminol	0.09
37	22.39	1237	Ascaridole	0.08
38	22.48	1239	Cumin aldehyde	0.28
39	22.55	1241	Carvone	0.05
40	22.73	1246	Carvotanacetone	0.05
41	22.92	1252	Piperitone	0.20
42	23.46	1267	ND	0.06
43	23.77	1275	2-Methyl isoborneol	0.05
44	23.98	1281	Isobornyl acetate	1.22
45	24.3	1290	Thymol	0.16
46	24.56	1297	Carvacrol	0.40
47	24.88	1307	ND	0.23
48	25.09	1313	Patchenol	0.69
49	25.85	1335	Nd	0.06
50	26.12	1344	α -Terpinyl acetate	0.16
51	27.66	1390	E-Jasmone	0.26
52	30.24	1471	ND	0.08
53	30.49	1480	Germacrene D	0.13
54	31.52	1513	Artedouglasiaoxide C	0.05
55	31.89	1526	Artedouglasiaoxide A	0.06
56	32.84	1558	Geranyl butanoate	0.07
Continued				

No	RT	RI	Name	%
57	33.14	1568	γ-Undecalactone	0.04
58	33.39	1578	Spathulenol	0.38
59	33.59	1583	ND	0.17
60	33.84	1592	Salvial-4(14)-en-1-one	0.14
			Total Identified%	98.97
			Phytochemical Groups	
			Monoterpene hydrocarbons	4.12
			Oxygenated monoterpenes	90.32
			Sesquiterpene hydrocarbons	0.13
			Oxygenated sesquiterpenes	1.32
			Others	3.08

Table 1. The identified phytochemical components in *A. herba Alba* essential oil by GC-MS.

alba, obtained from Jordan, revealed 40 components[20]. The main components comprised α- and β-thujones (24.7%), santolina alcohol (13.0%), artemisia ketone (12.4%), trans-sabinyl acetate (5.4%), germacrene D (4.6%), α-eudesmol (4.2%), and caryophyllene acetate (5.7%). The primary component, 1,8-cineole, was absent in the oil sourced from Jordan. Recently, Dmour et al. reported the identification of 22 compounds in the EO derived from the dried leaf powder of *A. herba alba*, sourced from Jordan, with α-pinene (17.20%), α-thujone (11.40%), 1,8-cineole (10.40%), and sabinene (8.40%) as major components[16]. The GC-MS analysis of 16 populations of *A. herba-alba* from ecologically diverse regions in southern Spain identified 60 compounds, accounting for 80.6–95.0% of the oils[21]. The predominant constituents across most populations were the oxygenated sesquiterpene davanone and the oxygenated monoterpene 1,8-cineole, followed by chrysanthenone, cis-chrysanthenol, and cis-chrysanthenyl acetate. The study revealed that essential oils sourced from Spain do not contain thujones or camphor. The essential oil profiles of *A. herba alba*, sourced from various regions in North Africa, exhibited various chemotypes, with one instance containing 1,8-cineole. Hajli et al. recently reported the identification of 35 compounds in the essential oil of *A. herba-alba* from the Taza region of Morocco, accounting for 99.7% of the total composition. The primary components included camphor (46.57%), endo-borneol (5.65%), eucalyptol (5.64%), and thymol (3.85%)[17]. The examination of *herba-alba* essential oil profiles from eight distinct locations in Morocco uncovered 52 components, accounting for 80.5–98.6% of the total oil. The primary constituents identified were camphor, chrysanthenone, α-thujone, and β-thujone, highlighting significant qualitative and quantitative variations[19]. Fifty constituents were identified in *A. herba-alba* oils obtained from four distinct locations in Algeria, with oxygenated monoterpenes predominating in all instances (72–80%)[22]. The principal components included camphor (17–33%), α-thujone (7–28%), and chrysanthenone (4–19%), resulting in three chemotypes of oils: α-thujone: camphor; camphor : chrysanthenone; and α-thujone: camphor: chrysanthenone. Younsi et al. documented the identification of 46 compounds from *A. herba alba* essential oils obtained from eight distinct eco-geographical regions of Tunisia, which were categorized into four chemotypes: trans-sabinyl acetate, α-thujone/trans-sabinyl acetate, camphor, and α-thujone/camphor/β-thujone. Oxygenated monoterpenes constituted the predominant class, comprising 73.6–89.7% of the total compounds[19]. The notable discovery of elevated cis-thujone levels in our study is crucial, given the compound's associated health risks, as indicated by the World Health Organization. The World Health Organization has brought attention to the neurotoxic effects of cis-thujone, raising concerns about its presence in various products. This finding emphasizes the value of monitoring and regulating this compound to ensure public health safety[23]. The European Parliament and Council regulation prohibits the direct addition of chemically pure thujone to foods, while it may be indirectly incorporated via thujone-containing plants. Moreover, thujone is prohibited as a food ingredient in the USA and is regulated in several countries[24]. As a result, it is important to conduct further studies on *A. herba alba* EO and, in general, for caution in the practical application of EOs and adherence to correct application practices.

