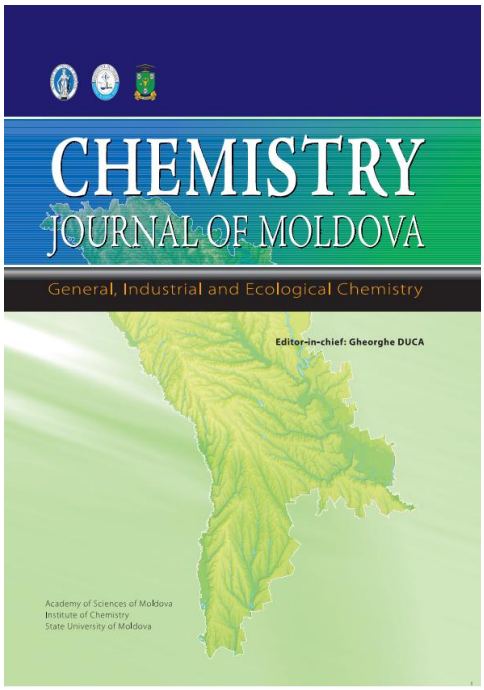


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Abstract. *Ephedra alata* subsp. *alenda* (Stapf) Trab., a resilient shrub known in Algeria as "Alenda", widely used in traditional Saharan medicine, yet its volatile fraction remains poorly characterized along the bioclimatic gradients of North Africa. This study investigated the phytochemical and biological properties of the essential oil (EO) harvested from wild populations in Bechar (Southwest Algeria). The EO was obtained *via* hydrodistillation, analyzed by GC-MS, and evaluated for antioxidant capacity (DPPH, FRAP, *β*-carotene) and anti-inflammatory potential (albumin denaturation and HRBC membrane stabilization). GC-MS analysis showed 94.70% of the total composition, which was primarily characterized by *β*-Pinene (18.30%), *α*-Terpinyl acetate (13.80%), and Limonene (9.20%). Biological results revealed a DPPH IC_{50} of 28.45 µg/mL, an EC_{50} for FRAP of 42.15 µg/mL, and an IC_{50} for protein denaturation inhibition of 92.30±3.45 µg/mL. Further significant findings included an IC_{50} for HRBC heat lysis of 84.50±2.90 µg/mL and hypotonic lysis of 68.15±2.20 µg/mL. One-way ANOVA confirmed significant quantitative disparities in the distribution of chemical classes compared to regional populations from Aïn Sefra, El Oued, and Tunisia. Statistical comparisons indicate that Bechar species exhibits intermediate chemical characteristics between highland and lowland populations, suggesting a transitional chemotype with relevant *in vitro* biological activities. providing a preliminary scientific support for its traditional therapeutic use in Saharan medicine.

Keywords: *Ephedra alata* subsp. *alenda*, essential oil, antioxidant activity, anti-inflammatory activity, Sahara.

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Introduction

Ephedra alata subsp. *alenda* is a resilient, dioecious gymnosperm shrub that defines much of the arid and hyper-arid landscapes across north Africa. Known locally as "Alenda", this species has long been a pillar of traditional Saharan medicine, where it is utilized for its stimulating, bronchial, antimicrobial, and anti-inflammatory properties [1]. Modern phytochemical interest in the plant centres primarily on its secondary metabolites, which have been extensively documented in studies focusing on various solvent extracts [1-3]. While these investigations have successfully identified a rich profile of polyphenols in the plant's tissues, research specifically addressing the volatile fractions remains notably scarce [4-6]. This significant gap in the literature regarding the essential oil served as the primary motivation to initiate the present study.

In many aromatic and medicinal plants, the biosynthesis of volatile constituents has been reported to vary with climatic and ecological conditions, contributing to plant adaptation and survival [7]. In the vast Saharan ecozone, plants are subjected to a complex gradient of environmental pressures, ranging from the high-altitude cold stress of the Saharan Atlas to the extreme thermal amplitudes and high solar radiation of low-lying desert basins [8,9]. It has been hypothesized that localized climatic factors, such as elevation, temperature fluctuations, and solar radiation, may induce metabolic shifts in secondary pathways, potentially modulating the proportion of simple terpenoid hydrocarbons relative to oxygenated or aromatic constituents [10-12].

