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Essential oil composition of *Cladanthus eriolepis* (Coss. ex Maire) Oberpr. & Vogt, an endemic species to Morocco

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ABSTRACT

Cladanthus eriolepis (*C. eriolepis*) (Coss. ex Maire) Oberpr. & Vogt. is endemic to High Atlas (Dades Gorge and Todgha Gorge), the Anti Atlas and Saharan Morocco. It is known under the vernacular names 'Alougjim, gtaa-eddib, laatetecha' and 'Lamghizal'. Two essential oil samples have been isolated from aerial parts and analyzed by combination of chromatographic and spectroscopic techniques [gas chromatography (GC) in combination with retention indices (RI), gas chromatography-mass spectrometry (GC/MS) and ¹³C-NMR spectroscopy]. The compositions of both oil samples were dominated by hemiterpene esters such as isobutyl isobutyrate (21.2% and 20.8% respectively) and isobutyl angelate (22.0% and 22.4% respectively). Other esters present at appreciable contents were 2-methylallyl isobutyrate (5.3% and 5.5%), 2-methylbutyl isobutyrate (5.7% and 5.8%), 2-methylallyl angelate (4.6% and 4.9%) and 2-methylbutyl angelate (7.7% and 7.2%) beside α -pinene (9.5% and 5.8%) and β -bisabolene (2.8% and 4.0%). The compositions of the investigated oil samples from *C. eriolepis* differed substantially with literature data and with those of other *Cladanthus* species growing wild in Morocco.

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Introduction

The genus *Cladanthus* (Asteraceae) is a small genus that includes only five species, all native of the Mediterranean basin and South-Western Europe (1). Moroccan flora records five species for this genus, including *Cladanthus mixtus* (L.) Chevall. and *C. arabicus* (L.) Cass. which are also common to the Mediterranean basin while three of these species are endemic: *Cladanthus scariosus* (Ball.) Oberpr. & Vogt, *C. flahaultii* (Emb.) Oberpr. & Vogt and *C. eriolepis* (Coss. ex Maire) Oberpr. & Vogt (2).

Cladanthus eriolepis (Coss. ex Maire) Oberpr. & Vogt [synonyms: *Ormenis eriolepis* Coss. ex Maire; *Chamaemelum eriolepis* (Coss. ex Maire) Benedi] (2) is known under the vernacular names 'Alougjim, gtaa-eddib, laatetecha' and 'Lamghizal' in the region of Zagora (2).

C. eriolepis is an annual, spontaneous plant, 30 to 50 cm high. The stems are erect, branched from the base, with a basal leaf rosette quickly deciduous, ending in solitary, pedunculated flower heads from 1 to 5 cm. The peripheral flowers are sterile hemiligulated, with orange blade with three apical teeth, yellow tubulars, all fertile. The fruit is an achene, ovoid, hairless and longitudinally striated.

This species occupies the stony environments, sands and steppes of dry to arid regions. The geographic

repartition area includes the High Atlas (Dades Gorge and Todgha Gorge), the Anti Atlas and Saharan Morocco (2). This plant is frequently used in traditional medicine as stomachic, anthelmintic and antidiabetic (3). In the region of Zagora, *C. eriolepis* is especially used to treat gastrointestinal diseases.

Previous studies on solvent extracts reported antibacterial (4), antileishmania (4,5) and antitumor activities (6). The methanolic extract of *C. eriolepis* displayed low cytotoxic effect on SiHa and HeLa cervical cancer cell lines (6,7). Otherwise, the antimicrobial activity of *C. eriolepis* essential oil was checked against bacterial and fungal strains. The composition of this oil sample was dominated by camphor (37.0%), beside monoterpene hydrocarbons: sabinene (10.3%), α -pinene (6.3%) and *p*-cymene (6.1%) (8). After the submission of our results, another paper (9) reported on the composition of an oil sample isolated from *C. eriolepis* whole plant, dominated by α -pinene (13.0%), isobutyl angelate (10.7%), 2-methylbutyl angelate (9.5%) and germa-crene D (7.1%).

