FULL PAPER

Artemisia arborescens Essential Oil Composition, Enantiomeric Distribution, and Antimicrobial Activity from Different Wild Populations from the Mediterranean Area

by **Mohammed El-Amin Said**[a]), **Marcello Militello***[b]), **Sergio Saia**[c]), **Luca Settanni**[b]), **Aurora Aleo**[d]), **Caterina Mammina**[d]), **Isabelle Bombarda**[a]), **Pierre Vanloot**[a]), **Christian Roussel**[e]), and **Nathalie Dupuy**[a])

[a]) EA4672 LISA Equipe METICA, Aix-Marseille Université, Case 451, Av. Escadrille Normandie Niémen, FR-13397 Marseille Cedex 20

[b]) Department of Agricultural and Forest Sciences (SAF), Università degli Studi di Palermo, Viale delle Scienze, Ed. 4, IT-90128 Palermo (phone: +393294906241; fax: +390916515531; e-mail: marcellomilitello@tiscali.it)

[c]) Council for Agricultural Research and Economics (CREA) – Cereal Research Centre (CREA-CER), S.S. 673 km 25,200, IT-71122 Foggia

[d]) Department of Health Promotion Sciences, "G. D'Alessandro'', Università degli Studi di Palermo, Via del Vespro, IT-90127 Palermo

[e]) Ecole Centrale, CNRS, iSm2 UMR 7313, Aix-Marseille Université, FR-13397 Marseille Cedex 20

Aerial parts of *Artemisia arborescens* were collected from different sites of the Mediterranean area (southwestern Algeria and southern Italy) and the chemical composition of their essential oil (EO) extracted by hydrodistillation was studied by both gas chromatography (GC) equipped with an enantioselective capillary column and GC/mass spectrometry (GC/MS). The EOs obtained were tested against several *Listeria monocytogenes* strains. Using GC and GC/MS, 41 compounds were identified, accounting for 96.0 – 98.8% of the total EO. All EOs showed a similar terpene profile, which was rich in chamazulene, *β*-thujone, and camphor. However, the concentration of such compounds varied among the EOs. *A. arborescens* EO inhibited up to 83.3% of the *L. monocytogenes* strains, but the inhibitory spectrum varied among the EOs, with those from Algeria showing a higher inhibition degree than the Italian EOs. Such effect likely depended on the ketone (*β*-thujone + camphor) content of the EO. The differences in the EO composition support the hypothesis that *A. arborescens* has at least two different chemotypes: a *β*-thujone and a chamazulene type. The EO inhibitory spectrum indicates the *A. arborescens* EO as a valuable option in the control of the food-borne pathogens.

Keywords: Enantiomeric distribution, Biological activity, *Gram*-negative bacteria, Volatile composition, *Listeria monocytogenes*.

Introduction

Artemisia arborescens L. is an aromatic and medicinal species belonging to the Asteraceae family [1][2]. It is widespread in the Mediterranean coast as an endemic species in many African and European countries [1] [3 – 6]. This species was used in the folk medicine for skin, bronchial catarrh, asthma, and insufficient bile production [7][8]. In addition, some of such effects were confirmed for antidiabetic; antipyretic; anti-inflammatory [1] [5][9]; antiangiogenic [10]; and antiviral activity against herpes simplex virus 1 and 2 [11]. From a biotechnological perspective, *A. arborescens* EO reduced the germination of some plant species belonging to distant taxa (*Triticum*, *Brassica*, *Amaranthus*) [12][13]. Similar effects were found when its methanolic and aqueous extracts were investigated for their allelopathic potential on *Lactuca sativa* seeds and plantlets [14]. This suggests that such an activity can also be played from some of its water-soluble compounds. *A. arborescens* EO slightly reduced the hatching of eggs and activity of juvenile of the nematode *Meloidogyne javanica* [15]. Similarly, *A. arborescens* EO showed a pesticidal (against *Lymantria dispar*, *Aphis gossypii*, *Bemisia tabaci*) [16] and an antifungal activities against several *Candida albicans* strains [17].

The effects of *A. arborescens* EO on some food-borne bacterial pathogens were also tested on few strains of *Listeria monocytogenes* and *Escherichia coli* [5][18] and the degree of inhibition varied among the strains. Such effects likely depended on the composition of the EO, since the EO of other *Artemisia* species strongly reduced *M. javanica* growth and such effect was mainly due to the concentration of the *Artemisia* ketone [15].