To test this hypothesis and bridge the current gaps in regional phytochemical data, the present study focuses on the characterization of *E. alata* essential oil harvested from the Bechar region of

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Southwestern Algeria. A central objective of this research is to perform a rigorous comparative analysis of this chemotype against three geographically distinct populations: Aïn Sefra (Highlands, Algeria) [4], El Oued (Low Basin, Algeria) [5], and Kebili (Chott region, Tunisia) [6]. While previous studies have characterized the essential oil composition of *E. alata*, each focused on a single population using its own methodology, no essential oil composition data currently exist for the Bechar population, which has previously been studied only for the extract-based antifungal activity of its aerial parts [1,3]. To our knowledge, this is the first study to characterize the Bechar essential oil and to directly compare it with these previously reported populations within a unified analytical framework, statistically relating chemical-class distribution to quantitative climatological gradients across the four sites. By integrating localization data and climatological parameters derived from NASA POWER [13], the study aims to elucidate the precise influence of geographical origin on the quantitative distribution of major chemical classes.

The specific objectives of this study were: (i) to characterize the chemical composition of the Bechar essential oil by GC-MS and compare it with the three previously reported populations (Aïn Sefra, El Oued, and Tunisia) within a common statistical framework; (ii) to examine the relationship between this chemical-class distribution and quantitative climatological parameters (altitude, temperature, UV index, and precipitation) derived from NASA POWER [13] across the four sites; (iii) to evaluate the antioxidant activity of the Bechar essential oil using three complementary assays (DPPH radical scavenging, reducing power/FRAP, and β-carotene bleaching); and (iv) to assess its anti-inflammatory potential using albumin denaturation and erythrocyte membrane stabilization (HRBC) assays.

Experimental

Plant material

Aerial parts of *E. alata* were collected from wild populations in Bechar, southwestern Algeria (31.7673°N, 2.2326°W; elevation 728.77 m) in March 2022, during the late vegetative-early flowering phenophase, corresponding to optimal essential oil accumulation [14] (Figure 1). Plant material was authenticated by Prof. Abdelkader Guenaia, Department of Biology, University of Bechar, using macro-morphological characteristics and comparison with reference specimens; voucher specimen (Gr-0100-2022) was deposited in the herbarium. Fresh aerial parts were transported to the laboratory within 2 hours, cleaned with tap water, chopped into 1–2 cm fragments and shade-dried for 5 days until constant weight (final moisture content: 8.2±0.8%, n= 3) [15,16].

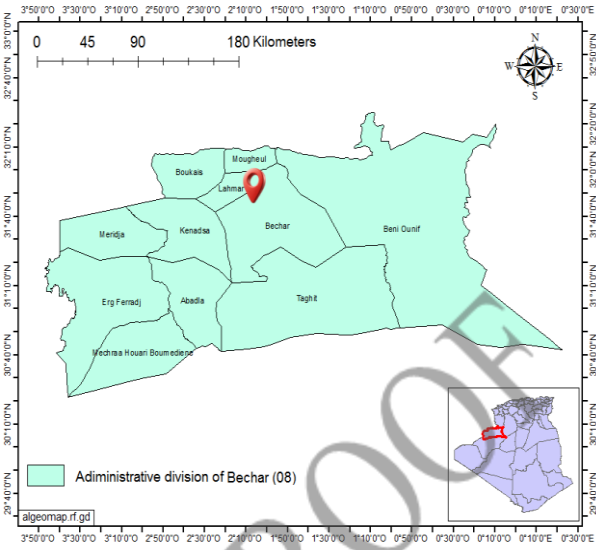


Figure 1. Geographic localization of the sampling area.

Essential oil extraction

The dried plant material was subjected to hydrodistillation using a glass Clevenger-type apparatus (Dean and Stark & Clevenger Systems). For each extraction, the dried plant material (100 g) was mixed with distilled water at a ratio of 1:20 (w/v) and hydrodistilled for 3 h. Three independent extractions were performed. Following distillation, the recovered essential oil was separated from the aqueous phase, dried over anhydrous sodium sulphate (Na_2SO_4), filtered, and stored in sealed amber glass vials at 4°C until further analysis by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS).