We report herein the chemical compositions of two oil samples isolated from plants harvested in Southern Morocco that differ drastically from the first recently published (8) and substantially from the second one (9).

Experimental

Plant material and essential oil isolation

Samples of wild growing *C. eriolepis* were harvested in May 2019, during flowering, from two sites in Zagora region (South of Morocco), S1: Jbal zagora, 30° 19'28.54"N, 005°48'47.33"W; 768 m above sea level; S2: Ouled ouchah, 30°24'59.21"N, 005°53'24.54"W; 749 m above sea level. Collective samples (five individual plants) were collected in a limited area.

Specimens of plants were taxonomically identified by Curator Cyrille Chatelain from the Conservatory and Botanical Garden, Department of Culture and Sport, Geneva, Switzerland. Voucher specimens from each locality are kept at the Herbarium of National Institute of Agriculture Research (INRA).

Essential oil isolation

The fresh raw material was air-dried in the shade for 5 days and then submitted to hydrodistillation in a Clevenger-type apparatus for 3 hours (mass). The yellowish essential oil (mass) was kept in glass tubes at 4°C.

GC-FID analysis

GC-FID analyses were carried out using a Clarus 500 Perkin Elmer (Perkin Elmer, Courtaboeuf, France) system equipped with a FID and two fused-silica capillary columns (50 m x 0.22 mm, film thickness 0.25 µm), BP-1 (polydimethylsiloxane) and BP-20 (polyethylene glycol). The oven temperature was programmed from 60°C to 220°C at 2°C/min and then held isothermal at 220°C for 20 min; injector temperature: 250°C; detector temperature: 250°C; carrier gas: H₂ (0.8 mL/min); split: 1/60; injected volume: 0.5 µL. The relative proportions of the oil constituents were expressed as percentages obtained by peak-area normalization, without using correcting factors. Retention indices (RI) were determined relative to the retention times of a series of n-alkanes with linear interpolation (Target Compounds software from Perkin Elmer).

GC/MS analysis

GC/MS analyses were performed on a Clarus SQ8S Perkin Elmer TurboMass detector (quadrupole), directly coupled to a Clarus 580 Perkin-Elmer Autosystem XL, equipped with a BP-1 (polydimethylsiloxane) fused-silica capillary column (60 m x 0.22 mm i.d., film thickness 0.25 µm). The oven temperature was programmed from 60°C to 230°C at 2°C/min and then held isothermal at 230°C for 45 min; injector temp., 250°C; ion-source temp., 150°C; carrier gas, He (1 ml/min); split ratio, 1:80;

injection volume, 0.2 µL; ionization energy, 70 eV. The electron ionization (EI) mass spectra were acquired over the mass range 35–350 Da.

Nuclear magnetic resonance

¹³C-NMR spectra were recorded on a Bruker AVANCE 400 Fourier Transform spectrometer operating at 100.63 MHz for ¹³C, equipped with a 5 mm probe, in CDCl₃, with all shifts referred to internal TMS. The following parameters were used: pulse width = 4 µs (flip angle 45°); acquisition time = 2.7 s for 128 K data table with a spectral width of 25 000 Hz (250 ppm); CPD mode decoupling; digital resolution = 0.183 Hz/pt. The number of accumulated scans was 3000 for each sample (40 mg of essential oil in 0.5 mL of CDCl₃).

Identification of individual components

Identification of the individual components was carried out: (i) by comparison of their GC retention indices (RI) on polar and apolar columns with those of reference compounds compiled in a laboratory-built library and with literature data (10); (ii) on computer matching against commercial mass spectral libraries (10–12); (iii) on comparison of the signals in the ¹³C-NMR spectra of the samples with those of reference spectra compiled in the laboratory spectral library, with the help of a laboratory-made software (13,14).

