

Article

Integrated Analysis by GC/MS and ^{13}C NMR of Moroccan *Cladanthus mixtus* Essential Oil; Identification of Uncommon Epoxyfarnesanes

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Abstract: *Cladanthus mixtus* (L.) Chevall., Asteraceae, also known as Moroccan chamomile, is a spontaneous, annual plant growing wild in North-Western Morocco. Economically, the essential oil of *C. mixtus* is of high interest, Morocco being the only supplier on the international market. Two essential oil samples (EO) were isolated from aerial parts of *Cladanthus mixtus* (L.) Chevall., and analyzed by a combination of chromatographic and spectroscopic techniques (gas chromatography (GC) in combination with retention indices (RI), gas chromatography-mass spectrometry (GC/MS), and ^{13}C NMR spectroscopy). Computer matching against the in-house ^{13}C NMR library allowed the identification of the eight components at appreciable contents, namely 3,6,6,9-bis-epoxy-farnesa-1,7(14),10-triene, and its 3-epi, 9-epi, and 3,9-diepi epimers, and 6,9-epoxy-farnesa-1,7(14),10-trien-3-ol and its 3-epi, 6-epi, and 3,6-diepi epimers. Our results confirm the tremendous chemical variability of Moroccan *C. mixtus* essential oil and the usefulness of ^{13}C NMR analysis, in combination with GC(RI), for the identification of uncommon oxygenated sesquiterpenes that induce an original composition.

Keywords: *Cladanthus mixtus*; chemical variability; bis-epoxy-farnesa-1,7(14),10-triene; 6,9-epoxy-farnesa-1,7(14),10-trien-3-ol; ^{13}C NMR



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1. Introduction

Cladanthus mixtus (L.) Chevall. (synonyms: *Anthemis mixta* (L.), *Chamaemelum mixtum* (L.) All., *Ormenis mixta* (L.) Dumort, *Ormenis multicaulis* Braun-Blanq. & Maire), also known as Moroccan chamomile, Asteraceae, is a spontaneous, annual plant 10 to 40 cm tall with numerous erect, lying, or ascending stems terminated by fragrant flower heads with ligulate, white, and sterile external flowers decorated with yellow at their base and fertile yellow tuberos internal flowers. This species is a sialophyte that abounds in the voids of semi-arid and sub-humid bioclimates on sandy soils of the thermomediterranean stage. It is generally found in open forests, fields, sand and stone agricultural landscapes, and low mountains. In Morocco, this plant is known by the vernacular name “Hellâla” [1] and is mainly distributed in two disjointed areas, the first between Tangier, Ouezzane, Souk Larbaa, Moulay Bouselham, and Azilah, and the second between Kenitra, Sidi Slimane, Khémisset, and Rabat [2]. Economically, the essential oil of *C. mixtus* is of high interest. Morocco is the world’s leading producer [3]. In terms of quality, the essential oil of *C. mixtus* was ranked ninth among the 20 best essential oils produced in Morocco. In Morocco, *C. mixtus* is advised as an anxiolytic for the rebalancing of the central nervous system; it has great value in nervous breakdowns and for mild hepatic and gastric insufficiency and

Colibacillary colitis [4]. Thanks to its pleasant smell, the essential oil of *C. mixtus* is sought after in perfumery, cosmetics, and medicine [4]. Concerning the chemical composition of the essential oil of *Cladanthus mixtus* (Moroccan chamomile), various studies have shown a very important chemical polymorphism.

The chemical composition of *C. mixtus* essential oil has been investigated, most of the time, by GC/MS in combination with retention indices on the non-polar or semi-polar chromatography column. Various chemical compositions have been reported describing an important chemical variability, and they have recently been reviewed [5]. The authors listed the 264 compounds that have been identified at least once in *C. mixtus* essential oil isolated from plants harvested all around the Mediterranean Sea (Algeria, Morocco, France, and Italy).

We focused our attention on Moroccan *C. mixtus* essential oil, which also displayed a fair chemical variability. According to the literature data, the reported essential oil compositions are distributed within two groups: (i) those that displayed a major component (Group 1) and (ii) those that contained various components with more or less similar content (Group 2).