Evaluation of the antioxidant

The antioxidant activity was assessed using a reliable, straightforward, and commonly used approach based on the DPPH assay[25]. Figure 2 illustrates that the EO derived from the desiccated leaves of *A. herba alba* exhibits significant antioxidant capacity, with an IC_{50} value of 22.17 ± 1.11 µg/mL, which is less effective than the Trolox (positive control) with an IC_{50} value of 1.94 ± 0.10 µg/mL. The enhanced antioxidant capacity of the EO can be attributed to its significant concentration of oxygenated monoterpenes, particularly 1,8-cineole and camphor, and/or to the synergistic effect of more than one oil compound[26]. Dmour et al. reported that EO extracted from the dried leaf powder of *A. herba-alba* from Jordan exhibited significant antioxidant activity, as demonstrated by the DPPH assay, which yielded an IC_{50} value of 64.57 ± 8.74 µg/mL. They attributed this potent antioxidant capacity to the high levels of oxygenated monoterpenes[16]. Using the DPPH assay, EO derived from Moroccan-sourced A. *herba alba*, rich in oxygenated monoterpenes like chrysanthenone and camphor, demonstrated strong radical scavenging activity with an IC_{50} value of 7.84 ± 0.72 µg/mL[27]. The essential oil of *A. herba alba*, sourced from Algeria and notable for its high camphor content, demonstrated an ability to reduce the stable free radical DPPH, exhibiting an IC_{50} value of 2.66 µg/mL[28]. Younsi et al. indicated that the EO derived from *A. herba-alba* in Tunisia, which is characterized by high levels of β- and α-thujone and a moderate concentration of camphor, exhibited limited antioxidant activity, as evidenced by an IC_{50} value of 5030 ± 30 µg/ml, determined