Results and discussion

Aerial parts of *C. eriolepis* harvested in two sites located at Jbal zagora and Ouled ouchah, in Zagora region (Southern Morocco), were dried and then separately submitted to hydrodistillation using a Clevenger-type apparatus. Yellowish essential oil was obtained with yields of 0.4% (S1) and 0.5% (S2) (v/dw), respectively. Both oil samples were subjected to chromatographic and spectroscopic analyses: GC(FID) on two columns of different polarity, GC/MS and ¹³C-NMR following a computerized method developed at the University of Corsica (13,14). In total, 44 compounds have been identified, they accounted for 94.9% (S1) and 93.2% (S2) of the whole composition (Table 1, Figure 1). The two compositions were similar and they were characterized by the occurrence of esters containing one or two hemiterpene sub-structures. Indeed, the major components were isobutyl isobutyrate (21.2% and 20.8%, respectively) and isobutyl angelate (22.0% and 22.4%, respectively). Other components present at appreciable contents were 2-methylallyl isobutyrate (5.3% and 5.5%), 2-methylbutyl isobutyrate (5.7% and 5.8%), 2-methylallyl angelate (4.6% and 4.9%),

Table 1. Chemical composition of *Cladanthus eriolepis* aerial parts essential oils.

N°	Components ^a	Rla lit ^b	Rla	Rip lit ^l	Rlp	S1 %	S2 %	Identification
1	Isobutyl acetate	755 ^c	753	1018 ^m	nd	0.2	0.2	GC(RI), GC/MS
2	Propyl isobutyrate	842 ^d	838	1054 ⁿ	1049	0.1	0.1	GC(RI), GC/MS
3	Isobutyl propionate ^e	851 ^e	848	1085 ^m	1083	0.9	0.8	GC(RI), GC/MS, NMR
4	2-Methylbutyl acetate	868 ^f	859	1145 ^o	1125	0.7	0.9	GC(RI), GC/MS
5	Isobutyl isobutyrate	899 ^g	901	1095 ^m	1096	21.2	20.8	GC(RI), GC/MS, NMR
6	(E)-2-Methylbutenyl propionate	-	903	-	1251	1.7	3.0	GC(RI), GC/MS, NMR
7	2-Methylallyl isobutyrate	914 ^h	913	-	1184	5.3	5.5	GC(RI), GC/MS, NMR
8	α -Thujene	932	924	1025	1019	0.1	tr	GC(RI), GC/MS
9	α -Pinene	936	932	1026	1019	9.2	5.8	GC(RI), GC/MS, NMR
10	2-Methylbutyl propionate	952 ^g	954	1192 ^p	1193	0.5	0.5	GC(RI), GC/MS
11	Sabinene	973	967	1122	1126	0.2	0.3	GC(RI), GC/MS
12	β -Pinene	978	973	1110	1115	0.1	0.1	GC(RI), GC/MS
13	Propyl angelate	980 ⁱ	976	-	nd	0.2	0.1	GC(RI), GC/MS
14	Myrcene	987	982	1161	1164	0.4	0.2	GC(RI), GC/MS, NMR
15	Isobutyl 2-methylbutyrate	991 ^j	990	1180 ^p	1183	0.8	1.2	GC(RI), GC/MS
16	Isobutyl isovalerate	989 ^g	992	1167 ^q	1179	0.5	0.7	GC(RI), GC/MS
17	3-Methylbutyl isobutyrate	994	996	1190 ^r	1195	0.1	0.1	GC(RI), GC/MS
18	2-Methylbutyl isobutyrate	1001 ^j	1003	1203 ^s	1201	5.7	5.8	GC(RI), GC/MS, NMR
19	p-Cymene	1015	1013	1270	1276	1.0	0.7	GC(RI), GC/MS, NMR
20	Limonene*	1025	1022	1198	1205	2.0	1.4	GC(RI), GC/MS, NMR