This protocol differs from that reported by Jerbi, A. *et al.* [6], in which fresh plant material was used and hydrodistillation was performed for 4 h, while it is comparable to the protocol reported by Chouitah, O. [4], except for the plant-to-water ratio used (1:20 vs. 1:25, w/v) and the absence of diethyl ether for essential oil recovery.

The recovered essential oil yield was 0.89%, calculated as the mass of essential oil obtained relative to the mass of dried plant material and expressed as a percentage (g/100 g dry plant material).

Gas chromatography/mass spectrometry (GC/MS) analysis

Volatile constituents were analysed using a Bruker SCION 436 GC/MS equipped with a quadrupole mass analyser (70 eV ionization

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energy) and an HP-5MS capillary column (30 m× 0.25 mm× 0.25 µm). The system was operated in splitless mode with helium carrier gas (1.2 mL/min), injector temperature 250°C, and oven temperature programmed from 50°C to 240°C at 5°C/min. Mass spectra were acquired in full-scan mode (m/z 40–300). Retention indices (RI) were calculated using *n*-alkane standards (C_7–C_{28}) according to the van den Dool and Kratz method. Compound identification required agreement on both criteria: an experimental RI within ±5 units of the literature value, and a mass spectral match of ≥85% against the Wiley 7N and NIST 2023 libraries. Compounds meeting both criteria were considered reliably identified, whereas compounds that did not meet both criteria, as well as compounds not detected in the sample, were classified as not determined. Literature RI values were cross-referenced with the literature of Adams, R.P. [17].

DPPH radical scavenging assay

The free radical scavenging capacity of the essential oil was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, following previously described protocols [18,19]. Briefly, 100 µL of essential oil solutions at different concentrations were added to 750 µL of a 0.004% (w/v) DPPH solution prepared in methanol. The mixtures were vortexed and incubated in the dark at 27 °C for 45 minutes, after which the absorbance was measured at 517 nm using a UV-Visible spectrophotometer, with methanol as blank. The percentage of DPPH radical scavenging was calculated, and results were expressed as IC_{50} values (µg/mL), corresponding to the concentration of essential oil required to inhibit 50% of the DPPH radicals. All measurements were performed in triplicate (n= 3), and butylated hydroxytoluene (BHT) and ascorbic acid were used as reference antioxidants.

Ferric reducing antioxidant power (FRAP) assay

The reducing capacity of the essential oil was assessed using the ferric-reducing antioxidant power (FRAP) method adapted from previously described procedures [19,20]. Reaction mixtures were prepared by combining 1 mL of essential oil solution at different concentrations (1-1000 µg/mL) with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixtures were incubated at 50°C for 25 minutes in a water bath, followed by the addition of 2.5 mL of 10% (w/v) trichloroacetic acid to stop the reaction. After centrifugation at 3000 rpm for 10 minutes, 2.5 mL of the supernatant were mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ solution. The absorbance was measured at 700 nm, and higher absorbance indicated greater reducing power. Results were expressed as EC_{50} values (µg/mL, mean± SD, n= 3) as the concentration at the absorbance of 0.5. Ascorbic acid and BHT were used as reference standards.

β-Carotene bleaching assay

Antioxidant activity in a lipidic medium was evaluated using the *β*-carotene/linoleic acid bleaching assay as previously reported [18,19], with slight modifications to be adapted for essential oils. An emulsion containing *β*-carotene, linoleic acid, and Tween-40 was prepared, and 2 mL of this emulsion were mixed with 100 µL of essential oil at various concentrations. The absorbance was recorded at 470 nm against a methanol blank at the initial time (t= 0) and after 2 hours of incubation at 50°C. A negative control consisted of 100 µL methanol instead of extract or reference antioxidants. The antioxidant activity (AA) was expressed as a percentage (%) according to the following Eq.(1).

$$AA(\%) = \frac{1 - (A_{S0} - A_{S120})}{A_{C0} - A_{C120}} \times 100 \qquad (1)$$

where, A_{S0}, A_{S120}, A_{C0}, and A_{C120} are the absorbances of the sample and the control, respectively, measured at 0 and 120 min. All tests were carried out in triplicate, and BHT and ascorbic acid were used as standard antioxidants.