- The compositions of essential oils of group 1 were dominated either by santolina alcohol (24.1–66.0%) [6–11], camphor (17.8–33.0%) [12–14], 2-methyl-2-trans-butenyl methacrylate (32.0–35.2%) [12,15,16], (E)- β -farnesene (35.5–50.3%) [12,15], or (E)-nerolidol (44.1%) [13];
- Samples of group 2 contained mainly monoterpenes (α -pinene, myrcene, 1,8-cineole, and camphor), irregular monoterpenes (santolina triene and santolina alcohol), sesquiterpene hydrocarbons (germacrene D and (E)- β -farnesene), or miscellaneous (2-tridecanone and (Z)-methyl isoeugenol) [12,13,17,18].

The aim of this paper that reports on the composition of two *C. mixtus* oil samples submitted to combined analysis by chromatographic and spectroscopic techniques is to demonstrate overall the importance of ^{13}C NMR in identifying uncommon oxygenated sesquiterpenes, the presence of which induces an unusual composition.

2. Materials and Methods

2.1. Plant Material and Essential Oil Isolation

Aerial parts of *C. mixtus* have been collected in two locations (Figure 1, Table 1). Hydrodistillation (2 h) using a Clevenger-type apparatus of *C. mixtus* aerial parts (300 g) in 2 L flask yielded 0.3 mL of essential oil for both samples. To avoid any damage, the samples were stored at 5 °C in amber vials.



Figure 1. *Cladanthus mixtus* (L.) Chevall (Souk Had location).

Table 1. Characteristics of localities of harvest.

Oil Sample	Location of Harvest	Elevation (m)	Longitude/Latitude	Date of Harvest
SH	Souk Had	14	N: 34°51'13.81" W: 6°39'04.82"	6 April 2022
SS	Ain Chkef forest Sidi Slimane	451	N: 33°98'57.60" W: 5°01'84.39"	12 April 2022

2.2. GC-FID Analysis

GC-FID analyses were carried out using a Clarus 500 Perkin Elmer (Perkin Elmer, Courtaboeuf, France) chromatograph equipped with two FID and two fused-silica capillary columns (50 m length \times 0.22 mm internal diameter, film thickness 0.25 μ m), 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; and injected volume, 0.5 μ L. The relative proportions of the essential 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 (C8–C28) with linear interpolation (Target Compounds software from Perkin Elmer, V1.2019, Courtaboeuf, France).

2.3. 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 chromatograph, equipped with a BP-1 (polydimethylsiloxane) fused-silica capillary column (50 m length \times 0.22 mm internal diameter, film thickness 0.25 μ m). The oven temperature was programmed from 60 to 220 °C at 2°/min and then held isothermal at 220 °C for 30 min; injector temp., 250 °C; ion source temp., 150 °C; carrier gas, He (1 mL/min); split ratio, 1:80; injection volume, 0.5 μ L; and ionization energy, 70 eV. The electron ionization (EI) mass spectra were acquired over the mass range of 35–350 Da.

2.4. Nuclear Magnetic Resonance

¹³C NMR spectra were recorded on a Bruker AVANCE 400 Fourier Transform spectrometer equipped with a 5 mm probe operating at 100.63 MHz for ¹³C, in CDCl₃, with all shifts referred to internal Tetramethylsilane (TMS) at room temperature (298 °C). The following parameters were used: pulse width = 4 μ s (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; and digital resolution = 0.183 Hz/pt. For each sample (40 mg of essential oil in 0.5 mL of CDCl₃), 3000 scans were recorded.

2.5. Identification of Individual Components

Identification of the individual components was carried out (i) by comparison of their GC retention indices (RI) on non-polar and polar columns with those of reference compounds compiled in the in-house library and with literature data [19–21]; (ii) on computer matching against commercial mass spectral libraries [21–23]; and (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 [24,25] (Figure 2).