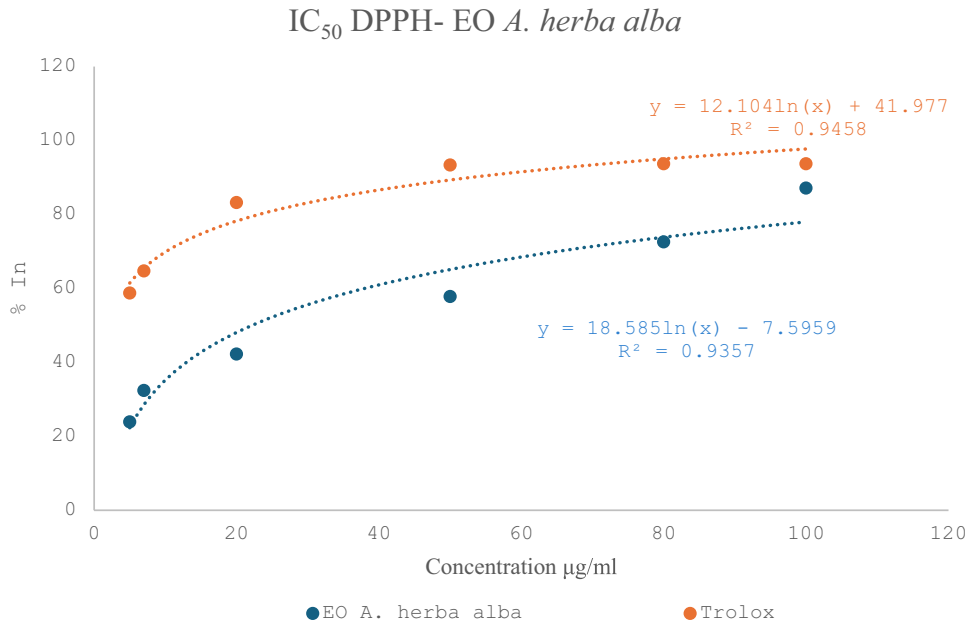

Fig. 2. Antioxidant activity of *A. herba alba* EO essential oils on (DPPH) free radicals and Trolox as a positive control.

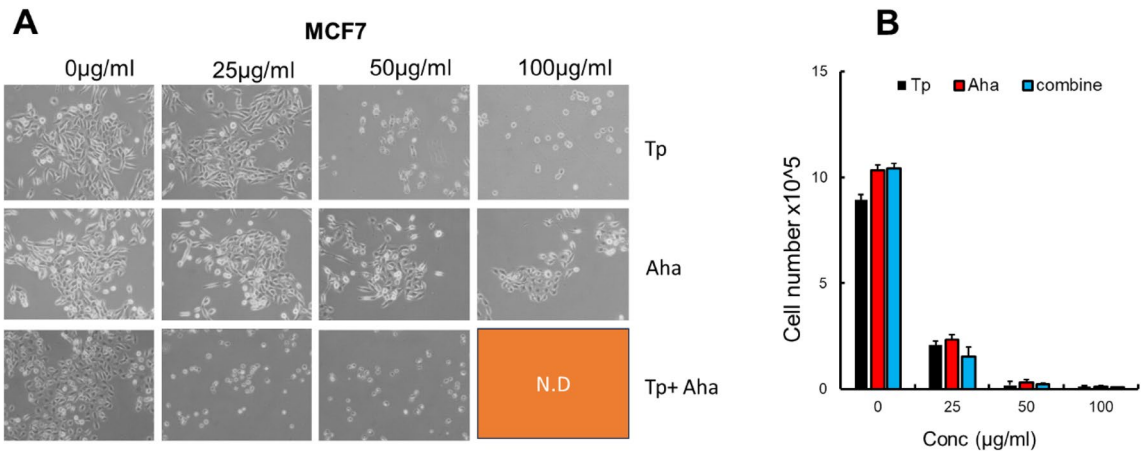

Fig. 3. (**A**) Macroscopic images of MCF-7 cells after 24 h of *T. polium* (Tp) -EO (black), *A. herba alba* (Aha)-EO (red) and Mix (blue) treatment or DMSO as a control ($n = 3$ groups). The indicated doses of EO's were applied to MCF-7 cells in (**B**). After 24 h treatment ($n = 6$). Viable cells were counted using Trypan blue after 24 h ($n = 6$/group). Data are expressed as mean +/- SEM. **p 0.01; *p 0.05 (Student's t-test).

using the DPPH assay[29]. The variation in the antioxidant activities is due to the differences in the component's quantities or the synergistic effect of some ingredients.

Cytotoxicity of *Artemesia herba Alba* EO

The quest for innovative therapeutic compounds that mitigate the adverse effects of chemotherapy remains a critical strategy for reducing toxicity and enhancing efficacy in cancer treatment. We evaluated the anticancer activity of EO extracted from the aerial parts of *Artemisia herba alba*, cultivated in Jericho, Palestine, alongside a 1:1 blend of essential oils from *Artemisia herba alba* and *Teucrium polium*. *Artemisia herba alba* is commonly used in herbal formulations in traditional medicine[30]. The in vitro cytotoxicity assessment of *Artemisia herba alba* oil and a 1:1 blend of essential oils from *A. herba alba* and *T. polium* exhibited notable anticancer efficacy against the B16F10 cell line, with IC_{50} values of 12.39 and 6.184 µg/mL, respectively (Fig. 3). The two experiments performed on the B16F10 cell line were similarly applied to the MCF-7 cancer cell line, demonstrating significant cytotoxicity with IC_{50} values of 13.60 and 9.427 µg/mL (Fig. 4; Table 2).