21	1,8-Cineole*	1025	1022	1211	1215	0.9	1.7	GC(RI), GC/MS, NMR
22	Isobutyl angelate	1027	1036	1293 ^t	1293	22.0	22.4	GC(RI), GC/MS, NMR
23	Prenyl isobutyrate	1053 ^k	1040	-	nd	1.0	1.0	GC(RI), GC/MS
24	2-Methylallyl angelate	1040	1047	-	1378	4.6	4.9	GC(RI), GC/MS, NMR
25	γ -Terpinene	1051	1050	1245	1249	0.3	0.1	GC(RI), GC/MS
26	Linalool	1086	1086	1544	1550	tr	0.1	GC(RI), GC/MS
27	2-Methylbutyl 2-methylbutyrate	1090 ^g	1091	-	1283	0.4	0.5	GC(RI), GC/MS, NMR
28	3-Methylbutyl angelate	1125	1133	-	1376	0.2	0.2	GC(RI), GC/MS
29	2-Methylbutyl angelate	1130	1138	1395 ^t	1397	7.7	7.2	GC(RI), GC/MS, NMR
30	Terpinen-4-ol	1164	1164	1601	1605	0.1	0.1	GC(RI), GC/MS
31	α -Terpineol	1176	1175	1694	1699	0.5	0.5	GC(RI), GC/MS
32	Bornyl acetate	1270	1272	1580	1574	tr	0.1	GC(RI), GC/MS
33	Myrtenyl acetate	1313	1308	1692	1691	0.2	0.2	GC(RI), GC/MS
34	Geranyl acetate	1362	1362	1752	1751	0.3	0.8	GC(RI), GC/MS, NMR
35	(E)- β -Farnesene	1446	1449	1664	1667	tr	0.1	GC(RI), GC/MS
36	<i>ar</i> -Curcumene	1473	1472	1773	1775	0.1	0.2	GC(RI), GC/MS
37	Germacrene D	1479	1478	1708	1709	0.7	0.1	GC(RI), GC/MS, NMR
38	β -Bisabolene	1503	1503	1728	1728	2.8	4.0	GC(RI), GC/MS, NMR
39	δ -Cadinene	1520	1517	1756	1758	0.2	0.1	GC(RI), GC/MS
40	Geranyl 2-methyl butyrate	1591	1591	1904 ^u	1920	0.5	0.4	GC(RI), GC/MS
41	<i>epi</i> -Cubanol	1623	1618	2068	2064	0.5	0.2	GC(RI), GC/MS, NMR
42	α -Cadinol	1643	1641	2227	2231	0.3	0.2	GC(RI), GC/MS
43	β -Bisabolol	1659	1655	2143	2152	0.2	0.2	GC(RI), GC/MS
44	α -Bisabolol	1673	1669	2214	2231	0.5	0.4	GC(RI), GC/MS, NMR
	Total					94.9	93.2	

^aComponents are listed following their order of elution on apolar column. Percentages measured on apolar column, except those with * (polar column); Rla, Rip: Retention indices on apolar and polar columns, respectively; NMR: compound identified by ¹³C NMR, at least in one oil sample. Rla lit, apolar literature retention indices; ^b Retention indices reported in Terpenoids Library Website (35) or in references: ^c (36); ^d (37); ^e (38); ^f (39); ^g (40); ^h (34); ⁱ (31); ^j (41); ^k (42); Rlp lit, polar literature retention indices ^l Retention indices reported in Babushok et al. (43) or in references ^m (44); ⁿ (45); ^o (46); ^p (47); ^q (48); ^r (49); ^s (50); ^t (51); ^u (52); nd: not determined.

2-methylbutyl angelate (7.7% and 7.2%). Beside hemiterpene esters, various monoterpene hydrocarbons, oxygenated monoterpenes sesquiterpene hydrocarbons and oxygenated sesquiterpenes have been identified at low content. Among these, only α -pinene (9.5% and 5.8%), on the one hand, and β -bisabolene (2.8% and 4.0%), on the other hand, exhibited a fair content.