Indeed, the composition of *A. arborescens* EOs from different environments was found to be variable and various putative chemotypes were suggested. For example, in

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North America, *A. arborescens* EOs was characterized by chamazulene and camphor [19][20]. In the Mediterranean region, the chemical composition of the EO of *A. arborescens* from Morocco was characterized by β-thujone and camphor [21], that from north-west of Algeria (Tlemcen) only by camphor, whereas that from north-east of Algeria (Bejaia) by chamazulene and β-thujone [4][5]. The chemical composition of the essential oil of *A. arborescens* from Lebanon was characterized by β-thujone and chamazulene [1]. Those from the central Mediterranean islands Sicily and Sardinia showed some similarities with those from north-east of Algeria and north-west of Algeria, respectively [3][18][22 – 24].

Previous reports indicate that environmental factors can drive natural selection toward chemotypes more adapted to local environmental conditions [25 – 27].

However, little information is available about the antimicrobial effects of the EO of *A. arborescens* from different environments. Thus, the aim of this study was to characterize *A. arborescens* EOs from some contrasting environments in the Mediterranean basin in terms of both chemical composition and enantiomeric distribution of the main chiral compounds. In addition, the inhibitory effect against a wide collection of *L. monocytogenes* strains was screened.

Results and Discussion

Climatic Index of the Collection Sites

Strong differences were found among the collection sites in term of aridity, especially from October to April (late fall to early spring), as also displayed by the *De Martonne* index (*Fig. 1*). In particular, Bechar showed 12 dry months according to *Bagnouls* and *Gaussen*, Ain Sefra eight dry months (from April to November) and those from Italy showed only four dry months (from May to August).

Essential Oil Composition

The identification of the compounds was achieved by comparing their mass spectra with those of Wiley275 and NIST05a libraries as well as by comparing their retention indices with those of authentic samples [28]. Forty-one compounds were identified (*Table 1*), representing 96.0%, 98.8%, 96.7%, and 96.5% of the total composition of the samples from Bechar, Ain Sefra, Capo Zafferano, and Termini Imerese, respectively. EOs were mostly composed by oxygenated monoterpene (35.91 – 84.79%) including α-thujone (0.28 – 4.41%), β-thujone (19.57 – 70.31%), camphor (1.12 – 25.70%), and terpinen-4-ol (1.89 – 7.71%), followed by sesquiterpenes hydrocarbons (8.44 – 49.42%) including β-caryophyllene (0.53 – 2.73%), germacrene D (0.62 – 4.26%), chamazulene (3.64 – 43.12%). Monoterpene hydrocarbons and oxygenated sesquiterpene were found in small fraction, lower than 12.74% and 1.85%, respectively. These compounds included α-pinene (0.35 – 1.12%), camphene (0.18 – 1.18%), sabinene (0.30 – 2.76%), myrcene (0.53 – 3.47%), *p*-cymene (0.64 – 1.25%), γ-terpinene (0.99 – 2.24%), elemol (lower than 0.62%), caryophyllene oxide (lower than 0.75%), and β-eudesmol (lower than 1.22%).