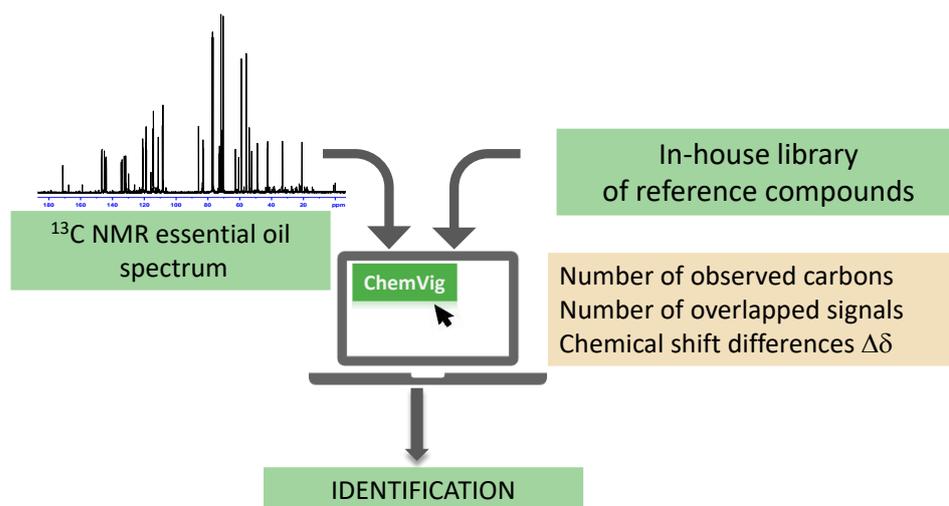


Figure 2. Identification of individual compounds using ¹³C NMR.

3. Results

3.1. Methods for Identification of Individual Components of Essential Oils

Identification of individual components of essential oils is routinely performed by using a fast-scanning mass spectrometer associated with a gas chromatograph. The mass spectrum of every component is compared with those of reference compounds compiled in commercial or in-house libraries. Two-dimensional GC coupled with MS may be useful for the analysis of complex essential oils in order to individualize components that are co-eluted when a unique column is employed and then to record more reliable mass spectra. Commercial libraries contain mass spectra of thousands of compounds (covering all fields of research); among these, a few thousand belong to volatile components of essential oils. In-house MS libraries are constructed with pure compounds or compounds whose identity is ascertained in one essential oil sample by spectroscopic techniques. Therefore, the reference MS spectra of various new compounds identified in essential oils and reported in the literature are not directly available to be introduced in a given MS library. In most analyses, identification of the compounds suggested by MS is confirmed by comparison of its retention indices (RI) on non-polar and polar chromatography columns with those of reference compounds compiled in the literature and/or homemade RI libraries.

In parallel, it has been shown that ¹³C NMR can be used for the non-destructive, non-separative identification of individual components of essential oils. In this computerized procedure developed at the University of Corsica, an individual component is identified by comparison of the signals of the mixture spectrum with those of reference spectra compiled in a library [24,25] (Figure 2). It should be pointed out that structural elucidation of every new compound proceeds, inter alia, via ¹³C NMR spectroscopy, and therefore, the ¹³C NMR spectrum is fully reported in the publication. It could be added that nowadays, a high-field spectrometer allows us to record the ¹³C NMR spectrum of isolated compounds at the mg level. The diluted solutions avoid intermolecular influence, and therefore, chemical shifts are perfectly reproducible. In practice, two spectral data libraries were constructed; the first one contains spectra recorded in the lab, and the second contains spectra reported in the literature for every new compound isolated from plants or obtained by synthesis. Both libraries are continuously implemented. Each component is identified considering three parameters directly available from the in-house computer program: (i) the number of observed carbons with respect to the number of expected signals, (ii) the number of overlapped signals of carbons that possess the same chemical shift, and (iii) the difference of the chemical shift of each signal in the mixture spectrum and in the reference (Figure 2). A compound is considered as identified when at least 50% of its signals belonging solely to that molecule are observed [24,25].

The benefit of using various chromatographic and spectroscopic techniques for the analysis of essential oil has been demonstrated and exemplified. For instance, the key role of ^{13}C NMR analysis in the identification of individual components of Ivoirian *Polyalthia longifolia* leaf oil and of *Xanthocyparis vietnamensis* wood oil has been highlighted [26,27]. ^{13}C NMR analysis also appeared suited for the identification of stereoisomers [28,29].

3.2. Chemical Composition of the Two Oil Samples

EOs were isolated from aerial parts of *C. mixtus* harvested in North-Western Morocco, at Souk Had (sample SH) and at Ain Chkef forest, near Sidi Slimane (Sample SS). Yields were measured as 0.10% (*v/w*) for both oil samples, which were submitted to GC(RI) on two column (non-polar and polar phases), GC/MS, and ^{13}C NMR analyses.

We will detail below the identification of individual components of two Moroccan *C. mixtus* essential oil samples (Table 2) by (i) GC(RI), GC/MS, and ^{13}C NMR (major components), (ii) GC(RI) and GC/MS (minor components), and (iii) by GC(RI) and ^{13}C NMR (uncommon oxygenated sesquiterpenes).

Table 2. Chemical composition of two oil samples from aerial parts of Moroccan *Cladanthus mixtus*.