Albumin denaturation inhibition assay

The *in vitro* anti-inflammatory activity of essential oil was evaluated via inhibition of heat-induced denaturation of bovine serum albumin (BSA), following the protocol of Hasan, M.M. *et al*. [21] with modifications. This assay is based on the principle that protein denaturation is a key mechanism in inflammatory processes, and anti-inflammatory agents can stabilize proteins against heat-induced structural changes. Essential oil samples at various concentrations (10–200 µg/mL in distilled water, with 10% DMSO as co-solvent) were mixed with BSA solution (0.2% w/v in phosphate-buffered saline, PBS, pH 7.4) in a 1:1 ratio (v/v) in test tubes. The reaction mixtures were incubated at 37± 2°C for 30 minutes in a water bath to allow stabilization of BSA structure, followed by heating at 70 ± 2°C for 15 minutes to induce protein denaturation. Control tubes containing BSA without sample were treated identically. Following cooling to room temperature, the absorbance was measured at 280 nm using a UV-Vis spectrophotometer

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(Shimadzu UV-1800) against a blank (buffer only). Diclofenac sodium (Sigma-Aldrich, ≥ 98% purity; 50–400 µg/mL) served as positive control. The inhibition percentage of protein denaturation was calculated as Eq.(2).

$$Inhibition\ \% = [(A_0 - A_1)/A_0] \times 100 \quad (2)$$

where, A_0 is the absorbance of the control (BSA without the sample), and
A_1 is the absorbance with the essential oil.
IC_{50} values were determined from dose-response curves using non-linear regression. All experiments were conducted in triplicate (n= 3).

Human Red Blood Cell (HRBC) membrane stabilization assay

The HRBC membrane stabilization assay was performed based on cellular membrane protection. Human fresh blood (blood group O-positive, O^+) was collected from healthy donors into heparinized tubes and centrifuged at 1300 g for 5 minutes. The supernatant was discarded, and red blood cells (RBCs) were washed three times with ice-cold saline (0.85% w/v NaCl in 10 mM sodium phosphate buffer, pH 7.4) until the supernatant remained clear. A 2% (v/v) RBC suspension was prepared by diluting the packed cells with saline buffer.

Two haemolysis protocols were employed, as described by Anosike, C.A. *et al*. and Gunathilake, K.D.P.P. *et al.*, with slight modifications [22,23]:

(A) Hypotonicity-induced haemolysis:

Test tubes containing 5 mL hypotonic saline (0.36% w/v NaCl in 10 mM sodium phosphate buffer, pH 7.4) were supplemented with 500 µL essential oil samples at concentrations ranging from 10 to 200 µg/mL, followed by 100 µL of 2% (v/v) RBC suspension. Reaction mixtures were incubated at 25°C for 30 min and subsequently centrifuged at 1300 g for 5 min. Supernatant haemoglobin content was determined spectrophotometrically at 540 nm.

(B) Heat-induced haemolysis:

Essential oil samples (500 µL) at the same concentrations were added to 5 mL isotonic saline (0.85% NaCl). After addition of 100 µL RBC suspension, the tubes were incubated at 54°C for 20 minutes in a regulated water bath, then cooled to room temperature and centrifuged at 1300 g for 3 minutes. Haemoglobin absorbance was measured at 540 nm.

In both protocols, Blank and control tubes containing, respectively, RBCs in isotonic saline (0% haemolysis) and RBCs in distilled water (100% haemolysis) were prepared in parallel. Diclofenac sodium (10–200 µg/mL) was used as a reference compound for comparison purposes.

$$Inhibition\ \% = 100 - [(A_S/A_C) \times 100] \quad (3)$$

where, A_S is the absorbance of the treated sample,
A_C is the absorbance of the stressed control.
IC_{50} values were expressed in µg/mL.

Statistical analysis

Data were analysed in triplicate and expressed as mean ± SD. Variations in chemical classes across the four geographical regions were compared using one-way ANOVA followed by Tukey's post-hoc test. Pearson's correlation coefficient was employed to evaluate the relationship between major constituents and biological activities. All analyses were performed using IBM SPSS Statistics Version 27.0, with statistical significance set at $p< 0.05$.