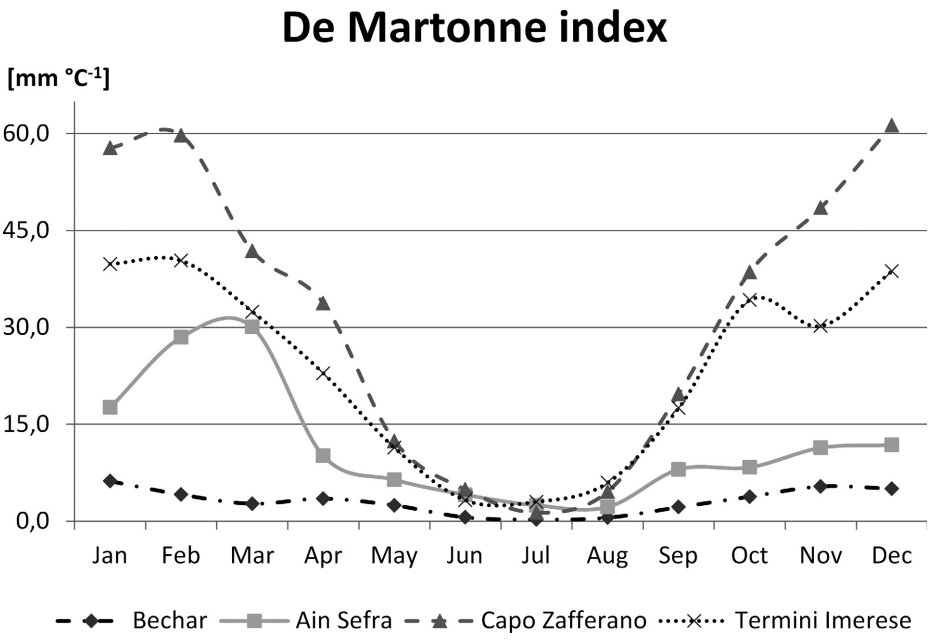

Fig. 1. *De Martonne* index, calculated on a monthly basis, of the collection sites from Algeria (Bechar and Ain Sefra) and Italy (Capo Zafferano and Termini Imerese). *De Martonne* index lower than 15, between 15 and 30, and higher than 30 indicate an arid month, semiarid to subhumid, and humid month, respectively.

Table 1. Chemical composition by GC and GC/MS (expressed as % of the total peak area) of the essential oil of *Artemisia arborescens* from two Algerian (Bechar and Ain Sefra) and two Italian (Capo Zafferano and Termini Imerese) sites

RI[a])	*RI*[b])	Compound	Origin			
			Bechar	Ain Sefra	Capo Zafferano	Termini Imerese
913	924	α-Thujene	0.36	0.42	0.32	0.37
925	932	α-Pinene	1.12	0.35	0.96	0.75
945	946	Camphene	1.18	0.18	0.80	0.85
974	969	Sabinene	1.07	0.30	1.49	2.76
981	971	6-Methylhept-5-en-2-one	0.33	0.28	0.24	0.27
982	974	β-Pinene	tr[c])	tr	tr	tr
985	988	Myrcene	2.23	0.53	2.08	3.47
1010	1002	α-Phellandrene	0.17	tr	0.34	0.27
1019	1014	α-Terpinene	0.77	0.34	0.87	0.60
1026	1020	*p*-Cymene	1.25	0.82	0.64	0.89
1030	1024	Limonene	0.35	0.15	0.17	0.32
1034	1026	Eucalyptol	0.41	0.66	0.26	0.21
1058	1054	γ-Terpinene	1.94	0.99	1.69	2.24
1087	1086	Terpinolene	0.39	0.18	0.30	0.22
1095	1095	Linalool	1.07	1.36	1.42	1.32
1099	1098	Sabinene hydrate	tr	tr	0.59	0.65
1107	1101	α-Thujone	1.15	4.41	0.28	0.41
1115	1112	β-Thujone	26.98	71.31	19.57	19.89
1123	1118	*p*-Menth-2-en-1-ol	0.31	0.19	0.16	0.25
1150	1141	Camphor	25.70	1.12	8.78	8.68
1173	1165	Borneol	tr	tr	0.21	0.23
1183	1174	Terpinen-4-ol	7.71	3.45	2.51	1.89
1196	1186	α-Terpineol	0.85	0.48	0.30	0.37
1236	1227	Nerol	0.33	0.65	0.56	0.55
1272	1261	Chrysanthenyl acetate	3.19	0.18	tr	tr
1282	1269	Perillaldehyde	0.18	0.29	0.31	0.37
1293	1284	Bornyl acetate	tr	tr	0.84	1.97
1298	1289	Thymol	0.22	0.15	0.12	0.26
1384	1374	α-Copaene	0.22	0.54	0.54	0.39
1396	1387	β-Bourbonene	tr	0.51	tr	0.26
1408	1403	Methyl eugenol	0.34	0.42	0.18	0.14
1432	1417	β-Caryophyllene	0.53	0.83	1.65	2.73
1464	1452	α-Caryophyllene	tr	tr	0.14	0.21
1491	1484	Germacrene D	0.62	2.61	3.59	4.26
1513	1505	α-Farnesene	0.15	0.12	0.18	0.24
1524	1513	γ-Cadinene	0.12	0.25	0.22	0.31
1534	1522	δ-Cadinene	tr	0.19	0.20	tr
1558	1548	Elemol	0.62	0.40	tr	tr
1595	1582	Caryophyllene oxide	tr	tr	0.60	0.75
1665	1649	β-Eudesmol	tr	0.25	1.22	1.10
1738	1730	Chamazulene	13.78	3.64	43.12	36.83
		Monoterpene hydrocarbons	10.47	3.84	9.66	12.74
		Oxygenated monoterpene	68.10	84.79	35.91	37.05
		Sesquiterpene hydrocarbons	15.42	8.69	49.64	45.23
		Oxygenated sesquiterpene	1.33	1.49	1.82	1.85
		Others	0.33	0.28	0.42	0.41
		Total identified	96.01	98.55	97.45	97.28