Obviously, the compositions of our oil samples differed drastically for the one recently reported, dominated by camphor and monoterpene hydrocarbons and characterized by the lack of hemiterpene esters (8). They differed also substantially from that of Chibane et al. (9) by their high content of hemiterpene esters, isobutyl

isobutyrate (21.2% and 20.8% vs. 1.9%), isobutyl angelate (22.0% and 22.04% vs. 10.7%) and 2-methylallyl isobutyrate (5.3% and 5.5% vs. 0.3%).

Otherwise, it looked opportune to compare with the composition of essential oils isolated from other Moroccan *Cladanthus* species:

- *C. scariosus* essential oil contained mainly germacrene D followed by (E)-chrysanthenyl acetate, chamazulene, sabinene, α -pinene, τ -muurolol and (E, E)-farnesyl acetate (15);
- The chemical composition of the essential oils of the aerial parts (stems and leaves/flowers) of *C. arabicus*

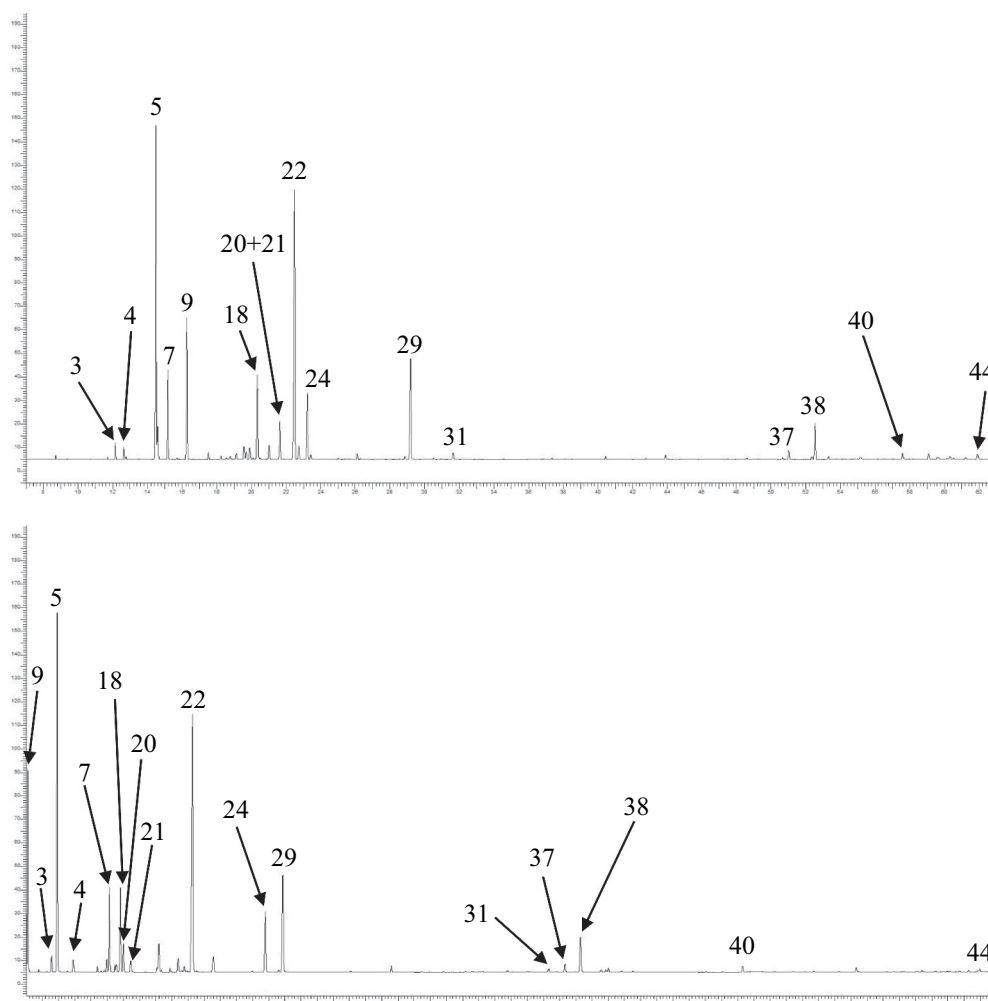


Figure 1. GC-FID chromatograms on apolar (top) and polar columns (bottom) of *Cladanthus eriolepis* essential oil (sample S1). Peak numbers correspond to ordinal numbers in Table 1.