N°	Components ^a	RIa Lit ^b	RIp Lit ^b	RIa	RIp	SH%	SS%	Identification Mode
1	3-Methyl-1-pentanol	825 ^c	1327 ^c	829	1326	-	0.1	RI, MS
2	Isobutyl isobutyrate	899	1095	901	1096	0.9	0.1	RI, MS, ^{13}C NMR
3	α -Thujene	932	1025	924	1019	0.7	0.8	RI, MS, ^{13}C NMR
4	α -Pinene	936	1026	932	1019	16.1	4.6	RI, MS, ^{13}C NMR
5	Camphene	947	1068	945	1066	0.2	0.2	RI, MS
6	Sabinene	973	1122	967	1126	3.2	1.0	RI, MS, ^{13}C NMR
7	β -Pinene	978	1110	972	1115	0.5	0.1	RI, MS
8	Myrcene	987	1161	982	1164	1.1	1.3	RI, MS, ^{13}C NMR
9	Isobutyl 2-methylbutyrate	990 ^d	1183 ^d	990	1183	0.2	-	RI, MS
10	Isobutyl isovalerate	992 ^d	1179 ^d	992	1179	0.1	-	RI, MS
11	Isopentyl isobutyrate	996 ^d	1195 ^d	999	1195	0.1	0.1	RI, MS
12	2-Methylbutyl isobutyrate	1003 ^d	1201 ^d	1003	1201	1.1	-	RI, MS, ^{13}C NMR
13	α -Terpinene	1011	1178	1010	1183	-	0.1	RI, MS
14	<i>p</i> -Cymene	1015	1270	1013	1276	2.2	0.2	RI, MS, ^{13}C NMR
15	Limonene	1025	1198	1022	1205	3.6 *	1.0 *	RI, MS, ^{13}C NMR
16	1,8-Cineole	1025	1211	1022	1215	20.8 *	3.9 *	RI, MS, ^{13}C NMR
17	Santolina alcohol	1019 ^e	1391 ^e	1022	1404	-	3.4 *	RI, MS, ^{13}C NMR
18	Isobutyl angelate	1036 ^d	1293 ^d	1035	1293	1.5	-	RI, MS, ^{13}C NMR
19	γ -Terpinene	1051	1245	1050	1249	0.3	0.2	RI, MS
20	Artemisia alcohol	1071	1510	1070	1507	-	0.2	RI, MS
21	Nonanal	1084	1391	1083	1389	-	0.1	RI, MS
22	Linalool	1086	1544	1084	1550	0.6	0.1	RI, MS
23	Hotrienol	1088	1602	1086	1611	-	0.6	RI, MS, ^{13}C NMR
24	2-Methylbutyl 2-methyl butyrate	1089 ^f	1279 ^f	1090	1283	0.5	-	RI, MS, ^{13}C NMR
25	3-Methylpentyl isobutyrate	1095 ^g	nd	1103	nd	0.8	0.2	RI, MS
26	α -Campholenal	1107	1496	1107	1486	-	0.1	RI, MS
27	trans-Pinocarveol	1126	1661	1125	1655	0.7	0.4	RI, MS, ^{13}C NMR
28	Pinocarvone	1140	1575	1142	1569	0.2	0.5	RI, MS
29	Borneol	1153	1700	1152	1703	1.8	1.2	RI, MS, ^{13}C NMR
30	Terpinen-4-ol	1164	1601	1164	1605	3.5	0.5	RI, MS, ^{13}C NMR
31	α -Terpineol	1176	1694	1175	1699	2.1	0.3	RI, MS, ^{13}C NMR
32	Myrtenol	1182	1790	1180	1790	-	0.4	RI, MS
33	Bornyl acetate	1270	1580	1271	1583	-	0.1	RI, MS
34	(Z)-2-Hexenyl hexanoate	1333 ^h	1653 ⁱ	1327	1655	-	0.1	RI, MS
35	δ -Elemene	1340	1469	1335	1470	-	0.5	RI, MS, ^{13}C NMR
36	7 α -Silphiperfol-5-ene	1348 ^j	1454 ^j	1347	1452	0.4	tr	RI, MS
37	Geranyl acetate	1362	1752	1360	1742	-	0.1	RI, MS
38	Cyclocopacamphene	1368	1483	1362	1483	0.4	0.6	RI, MS
39	α -Copaene	1375	1491	1375	1491	-	0.3	RI, MS

Table 2. Cont.