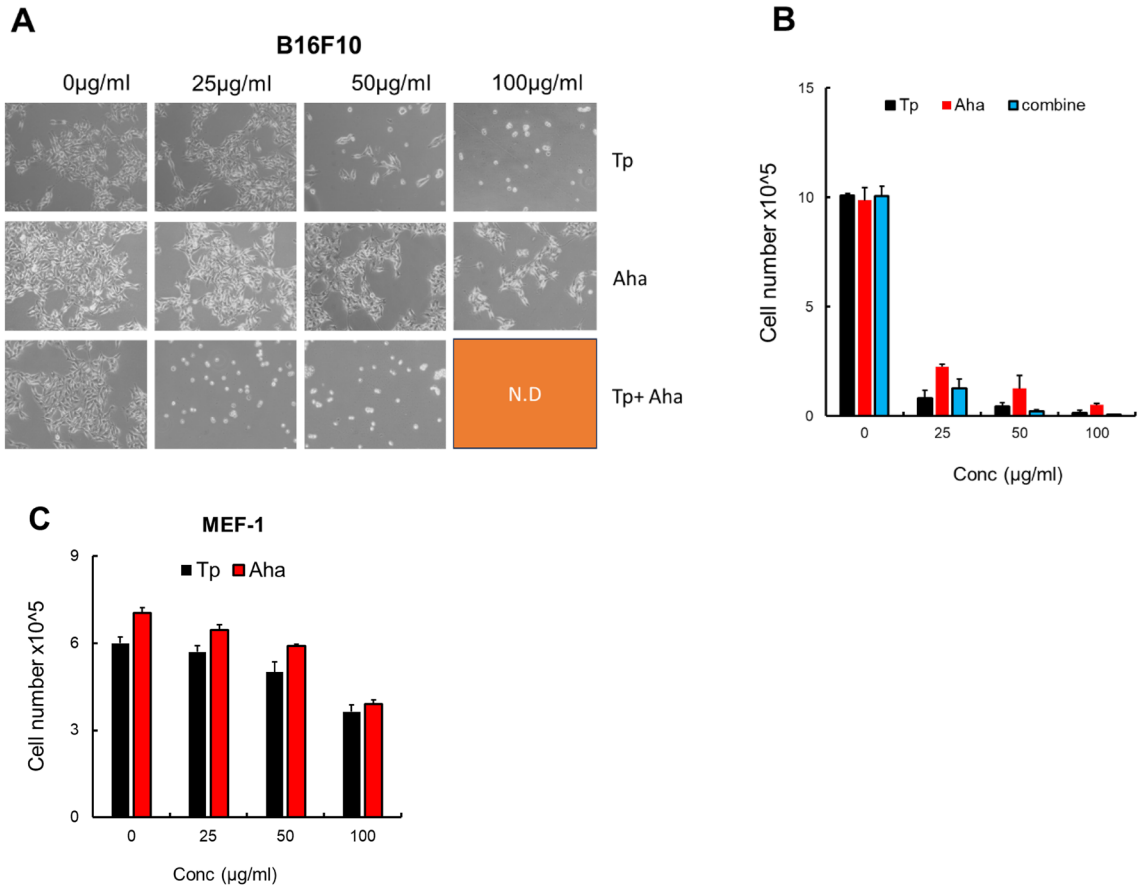

Fig. 4. (**A**) Macroscopic images of B16F10 cells after 24 h of *T. polium* (Tp) EO (black), *A. herba alba* (Aha) EO (red), and Mix (blue) treatment or DMSO as a control ($n = 3$ groups). The indicated doses of EO's were applied to B16F10 cells in (**B**). (**C**). Mouse embryonic fibroblast-1 (MEF-1) cell line was treated with indicated concentrations of EOs; after 24 h, viable cells ($n = 3$/group) were counted using Trypan blue. Data are expressed as mean +/− SEM. **p 0.01; *p 0.05 (Student's t-test).