Results and discussion

The four studied populations of *E. alata* are distributed across a pronounced altitudinal and climatological gradient (Table 1). Aïn Sefra, compared to Bechar and the two other areas, represents the highest environment (1248.65 m) with a cold semi-arid climate (BSk), characterized by the highest annual precipitation (151.7 mm) and the lowest absolute maximum temperatures.

Table 1

Comparative climatological and geographical profiles.

Study Location	*Köppen Climate Classification*	*Altitude (Elevation, m)*	*Absolute Max Temp (°C)*	*Absolute Min Temp (°C)*	*Average Annual Precipitation (mm/year)*
Bechar	BWk	728.77	46.82 (July 2023)	-3.46 (Jan 2017)	84.10 ± 38
Ain Sefra	BSk	1248.65	41.98 (July 2024)	-5.20 (Jan 2023)	151.70 ± 62
El-Oued	BWh	62.57	49.94 (July 2023)	-1.19 (Jan 2021)	61.10 ± 30
Kebili, Tunisia	BWh	41.01	49.65 (July 2023)	-1.07 (Jan 2017)	69.40 ± 19

* BWk: Cold desert climate; BSk: Cold semi-arid climate; BWh: Hot desert climate (according to Köppen climate classification). Temp: Temperature.

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In stark contrast, the low-lying eastern basins of El Oued (62.57 m) and Kebili (41.01 m) experience hyper-arid, hot desert conditions (BWh) with severe rainfall deficits and extreme summer thermal peaks approaching 50°C. Bechar occupies a transitional ecological niche; situated at a moderate altitude (728.77 m) within a cold-hot desert climate (BWk), it subjects the plant to a high thermal amplitude, combining freezing winter minimums (-3.46°C) with intense summer heat (46.82°C).

The chemical profile of Bechar's *E. alata* essential oil (BEO) is defined by several dominant markers and chemical classes as detailed in Tables 2 and 3. The monoterpene hydrocarbons represent the primary fraction at 30.50%, with *β*-pinene (18.30%) and limonene (9.20%) appearing as the most significant constituents, followed by a substantial ester content of 16.90%, where *α*-terpinyl acetate stands out as a major component at 13.80%. Within the oxygenated monoterpene class (14.90%), linalool is the most prominent at 7.60%. Furthermore, the oil contains a distinct aromatic and phenolic suite, specifically thymol (7.10%), cuminaldehyde (6.50%), and benzaldehyde (4.40%). Finally, the sesquiterpene hydrocarbon fraction reaches 6.70%, largely represented by *α*-selinene (5.80%).

The chemical composition of the Bechar population stands in contrast to the Aïn Sefra highlands, which exhibit the highest concentration of monoterpene hydrocarbons (47.8%), and a significantly higher *β*-pinene content (42.57%). While BEO maintains a moderate hydrocarbon fraction, this class is entirely absent in the hyper-arid populations of Kebili and El Oued. In the aromatic and phenolic categories, El Oued EO shows much higher concentrations of thymol (13.00%) and cuminaldehyde (12.50%) compared to BEO 7.10% and 6.50%, respectively. Kebili EO is distinguished by a high proportion of aliphatic hydrocarbons (23.10%), such as (Z)-3-tridecene (7.80%), which are not detected in the BEO. Furthermore, *β*-linalool is most significant in Kebili EO (19.30%), whereas it reaches only 7.60% in BEO and 2.50% in El Oued EO. Finally, while BEO and Aïn Sefra EO both contain *α*-terpinyl acetate (13.80% and 18.85%, respectively), this ester is absent in the El Oued and Kebili profiles.

Table 2

Comparative chemical profile of *E. alata* essential oils from Bechar and other geographical origins.