was dominated by monoterpene hydrocarbons: sabinene; β -pinene, myrcene and at lesser extent, α -pinene, α -phellandrene and p-cymene (16,17); - lastly, *C. mixtus*, mentioned in the literature under this name or one of its various synonyms, displayed a large chemical variability. Indeed, it seems that the chemical composition of the oil samples was dependent of the locality of harvest of the plant. Two types of essential oils could be differentiated i) those containing a major component such as bornyl acetate (45%) (18), santolina alcohol (32–55%) (19–21), (E)- β -farnesene (35.5–50.3%) (22,23); *trans*-nerolidol (44.1%) (24) or 2-methyl-2-*trans*-butenyl methacrylate (32.8–35.2%) (22,23,25) ii) those containing two or more components in the same range of percent: camphor (14.4–29%), (E)- β -farnesene (8.3%), 2-tridecanone (21.5%) (24); α -pinene (11.6%), santolina alcohol (10.2%), (E)- β -farnesene (8.6%) (26); camphor (13.6–26.3%), β -myrcene (2.5–17.4%) and santolinatriene (2.5–15.3%) (22); myrcene (26.5%), (E)-

β -farnesene (17.9%) and 2-tridecanone (15.5%) (22); santolina alcohol (17.4%) and 1,8-cineole (11.6%) (22); camphor (21.4%), santolinatriene (10.1%) (27); 2-tridecanone (21.5%), camphor (14.4–29.0%) (26), germacrene D (11.5%), 1,8-cineole (10.3%), and *cis*-methyl isoeugenol (9.0%) (28).

Otherwise, hemiterpene esters-rich essential oils have been reported, particularly essential oils from Roman chamomile (*Chamaemelum nobile*, synonym *Anthemis nobilis*). For instance, the composition of two oil samples from *C. nobile* grown in Northern Italy was dominated by isobutyl angelate (38.5/6.3%) and 2-methylbutyl angelate (20.3/18.2%) (29). The same esters were the main components (21.6% and 14.4%, respectively) of an oil sample isolated from plants cultivated in Slovak republic, accompanied by 3-methylamyl angelate (8.4%) (30). Isobutyl angelate (25.9–29.8%) and 2-methylbutyl angelate (11.6–17.2%) dominated the composition of oil samples isolated

from plants cultivated in Iran, a third main component being propyl tiglate (10.8–13.1%) (31). Recently, a commercial oil sample contained mainly various esters of angelic acid: isobutyl angelate (44.8%), isoamyl angelate (27.4%), 2-methylallyl angelate (9.5%) and 2-methylbutyl angelate (6.6%) (32). In parallel, oil samples of Spanish *Chamaemelum fuscatum* exhibited (E)-2-methyl-2-butenyl methacrylate (18.5–27.6%) and 2-methylallyl isobutyrate (7.5–9.8%), as major components (33) while another oil sample contained mainly 2-methylallyl isobutyrate (43.1%) and (E)-2-methyl-2-butenyl methacrylate (27.6%) (34).

Conclusions

The compositions of *C. eriolepis* essential oils, isolated from plants harvested in Southern Morocco, and reported in this study differed substantially from the recently reported compositions (8,9) by their high contents (more than 75%) of hemiterpene esters. They present some originality vs. other Moroccan *Cladanthus* spp. oils. The combination of chromatographic and spectroscopic techniques appeared efficient for the identification of esters bearing one or two hemiterpene substructures.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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