	Inhibition% without taxol		Inhibition% with taxol (10ng)	
Aha-EO (µg/mL)	B16F10	MCF-7	B16F10	MCF-7
0	0	0	34.7	23.6
5	13.2	22.3	70.4	55.8
25	77.2	77.5	96.7	90.5
50	87.3	96.9	98.7	99.7

Table 2. %Inhibition of viable B16F10 and MCF-7 cells were counted using Trypan blue after 24 h treatment of eo's and without taxol and with taxol (10ng/ml) ($n = 6$/group). Data are expressed as mean +/− SEM. **p 0.01; *p 0.05 (Student's t-test).

The EO of *A. herba alba* was less effective than that of *T. polium*, which demonstrated notable anticancer activity against the B16F10 and MCF-7 cell lines, with IC_{50} values of 8.145 and 12.43 µg/mL, respectively. The EO of *T. polium* is abundant in E-nerolidol (27.11%), geranyl acetone (23.26%), and germacrene D (19.08%), compounds known for their significant anticancer properties. The incorporation of 10 ng/mL of Taxol into *A. herba alba* EO at concentrations of 5 µg/mL, 25 µg/mL, and 50 µg/mL significantly enhanced the inhibition of MCF-7 and B16F10 cancer cells, resulting in an improved IC_{50} of 2.98 and 2.78 µg/mL, respectively (Table 2; Fig. 5). MCF-7 and B16F10 cancer cells demonstrated reductions of 23.6% and 34.7%, respectively, after 24 h of treatment with Taxol (10 ng/mL). A suppression of 90.5% and 99.7% of MCF-7 cancer cells was noted when 10 ng of Taxol was administered alongside 25 µg/mL and 50 µg/mL of EO, respectively. A 96.7% suppression of B16F10 cancer cells was achieved with Taxol (10 ng) combined with 25 µg/mL of essential oil, while a 98.7% inhibition was noted with 50 µg/mL of essential oil. The findings indicate that the synergistic characteristics of the essential oils augment their therapeutic effectiveness, rendering them suitable for further investigation

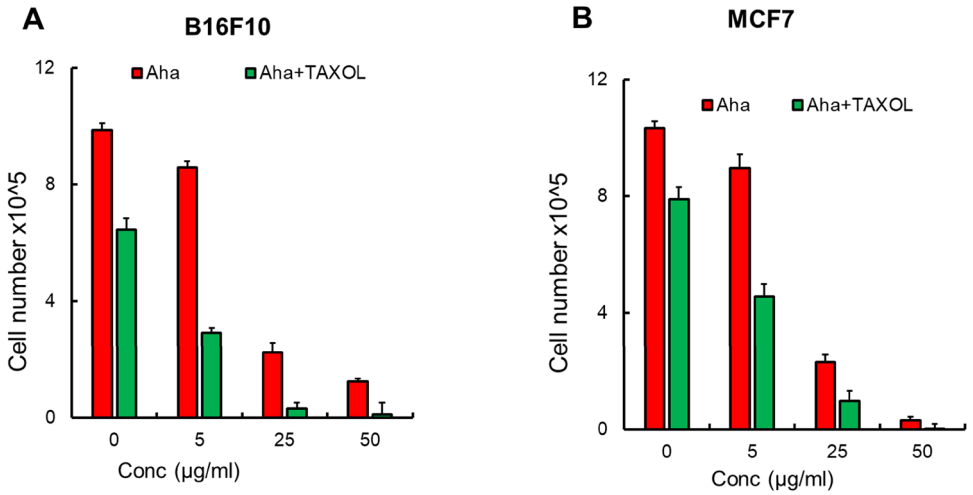

Fig. 5. (**A**,**B**) Viable B16F10 and MCF-7 cells were counted using Trypan blue after 24 h treatment of EO (red), and EO + Taxol (10ng/ml) (green) ($n = 6$/group). Data are expressed as mean +/- SEM. **p 0.01; *p 0.05 (Student's t-test).