N°	Components ^a	RIa Lit ^b	RIp Lit ^b	RIa	RIp	SH%	SS%	Identification Mode
40	β-Elementene	1388	1591	1389	1591	0.4	0.9	RI, MS, ¹³ C NMR
41	Bornyl isobutyrate	1402 ^k	1641 ^k	1402	1643	-	0.1	RI, MS
42	(E)-β-Caryophyllene	1419	1598	1416	1595	-	0.9	RI, MS
43	(E)-β-Farnesene	1446	1664	1446	1667	0.8	2.2	RI, MS, ¹³ C NMR
44	3,6,6,9-bis-epoxy-Farnesa-1,7(14),10-triene	1450 ^l	1831 ^l	1449	1828	4.3 *	5.8 *	RI, ¹³ C NMR
45	9-epi-3,6,6,9-bis-epoxy-Farnesa-1,7(14),10-triene	1450 ^l	1834 ^l	1449	1831	0.9 *	0.9 *	RI, ¹³ C NMR
46	3,9-di-epi-3,6,6,9-bis-epoxy-Farnesa-1,7(14),10-triene	1458 ^l	1865 ^l	1457	1867	0.9 *	1.9 *	RI, ¹³ C NMR
47	3-epi-3,6,6,9-bis-epoxy-Farnesa-1,7(14),10-triene	1458 ^l	1870 ^l	1457	1868	2.2 *	1.1 *	RI, ¹³ C NMR
48	Selina-4,11-diene	1474 ^m	1670 ^m	1470	1674	-	0.7	RI, MS, ¹³ C NMR
49	Germacrene D	1476	1708	1478	1709	0.3	4.0	RI, MS, ¹³ C NMR
50	β-Selinene	1481	1717	1481	1716	-	0.4	RI, MS
51	(Z,E)-α-Farnesene	1481	1728	1486	1714	-	0.2	RI, MS
52	Bicyclogermacrene	1490	1734	1490	1726	-	0.2	RI, MS
53	α-Muurolole	1491	1723	1492	1724	0.7	0.5	RI, MS, ¹³ C NMR
54	(E,E)-α-Farnesene	1496	1744	1495	1749	-	0.2	RI, MS
55	γ-Cadinene	1506	1763	1505	1755	-	0.2	RI, MS
56	δ-Cadinene	1520	1756	1516	1757	0.2	0.6	RI, MS, ¹³ C NMR
57	β-Elementol	1537	2088	1533	2053	-	0.7	RI, MS
58	(E)-Nerolidol	1550	2036	1550	2042	3.4	13.9	RI, MS, ¹³ C NMR
59	Spathulenol	1566	2126	1563	2119	-	0.7	RI, MS
60	Caryophyllene oxide	1570	1986	1572	1983	1.7	0.9	RI, MS, ¹³ C NMR
61	Fokienol	1577 ⁿ	2170 ⁿ	1577	2168	1.1	2.9	RI, MS, ¹³ C NMR
62	β-Oplophenone	1593	2084	1588	2077	-	0.3	RI, MS
63	Copabornol	1600 ^k	2181 ^k	1595	2176	1.3	1.0	RI, MS, ¹³ C NMR
64	6,9-epoxy-Farnesa-1,7(14),10-trien-3-ol	1601 ^l	2257 ^l	1598	2254	2.7	5.4	RI, ¹³ C NMR
65	3-epi-6,9-epoxy-Farnesa-1,7(14),10-trien-3-ol	1601 ^l	2255 ^l	1598	2249	1.1	4.3	RI, ¹³ C NMR
66	Junenol	1607 ^o	2055 ^o	1607	2046	-	0.6	RI, MS
67	6-epi-6,9-epoxy-Farnesa-1,7(14),10-trien-3-ol	1614 ^l	2276 ^l	1610	2270	0.9	2.3	RI, ¹³ C NMR
68	3,6-di-epi-6,9-epoxy-Farnesa-1,7(14),10-trien-3-ol	1614 ^l	2284 ^l	1612	2279	1.2	3.3	RI, ¹³ C NMR
69	epi-γ-Eudesmol	1608	2106	1614	2107	-	0.4	RI, MS
70	γ-Eudesmol	1616	2176	1614	2186	-	0.5	RI, MS
71	Alismol	1620 ^l	2295 ^l	1617	2291	0.8	0.4	RI, ¹³ C NMR
72	Caryophylla-4(12),8(13)-dien-5α-ol	1622 ^l	2294 ^l	1619	2291	-	0.5	RI, MS
73	τ-Muurolole	1631	2186	1625	2185	1.6	1.0	RI, MS, ¹³ C NMR
74	Torreyol (δ-Cadinol)	1631 ^p	2167 ^p	1629	2164	-	0.2	RI, MS
75	α-Cadinol	1643	2227	1637	2226	-	1.1	RI, MS, ¹³ C NMR
76	α-Bisabolol	1668	2213	1665	2201	-	0.8 *	RI, MS, ¹³ C NMR
77	epi-α-Bisabolol	1674	2214	1665	2212	-	0.8 *	RI, MS, ¹³ C NMR
78	Shyobunol	1687 ^q	1953 ^r	1675	1940	-	0.8	RI, MS
79	Neophytadiene	1827 ^s	1933 ^s	1835	1936	-	0.1	RI, MS
80	(Z)-Phytol	2077 ^t	2551 ^t	2096	2558	-	0.3	RI, MS
81	(E)-Phytol	2103 ^t	2613 ^t	2106	2609	-	0.2	RI, MS
Total						90.1	87.6	