No.	*Compounds*	*Bechar*		*Adams, R.P. [17]*		*Kebili, Tunisia* [6]		*El Oued, Algeria* [5]		*Ain Sefra, Algeria* [4]	
		KI	*%*	*AI*	*KI*	*KI*	*%*	*KI*	*%*	*KI*	*%*
1	*α*-Thujene	926	0.20	924	930	-	-	-	-	921	2.60
2	Camphene	940	0.70	946	954	-	-	-	-	940	1.25
3	Benzaldehyde	967	4.40	952	960	-	-	967	8.10	-	-
4	Sabinene	971	1.90	969	975	-	-	-	-	966	3.50
5	*β*-Pinene	976	18.30	974	979	-	-	-	-	969	42.57
6	*α*-Terpinene	1014	0.20	1014	1017	-	-	-	-	1014	0.22
7	Limonene	1025	9.20	1024	1029	-	-	-	-	1029	17.66
8	1,8-Cineole	1027	3.80	1026	1031	-	-	1032	7.10	-	-
9	*β*-Linalool	1093	7.60	1095	1096	1101	19.30	1125	2.50	-	-
10	Camphor	1145	2.10	1141	1146	-	-	1130	5.10	-	-
11	1-Phenylpropane-1,2-dione	-	-	-	-	-	-	1153	5.00	-	-
12	Terpinen-4-ol	1159	0.30	1174	1177	-	-	1159	0.50	-	-
13	Borneol	1168	5.20	1165	1169	-	-	1258	7.56	-	-
14	*α*-Terpineol	1170	0.90	1186	1188	-	-	1170	0.50	-	-
15	Estragole	-	-	1195	1196	-	-	1177	5.50	-	-
16	Dihydrocarvone	1195	0.20	1199-1202 [24]		-	-	1175	0.40	-	-
17	Cuminaldehyde	1232	6.50	1238	1241		-	1228	12.50	-	-
18	Linalyl acetate	1254	2.30	1254	1257	1259	4.30	-	-	-	-
19	(*E*)-Anethole	1282	0.60	1282	1284	1285	6.00	1269	10.20	-	-
20	(*Z*)-3-Tridecene	-	-	-	-	1283	7.80	-	-	-	-
21	Bornyl acetate	1285	0.30	1284	1285	-	-	1285	0.50	-	-
22	1-Tridecene	-	-	-	-	1293	2.30	-	-	-	-
23	Azulene	1290	-	1298	1298	-	-	1160	0.80	-	-
24	*α*-Terpinyl acetate	1348	13.80	1346	1349	-	-	-	-	1165	18.85
25	D-Carvone	-	-	1239	1243		-	1232	5.00	-	-

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Continuation of Table 2

No.	Compounds	Bechar		Adams, R.P. [17]		Kebili, Tunisia [6]		El Oued, Algeria [5]		Ain Sefra, Algeria [4]	
		KI	%	AI	KI	KI	%	KI	%	KI	%
26	*n*-Tridecane	-	-	1300	1300	1300	1.70	-	-	-	-
27	2-Caren-10-al	1265	0.30	1289	1289	-	-	1265	0.90	-	-
28	(*E*)-2-Tridecene	-	-	-	-	1305	2.10	-	-	-	-
29	(*Z*)-2-Tridecene	-	-	-	-	1316	2.90	-	-	-	-
30	2,3,5,8-Tetramethyldecane	-	-	-	-	1328	6.30	-	-	-	-
31	Thymol	1287	7.10	1289	1290	-	-	1287	13.00	-	-
32	2-Undecanone	-	-	1293	1294	-	-	1277	0.40	-	-
33	Eugenol	-	-	1356	1359	-	-	1345	2.00	-	-
34	(*E*)-Methyl cinnamate	1376	0.50	1376	1378	-	-	1367	4.10	-	-
35	Methyleugenol	-	-	1403	1403	-	-	1400	0.50	-	-
36	(*E*)-Geranylacetone	-	-	1434	1436	-	-	1445	0.90	-	-
37	Isomethyl-*α*-Ionone	-	-	1478	1479	-	-	1474	0.80	-	-
38	*α*-Curcumene	-	-	1479	1480	-	-	1472	0.90	-	-
39	*α*-Selinene	1495	5.80	1498	1498	-	-	-	-	1455.80	10.88
40	*δ*-Guaiene	-	-	1509	1509	-	-	-	-	1458.60	0.20
41	*δ*-Cadinene	1498	0.90	1513	1513	-	-	-	-	1483.80	4.23
42	Myristicin	1516	1.40	1517	1518	-	-	1516	4.30	-	-
43	Trans-calamenene	-	-	1521	1522	-	-	-	-	1488.80	0.12
44	Germacrene D	-	-	1559	1561	-	-	-	-	1492	6.50
45	*β*-Calacorene	-	-	1564	1565	-	-	-	-	1550	1.97
46	Spathulenol	1577	0.20	1577	1578	-	-	1567	1.20	1578	0.10
47	Caryophyllene oxide	-	-	1582	1583	-	-	1571	3.00	-	-
48	Globulol	-	-	1590	1590	-	-	-	-	1584	0.19
49	Caryophylla-dien-5-ol	-	-	1639	1640	-	-	1631	0.70	-	-
50	*α*-Eudesmol	1645	-	1652	1653	-	-	1645	0.50	-	-
51	Ar-Turmerone	1661	-	1668	1669	-	-	1661	4.80	-	-
52	Curlone	-	-	-	-	-	-	1695	0.50	-	-
53	Farnesol	-	-	1742	1743	-	-	1592	0.10	-	-
54	Hexahydrofarnesyl acetone	-	-	1845	1845	-	-	1844	0.90	-	-
55	Farnesyl acetone	-	-	1913	1913	-	-	1914	0.30	-	-