[a]) *RI*, Retention indices on *HP-5* capillary column. [b]) *RI*, Retention indices in [50]. [c]) tr, trace.

The EO from Ain Sefra was characterized by a high percentage of β-thujone (71.31%), whereas that from Bechar by β-thujone (26.98%), camphor (25.70%), and chamazulene (13.78%). The EOs from both Capo Zafferano and Termini Imerese were characterized by chamazulene (36.83 – 43.12%), β-thujone (19.57 – 19.89%), and camphor (8.68 – 8.78%). This suggests that the accessions from Algeria (Ain Sefra and Bechar) could be designated as β-thujone and intermediate (β-thujone + camphor) chemotypes, respectively, whereas those from southern Italy as chamazulene chemotype. These results agree with those reported from several authors [4][18][21][24][29] in which the EO of *A. arborescens* coming from collection sites nearby the sea showed similar β-thujone and chamazulene contents. The environmental conditions, especially drought, can strongly influence the composition

Table 2. Enantiomeric distribution (%) of α-Thujone, β-Thujone, Camphor, and Terpinen-4-ol in the EOs

Compound	t_R[a]) [min]	*RI*[b])	Origin			
			Bechar	Ain Sefra	Capo Zafferano	Termini Imerese
Camphor						
(−)	40.4	1177	1.76	1.85	1.20	1.23
(+)	41.0	1182	98.24	98.15	98.70	98.17
α-Thujone						
(−)	42.9	1200	100.00	100.00	100.00	100.00
(+)	–	–	0.00	0.00	0.00	0.00
β-Thujone						
(−)	–	–	0.00	0.00	0.00	0.00
(+)	43.8	1208	100.00	100.00	100.00	100.00
Linalool						
(−)	46.1	1230	0.88	0.76	0.80	0.68
(+)	46.8	1237	99.12	99.24	99.10	99.32
Terpinen-4-ol						
(+)	51.4	1280	70.40	72.15	69.49	67.45
(−)	52.0	1286	29.60	28.85	31.01	32.55

[a]) t_R, Retention time. [b]) *RI*, Retention indices on *HP-5* capillary column *Rt-bDEXse.*