^a Components are listed following their order of elution on non-polar column BP-1; percentages on non-polar capillary column, except those with * (%) on polar capillary column BP-20). RIa, RIp: Retention indices on non-polar and polar columns, respectively; ¹³C NMR: compound identified by ¹³C-NMR, at least in one oil sample; nd: not determined; and tr: traces. ^b RIa lit, RIp lit: literature retention indices [19] otherwise stated. ^c [30]; ^d [31]; ^e [32]; ^f [33]; ^g [20]; ^h [34]; ⁱ [35]; ^j [36]; ^k [37]; ^l [38]; ^m [39]; ⁿ [40]; ^o [41]; ^p [42]; ^q [43]; ^r [44]; and ^s [45]. ^t RI of pure compounds from Aldrich (Z/E mixture).

In total, 81 compounds were identified. They accounted for 90.1 and 87.6% of the whole composition, respectively (Table 2). The chromatogram of the SS sample (non-polar column) is reported in Figure 3.

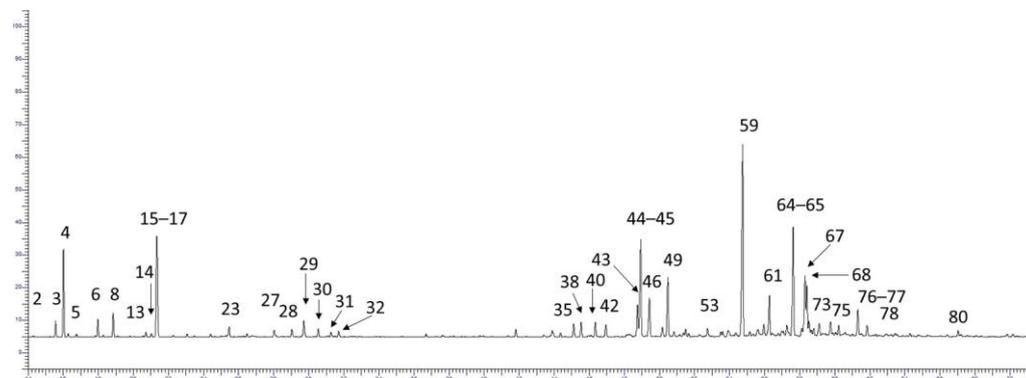


Figure 3. Chromatogram of SS sample (BP-1, non-polar column).

The identification of individual components was conducted as follows:

- Mass spectrometry in combination with retention indices on two capillary columns (non-polar and polar phases) allowed the identification of 73 components (1–43, 48–63, 66, 69–81) from traces accounting for more than 21%;
- In parallel, the identification of all the major components of both oil samples was ascertained by ^{13}C NMR following the computerized methodology developed at the University of Corsica (2–4, 6, 8, 12, 14–18, 23, 24, 27, 29–31, 35, 40, 43, 48, 49, 53, 56, 58, 60, 61, 63, 73, and 75–77) [25,26]. Alismol 71 was identified by ^{13}C NMR and RIs in both oil samples. Similarly, α -bisabolol 76 and epi- α -bisabolol 77 co-eluted on the non-polar column and were differentiated on the polar column. The occurrence of both epimers was confirmed by the observation of characteristic signals in the ^{13}C NMR spectra;
- Lastly, eight compounds (44–47, 64, 65, 67, and 68) that accounted for 0.2–8.3% each (percentages measured on the polar column due to overlapped GC signals on the non-polar column) remained unidentified regardless of the matching vs. MS commercial and homemade libraries at our disposal. Their retention indices were as follows: non-polar/polar column = 1451/1831, 1451/1834, 1460/1870, and 1460/1871 on the one hand, and 1600/2252, 1600/2255, 1613/2274, and 1613/2283 on the other hand. In parallel, computer alignment with the internal ^{13}C NMR library allowed the presence of eight components in significant content. Components 44–47 were identified as 3,6,6,9-bis-epoxy-farnesa-1,7(14),10-triene (IUPAC nomenclature (2*S*,5*S*,7*S*)-2-methyl-9-methylene-7-(2-methylprop-1-en-1-yl)-2-vinyl-1,6-dioxaspiro [4.4]nonane, relative stereochemistry), and its epimers, 3-epi (2*R*,5*S*,7*S*), 9-epi (2*S*,5*S*,7*R*), and 3,9-diepi (2*R*,5*S*,7*R*). Components 64, 65, 67, and 68 were identified as 6,9-epoxy-farnesa-1,7(14),10-trien-3-ol (IUPAC nomenclature (2*S*,3'*R*/*S*,5*S*)-(3'-hydroxy-3'-methylpent-4'-en)-3-methylene-5-isopropylidene tetrahydrofuran, relative stereochemistry)) and its epimers, 3-epi (2*S*, 3'*R*/*S*, 5*S*), 6-epi (2*S*, 3'*R*/*S*, 5*R*), and 3,6-diepi (2*R*, 3'*R*/*S*,5*R*) (Table 2, Figure 4).