KI: Kovats Index on HP-5MS column; *AI*: Arithmetic Index from literature (Adams, R.P.); %: Relative percentage based on peak area; "-": Not detected.

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Table 3

Distribution of chemical classes in *E. alata* essential oils from Bechar and the other geographical origins.

Chemical Class (%)	Bechar	Kebili, Tunisia [7]	El Oued, Algeria [6]	Ain Sefra, Algeria [5]
Monoterpene hydrocarbons	30.50	-	-	47.80
Oxygenated monoterpenes	14.90	-	16.10	-
Sesquiterpene hydrocarbons	6.70	-	0.90	23.90
Oxygenated sesquiterpenes	0.20	-	10.70	0.30
Esters	16.90	4.30	4.60	18.80
Phenylpropanoids	2.00	6.00	20.50	-
Phenols	7.10	-	15.00	-
Aromatic aldehydes	10.90	-	20.60	-
Non-terpenoids/Aliphatic HC	-	23.10	-	-
Others (Alcohols, Ketones, etc.)	5.50	-	22.60	-

* HC: Hydrocarbons; "-": Not detected or not reported in the respective studies.

Consequently, the statistical analysis suggests that these phytochemical shifts may reflect an adaptive environmental stress response rather than stochastic variation. One-way ANOVA and Tukey's post-hoc tests ($p< 0.05$) demonstrate significant variations in major chemical classes across regions, supporting the characterization of the Bechar species as a transitional chemotype, while Pearson's correlation ($r> 0.85$; $p< 0.01$) indicates a potential association between high thermal/UV indices and the upregulation of oxygenated and phenolic metabolites. These correlative findings are consistent with the hypothesis that as altitude decreases and oxidative pressure intensifies across the Saharan gradient [10,11], terpenoid biosynthesis may shift toward more oxygenated derivatives, a pattern reported for terpenoid metabolism in other aromatic species [25]; the concurrent increase in phenolic and aromatic aldehyde content may reflect additional, independently regulated biosynthetic routes not further examined in this study. Thus, the intermediate geographical position of Bechar coincides with the simultaneous presence of both highland-type terpene hydrocarbons and lowland-type oxygenated aromatics, giving BEO a comparatively diverse chemical profile that may contribute to its observed biological activity.

The biological evaluation of the Bechar *E. alata* EO exhibited measurable *in vitro* antioxidant and anti-inflammatory activity, which showed statistical association with several major constituents of its transitional chemical profile. The antioxidant capacity of the Bechar EO, as shown in Table 4, was consistently measurable across all three tested pathways, reaching an IC_{50} of 28.45±1.12 µg/mL for DPPH radical scavenging, an EC_{50} of 42.15±1.35 µg/mL for FRAP, and an IC_{50} of 55.80±2.10 µg/mL for the *β*-carotene bleaching assay. While these values are significantly higher than those of the pure reference antioxidants ascorbic acid and BHT ($p< 0.05$), indicating that the essential oil is less potent on a per-mass basis than these isolated standards, as expected for a complex mixture in which the active constituents represent only a fraction of the total mass. Statistical interpretation using Pearson's correlation coefficient identifies a strong positive relationship ($r> 0.85$, $p< 0.05$) between this antioxidant power and the presence of phenols (7.1%) and aromatic aldehydes (10.9%), which provide the necessary electron-donating properties to neutralize free radicals.