in cancer treatment methodologies. The finding underscores the need for further research to investigate the underlying mechanisms of action and evaluate the safety profiles of these essential oils. Subsequent studies ought to concentrate on in vivo models to enhance comprehension of their efficiency and any adverse effects within a more intricate biological context. We also recognize the significance of comparing the cytotoxic effects in normal and malignant cells in order to evaluate selectivity. Non-malignant cells' toxicity can be assessed using mouse embryonic fibroblasts (MEF-1). MEF-1 did not reduce proliferation when 50 µg/ml of oil was added (Fig. 4C). These results provide credence to the notion that both oils enhanced the anti-proliferative characteristics of cancer cells, reducing cytotoxicity and the need for less medicine.

The oil's safety profile and anticancer potential can be more accurately interpreted with the help of these extra data.

Despite the widespread use of *Artemisia herba alba* in traditional medicine, the anticancer properties of its EO have been infrequently studied. This lack of research is surprising, especially considering the increasing interest in natural compounds for cancer treatment. Tellaoui et al. documented that the EO of *A. herba alba*, characterized by its major constituents—verbenol (22%), bisabolone oxide (17.55%), E-farnesene epoxide (17.08%), and β-thujone—exhibited significant antiproliferative effects against the acute lymphoblastic leukemia (CEM) cell line, with an IC_{50} value of 3 µg/mL[31]. Subsequently, the same research group disclosed that the EO derived from *A. herba alba* leaves, comprising 2,5-octadecadiynoic acid methyl ester (22.48%), 1,8-cineole (20.37%), and bisabolone oxide (10.27%) as predominant constituents, exhibited a dose-dependent cytotoxic effect on P815 and BSR cell lines, with IC_{50} values of 15 µg/mL and 26 µg/mL, respectively[32].

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

The impact of EO on the inhibition of lipase and α-amylase was assessed utilizing established methodologies[14]. The *A. herba alba* essential oil, as illustrated in Fig. 6, exhibited moderate lipase inhibition activity, with an IC_{50} value of 543 ± 27.15 µg/mL, and a slightly lower α-amylase inhibition, with an IC_{50} of 569 ± 28.45 µg/mL (Fig. 7).

Research on the lipase and α-amylase inhibitory activity of essential oils from the *A. herba alba* species is limited. The essential oil of *A. herba alba* from Algeria, characterized by its primary constituents β-copaene (16.22%), limonene (14.56%), eucalyptol (14.49%), and camphor (13.74%), exhibited inferior α-amylase activity compared to our essential oil, with an IC_{50} value of 1.93 µg/mL. Jaradat et al. reported that the EO extracted from the *Artemisia jordanica* plant exhibited dose-dependent inhibitory activity against porcine pancreatic lipase and α-amylase, with IC_{50} values of 51.41 ± 0.91 and 14.17 ± 0.39, respectively[33].

Conclusion

A GC-MS analysis of the essential oil extracted from dried *A. herba alba* leaves through hydrodistillation identified 50 distinct components, accounting for 98.97% of the total oil. This EO is mainly composed of oxygenated monoterpenes, with 1,8-cineole as the primary constituent, followed by cis-thujone, camphor, chrysanthenone, terpinen-4-ol, borneol, and α-terpineol. The monooxygenated hydrocarbons exhibited significant antioxidant activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) at a concentration of 22.17 ± 1.11 µg/mL, although they showed only moderate lipase and α-amylase activity. Moreover, the EO of *A. herba alba*, when combined with *T. polium* in a 1:1 ratio, demonstrated notable anticancer efficacy against the MCF-7 and B16F10 cancer cell lines. Additionally, incorporating *A. herba alba* EO into the chemotherapeutic agent Taxol enhanced the suppression of both cancer cell lines under investigation. Furthermore, the unique properties of *A. herba alba* essential oil merit exploration in various therapeutic contexts, potentially leading to innovative strategies for cancer treatment and improved formulations in food safety and cosmetic products. Finally, more research is necessary to determine