The eight components were identified by comparison of their ^{13}C NMR chemical shifts, measured in the recorded ^{13}C NMR spectra of both oil samples with those of reference spectra compiled in our library (Figure 5, Table S1).

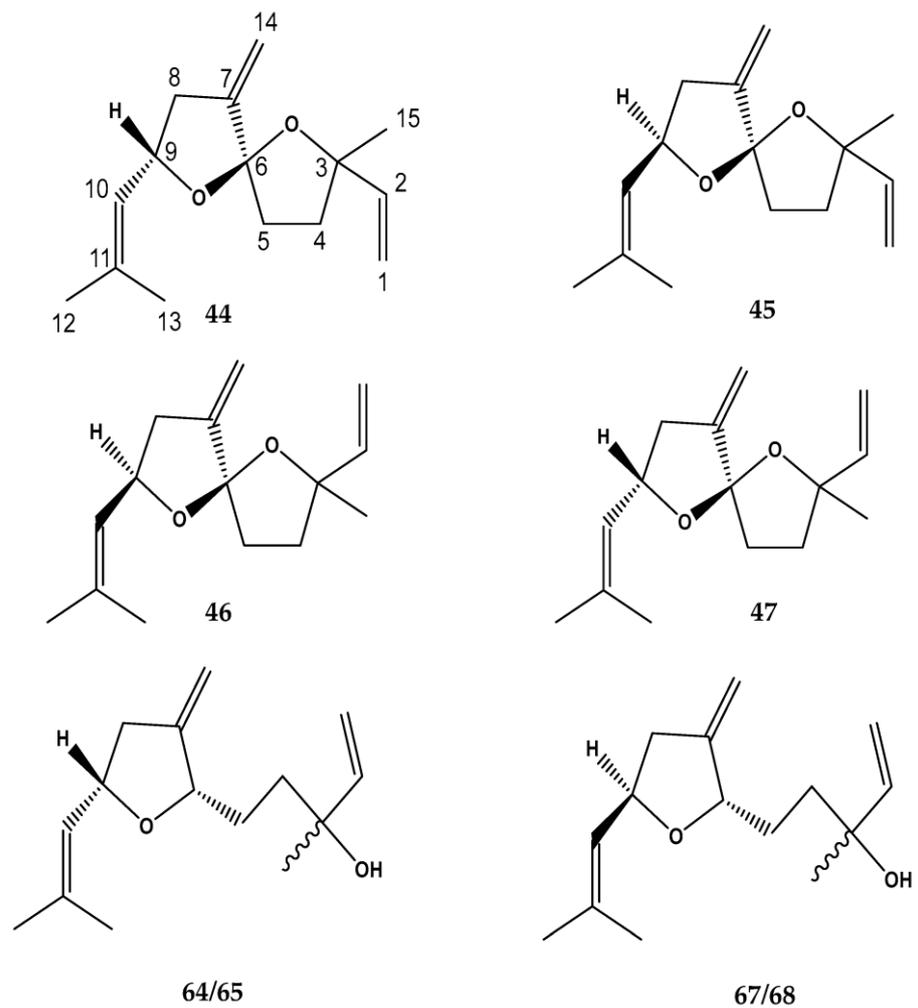


Figure 4. Structures of 3,6,6,9-bis-epoxy-farnesa-1,7(14),10-triene 44 and its epimers, 3-epi, 9-epi, and 3,9-diepi, and 6,9-epoxy-farnesa-1,7(14),10-trien-3-ol 64 and its epimers, 3-epi, 6-epi, and 3,6-diepi.