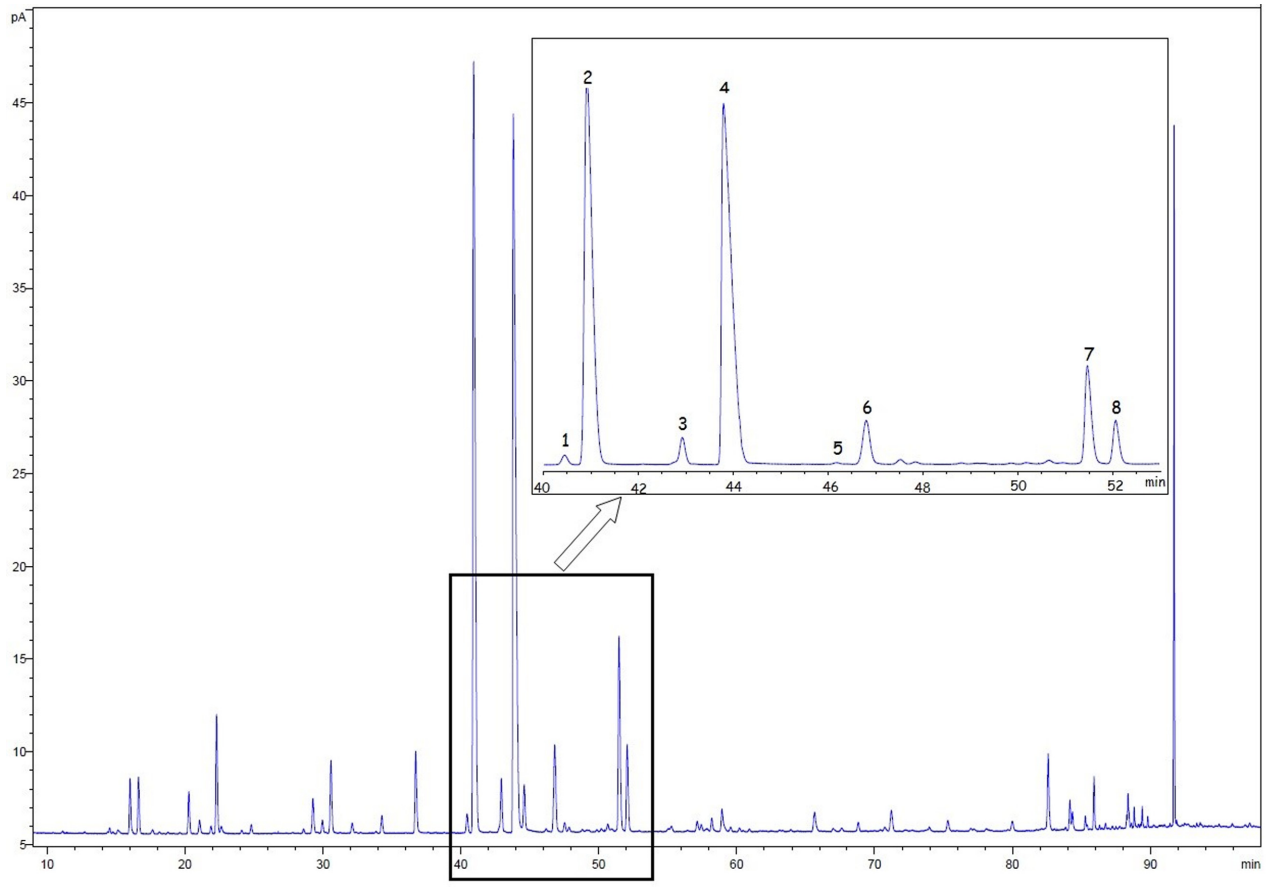

Fig. 2. Enantioseparation of the essential oil of *A. arborescens* by GC-FID on *Rt-bDEXse* capillary enantioselective column *RESTEK* (30 m × 0.25 mm; film thickness 0.25 μm). 1, (+)-camphor; 2, (−)-camphor; 3, (−)-α-thujone; 4, (+)-β-thujone; 5, (−)-linalool; 6, (+)-linalool; 7, (+)-terpinen-4-ol; 8, (−)-terpinen-4-ol.

of plant lipophilic fraction [30 – 33] due to their influence on the physiological mechanisms linked to the secondary metabolism [33 – 37]. In the present study, we found that sesquiterpenes were lower and monoterpenes higher in the EO coming from the relatively arid (Algeria) than the relatively humid (Italy) environments. Similar differences

Table 3. Biocidal activity of essential oil of *Artemisia arborescens* from two Algerian (Bechar and Ain Sefra) and two Italian (Capo Zafferano and Termini Imerese) sites against 42 strains of *Listeria monocytogenes*

Strain	Bechar	Ain Sefra	Capo Zafferano	Termini Imerese
13BO	+[a])	+	+	+/–
16BO	+	+	+	+/–
8BO	+	+	+	–
3BO	+	+	+	+
2BO	++	++	+	++
135	+	++	+	–
188	+	+	+	+
132	–	–	–	+/–
14BO	–	–	–	–
12BO	–	–	–	+/–
139	++	+	+/–	+
1BO	–	–	–	+
11BO	++	+	+/–	+/–
131	+	+	+	–
138	+	+	++	+
129	–	–	–	+
23BO	++	++	+	+
21BO	+	+	+/–	+/–
19BO	+	++	+	N.A.[b])
20BO	+	++	+	+/–
185	++	++	+	+/–
1BO	+	+	+/–	+
137	+	+	+	+
186	+	++	+	+
136	+	+	+/–	+/–
182	+	+	+	+
187	++	+	+	+
184	–	–	–	++
133	++	++	+/–	++
130	+	+	+	+
4BO	++	++	+	+/–
22BO	+	+	+	+
15BO	++	++	+	+/–
179	+	+	+/–	+/–
134	+	+	+/–	–
10BO	++	++	+/–	+/–
17BO	–	–	–	++
24BO	+	+	+	–
5BO	++	++	+	+
6BO	+	++	++	++
7BO	++	++	+	+
140	+	++	+/–	++
Total	35 (83.3%)[c])	35 (83.3%)	35 (83.3%)	35 (83.3%)
+/–	0 (0.0%)	0 (0.0%)	10 (23.8%)	13 (30.9%)
+	23 (54.8%)	20 (47.6%)	23 (54.8%)	16 (38.1%)
++	12 (28.6%)	15 (35.7%)	2 (4.8%)	6 (14.3%)