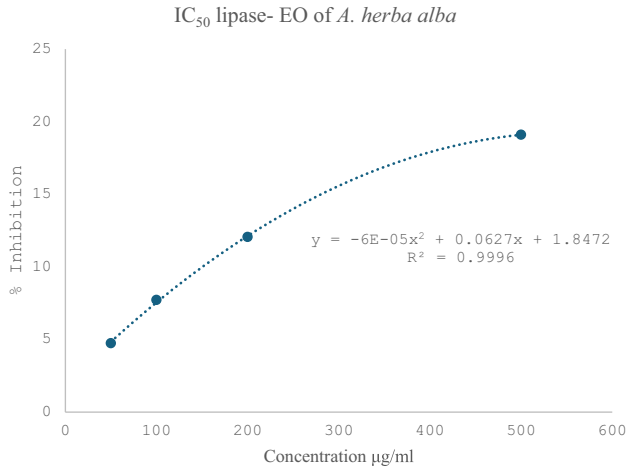

Fig. 6. A plot of percent lipase inhibition against the concentrations of EO extracted from *A. herba alba* dried leaves.

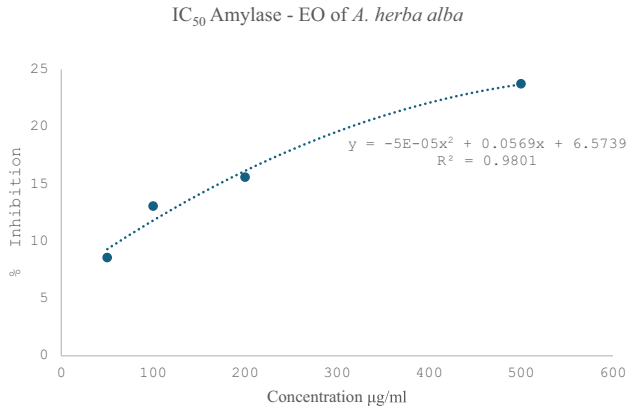

Fig. 7. A plot of percent α-amylase inhibition against the concentrations of essential oil extracted from *A. herba alba* dried leaves.

mechanisms of action, assess off-target effects, and validate efficacy and safety in more physiologically relevant models, even though the data point to promising cytotoxic activity. Therefore, it is appropriate to consider the present findings as foundational and hypothesis-generating, directing the development of more thorough future research.

Data availability

All data generated or analyzed during this study are included in this manuscript.

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Author contributions

Conceptualization (Al-Maharik N.); methodology (Bsharat O., Salams Y., Saed E., and Al-Maharik N.); software, (Bsharat O., Salama Y., Saed E., and Al-Maharik N.); validation (Bsharat O, Al-Hajj N, Jaradat, N, Warad, I, and Al-Maharik N.); formal analysis (Bsharat O, N Al-Hajj, and N Al-Maharik.); investigation (Bsharat O., Al-Hajj N., and Al-Maharik N.,); resources (Bsharat O., Al-Hajj N., and Al-Maharik N.); data curation (Bsharat O., Salama Y., and Al-Maharik N.); writing—original draft preparation (Bsharat O., and Al-Maharik); writing—review and editing (Bsharat O., Al-Hajj N., Salama Y and Al-Maharik N.); visualization (Bsharat O., Al-Hajj N., Salama Y and Al-Maharik N.) supervision (Al-Maharik N.) project administration (Al-Maharik N.). All authors read and approved of the final manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Permission

Prior to the collection of *Artemisia herba-alba*, all required permissions and licenses were obtained from the relevant authorities.

Voucher specimens

The formal identification of the plant was done by Dr. Nidal Jaradat, and voucher specimens were deposited in

An-Najah National University's Herbal Products Laboratory with the code Pharm-PCT-2815.

Additional information

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