Remarkably, BEO (28.45 µg/mL) demonstrates a high DPPH scavenging potential that is statistically comparable ($p> 0.05$) to the most potent polar solvent extracts reported in other studies, such as the ethanolic extract of stems of the Tunisian population, as reported by Jerbi, A. *et al.* [6], which showed an IC_{50} of 27.0 µg/mL, while its essential oil showed no noticeable activity. Belazougui, K. [5] reported a lower potential of ethanolic extract of *E. alata* (35.0 µg/mL).

Furthermore, BEO exhibited strong anti-inflammatory potential (Table 5), effectively inhibiting heat-induced protein denaturation (IC_{50}= 92.30±3.45 µg/mL) and stabilizing human red blood cell membranes against heat lysis (IC_{50}= 84.50±2.90 µg/mL). Although the pharmaceutical standard Diclofenac sodium remains two times more potent ($p< 0.05$), the EO efficacy is may be associated with the presence of Esters (16.90%) and Monoterpene Hydrocarbons (30.50%) [26]. These lipophilic constituents are documented to integrate into lipid bilayers, enhancing membrane resilience against thermal and osmotic stress [12,27].

Table 4

***In vitro* antioxidant capacity of Bechar's *E. alata* essential oil.**

Sample	*DPPH, IC_{50} (µg/mL)*	*FRAP, EC_{50} (µg/mL)*	*β-Carotene, IC_{50} (µg/mL)*
Essential oil	28.45 ± 1.12^{c}	42.15 ± 1.35^{c}	55.80 ± 2.10^{c}
Ascorbic acid	4.80 ± 0.15^{a}	6.20 ± 0.22^{a}	18.50 ± 1.05^{b}
BHT	8.10 ± 0.35^{b}	11.40 ± 0.45^{b}	9.30 ± 0.40^{a}

* Superscript letters indicate statistically significant differences ($p< 0.05$) according to Tukey's post-hoc test.

Table 5

***In vitro* anti-inflammatory potential of Bechar's *E. alata* essential oil.**

Sample	*Protein denaturation IC_{50} (µg/mL)*	*HRBC hypotonic lysis IC_{50} (µg/mL)*	*HRBC heat lysis IC_{50} (µg/mL)*
Essential oil	92.30 ± 3.45^{b}	68.15 ± 2.20^{b}	84.50 ± 2.90^{b}
Diclofenac sodium	48.10 ± 1.25^{a}	38.40 ± 1.15^{a}	44.25 ± 1.30^{a}

* HRBC: Human Red Blood Cell; Different superscript letters indicate statistically significant differences ($p< 0.05$) according to Tukey's post-hoc test.

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As previous regional studies focused primarily on antimicrobial or antioxidant properties of polar extracts, these results provide the first statistically validated evidence for the *in vitro* anti-inflammatory potential of the subspecies' volatile fraction, distinguishing the Bechar population as a source of natural bioactive compounds.

Conclusions

This study provides a comprehensive phytochemical and biological characterization of the essential oil of *E. alata* from the Southwestern Algerian Sahara. Through a comparative analysis with regional populations, the population of Bechar was identified as a statistically distinct chemotype. These findings support the hypothesis that Saharan bioclimatic gradients, specifically altitude, thermal extremes, and UV intensity, may influence secondary metabolism, although the present study does not experimentally establish the underlying environmental mechanisms. Across the gradient from the cooler highlands of Aïn Sefra to the hyper-arid basins of El Oued and Tunisia, the chemical profile shifts from simple terpene hydrocarbons toward more oxygenated and phenolic compounds. Bechar essential oil combines features of both extremes, giving it a comparatively diverse chemical profile relative to the other populations examined.

These results provide supportive *in vitro* evidence for the traditional use of *E. alata* in Saharan medicine and underscore the importance of geographical origin in the standardization of natural products; extrapolation to therapeutic efficacy in humans would require further pharmacological and clinical investigation. The Bechar population's transitional chemotype identifies the Southwest Algerian Sahara as a potentially valuable source of bioactive essential oils for future pharmaceutical and cosmeceutical applications, pending further investigation. Future research should focus on the molecular mechanisms of this environmental adaptation and on whether interactions between these volatile markers contribute to the observed biological activity.

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