[a]) Degree of inhibition: – no inhibition; +/– inhibition hint; + inhibition (8 – 10 mm); ++ hard inhibition (> 10 mm). [b]) N.A., Not available. [c]) Percentage of the number of strains inhibited per each inhibition class.

were found in other plant species [38][39]. The accumulation of monoterpenes can be derived from an oxidation of the sequiterpene fraction and this can be the outcome of the plant response to the oxidative conditions, as also observed in other species [40 – 42].

The enantiomeric distribution of α-thujone, β-thujone, camphor, linalool, and terpinen-4-ol in the EOs is reported in *Table 2* and *Fig. 2*. We detected enantiomerically pure (–)-α-thujone (100%) and (+)-β-thujone (100%) in all samples. Enantiomerically pure (+)-β-thujone was previously reported by *El Montassir* et al. [21] in the EO of *A. arborescens* from Morocco using enantioselective HPLC with polarimetric detection. Pure (–)-α-thujone and (+)-β-thujone were also the main enantiomer in the oil of *A. herba-alba* [43] and *Thuja occidentalis* [44]. In *A. arborescens* EO, the enantiomers (+)-α-thujone and (–)-β-thujone were not found [29]. Enantiomerically almost pure (–)-camphor (between 98.15% and 98.77%) and (+)-linalool (between 99.12% and 98.32%) were also found in the EO. (+)-Terpinen-4-ol was found in amount exceeding 67%. It has been showed that different enantiomers can be used to trace the fate of compounds in the plant/soil/atmosphere continuum [45], have different flavors [46], and activity on human health [47]. The results of the enantiomeric distribution of camphor, linalool, and terpinen-4-ol are similar with those previously reported for EO of *A. arborescens* from southern Italy [29], which showed that chiral compounds can differ in plants coming from different environments.

Inhibitory Activity

In this study, the antibacterial activity of *A. arborescens* EOs was tested against several *L. monocytogenes* strains. Previous investigations of EOs from this species growing wild in Sicily [18] indicated that *L. monocytogenes* is particularly sensitive to the EO, but the degree of inhibition can vary according to composition of the EO. Algerian EOs were more effective than Italian EOs both in terms of number of strains inhibited and for the extent of such an inhibition (*Table 3*). In particular, 35 strains were inhibited by the Algerian EOs, whereas less than 25 strains by the Italian EOs. Furthermore, a massive inhibition, measured as a diameter of the growth inhibition larger than 10 mm, was displayed against 12 and 15 strains by the EO from Bechar and Ain Sefra, respectively. The EO from Termini Imerese showed a substantial inhibitory effect only against six strains, whereas a strong inhibition of *L. monocytogenes* by Capo Zafferano EO was found only against two strains.

The inhibitory activity of the EO against *Gram*-negative bacteria can depend upon several compounds and the interaction among single compounds. In our experiment, EOs from Algerian *A. arborescens* showed a higher ketone (β-thujone + camphor) content, which also are the most abundant compounds in the EO, than the Italian EOs. Thus, it is likely that the ketones are responsible for the inhibitory activity of the EO, as also observed in another ketone-rich *A. arborescens* EO [18]. Nonetheless, such effect could also depend on other compounds including α-pinene, 1,8-cineole, borneol, and thymol [48 – 50] or

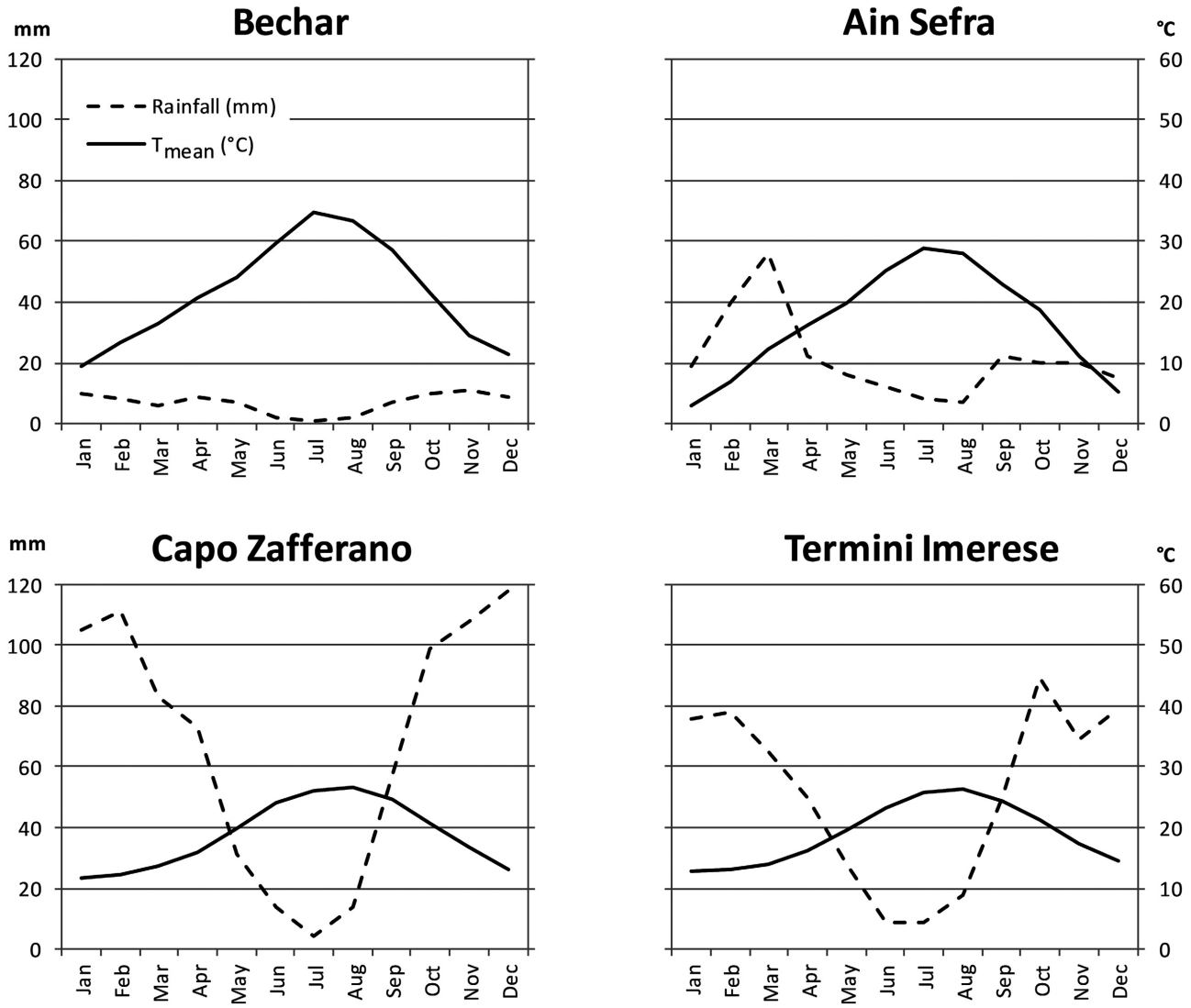

Fig. 3. *Bagnouls–Gaussen* diagrams of the average temperature (°, T_{mean}) and rainfall (mm) of the collection sites from Algeria (Bechar and Ain Sefra) and Italy (Capo Zafferano and Termini Imerese).

the enantiomeric distribution of some compounds, as also found by *Tabanca et al.* [51], whereas few or no inhibitory activity could be attributed to chamazulene and germacrene D, which are likely active against fungi and *Gram*-positive bacteria [18][52][53].

Conclusions

In conclusion, the results of this study showed that the EO of *A. arborescens* was characterized by a high inhibitory spectrum against *L. monocytogenes*. This implies that such EOs can be designated as a valuable option in the control of the food-borne pathogens. In addition, our results strongly support the hypothesis that *A. arborescens* has at least two different chemotypes: a *β*-thujone and a chamazulene type [19][22]. Further studies are needed to elucidate the role of the single compounds in the *A. arborescens* EO on the inhibition of bacteria.

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Experimental Part

Plant Material

The above-ground biomass of plants from four natural populations of *Artemisia arborescens* was collected from western Algeria (Bechar and Ain Sefra) and southern Italy (Sicily: Capo Zafferano and Termini Imerese) during the blossom stage of each population. The coordinates of the collection sites are: Bechar 31°54′59″ N, 2°18′0″ W, 870 m above sea level (a.s.l.); Ain Sefra 32°37′33′ N, 0°24′39″W, 1238 m a.s.l; Capo Zafferano 38°6′48″N, 13°31′14″E, 10 m a.s.l.; and Termini Imerese 37°58′26″N, 13°44′4″E, 25 m a.s.l.. The Bagnouls and

Gaussen diagram of the collection sites are shown in *Fig. 3*.

Essential Oil Extraction

The biomass collected was subjected to hydrodistillation for 3 h using a *Clevenger* apparatus. The EO yields were 0.41, 0.40, 0.32, 0.30 (% *v*/*w*) for sample from Bechar, Ain Sefra, Capo Zafferano, and Termini Imerese, resp. The EOs obtained were dried (Na_2SO_4) and stored in a sealed vial in the dark at 4° before analysis.

GC/MS Analysis

GC/MS analyses were performed on a *7890A* GC system coupled to a *5975C VL* mass spectrometer detector (*Agilent Technologies*) equipped with a *HP-5MS* capillary column (*J&W Scientific*, 30 m × 0.25 mm × 0.25 μm). Data acquisition and processing were performed using the MSD Chemstation E.01.01.335 (*Agilent*) software [21] [44]. A quantity of 1 μl of diluted EO (0.05 g in 1.5 ml of CH_2Cl_2) was injected. The experimental conditions developed in the laboratory were solvent delay, 2 min; column temp. program, 2 min at 80 °C, then 80 °C to 200 °C (5 °C/min), then 200 °C to 260 °C (20 °C/min), and held at final temp. for 5 min; temps. of injector (split ratio 1:60) and detector were 250 °C; carrier gas was He at a flow rate of 1.2 ml/min; ionization voltage 70 eV; electron multiplier, 1 kV.

GC Analysis

GC Analyses were performed on a *7890A* GC (*Agilent Technologies*) system with a flame-ionization detector (FID) equipped with a *HP5* capillary column (*J&W Scientific*, 30 m × 0.25 mm × 0.25 μm) and *Rt-bDEXse* enantioselective capillary column (*Restek*, 30 m × 0.25 mm × 0.25 μm). Data acquisition and processing were performed using the Chemstation B.04.03-SP1 (87) (*Agilent*) software. Experimental conditions for HP5 were: oven temp. 2 min at 80 °C, then 80 °C to 200 °C (5 °C/min), then 200 °C to 260 °C (20 °C/min), and held at final temp. for 5 min. Injector and detector temps. were set at 250 °C. Experimental conditions for Rt-bDEXse were: oven temp. 1 min at 40 °C, then 40 °C to 120 °C (1 °C/min), then 120 °C to 200 °C (5 °C/min), and held at final temp. for 5 min. Injector and detector temps. were set at 230°.

H_2 was the carrier gas at a flow rate of 1.2 ml/min. Linear retention indices were calculated with reference to *n*-alkanes (C_8 – C_{28}). EOs compositions were given as relative area percentages and for peak accounting for more than 0.1% of the total peak's area. The identification of the compounds was based on the comparison of their retention indices with those of authentic samples [28].

Antimicrobial Activity

The inhibitory properties of *A. arborescens* EOs on bacterial growth was tested against 42 *L. monocytogenes* strains. All strains were retrieved from the culture collection of the Department of Sciences for Health Promotion and Mother-Child Care "G. D'Alessandro'' (Palermo, Italy). All strains were isolated from foodstuff or human stools, subcultured in brain-heart infusion (BHI) agar (*Oxoid*) and incubated overnight at 37°.

The antibacterial activities were tested applying the paper disc diffusion method reported by *Militello et al.* [18]. The indicator strains were inoculated at a final concentration of 10^7 CFU/ml in soft agar (0.7 g/l). Sterile water and streptomycin (10%, *w*/*v*) were used as negative and positive controls, resp. Plates were incubated at 37° for 24 h and the inhibitory activity was evaluated as positive if a definite clear area was detected around the paper disc.

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