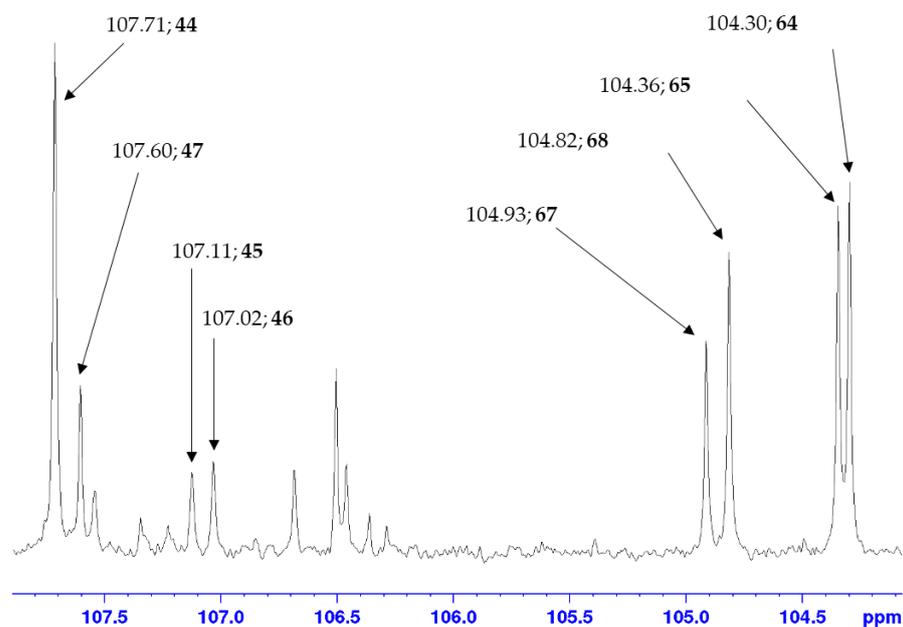


Figure 5. Part (104–108 ppm) of ^{13}C -NMR spectrum of *C. mixtus* (C14, Csp₂, methylene).

4. Discussion

Despite the similarity of the structures and the low percentage of some components, the chemical shifts of all carbons were observed, except those of the quaternary carbons of the minor isomers. The difference in chemical shifts between the experimental spectra and the reference data was always acceptable, as well as the number of overlapped signals. It could be noticed that the eight components were first isolated from the essential oil of *Tanacetum fruticosum* and spectroscopically characterized by Weyerstahl et al., who reported, inter alia, their ^{13}C NMR data [46]. To the best of our knowledge, since that time, the eight compounds have only been identified, by using ^{13}C NMR spectroscopy, in essential oil isolated from aerial parts of Corsican *Dittrichia viscosa* [38].

The composition of the SH oil sample was dominated by monoterpenes, 1,8-cineole (20.8%), and α -pinene (16.1%), followed by limonene (3.6%), terpinen-4-ol (3.5%), sabinene (3.2%), and α -terpineol (2.1%). Sample SS contained mainly (E)-nerolidol (13.9%), besides α -pinene (4.6%) and santolina alcohol (3.4%). A few compounds were present at appreciable content in both oil samples: myrcene (1.1/1.3%), 3-methylpentyl isobutyrate (0.8/0.2%), borneol (1.8/1.2%), caryophyllene oxide (1.7/0.9%), fokienol (1.1/2.9%), copaborneol (1.3/1.0%), and τ -muurolol (1.6/1.0%). Some components were noticeable in the SH oil sample, as follows: isobutyl isobutyrate (0.9%), α -thujene (0.7%), β -pinene (0.5%), 2-methylbutyl isobutyrate (1.1%), p-cymene (2.2%), isobutyl angelate (1.5%), linalool (0.6%), and 7 α -silphiperfol-5-ene (0.4%). Components in the SS oil sample were as follows: santolina alcohol (3.4%), β -elemene (0.9%), (E)- β -caryophyllene (0.9%), and (E)- β -farnesene (2.2%). It is noticeable that irregular monoterpene alcohols, santolina alcohol (3.4%) and artemisa alcohol (0.2%), and diterpenes, neophytadiene (0.1%), (Z)-phytol (0.3%), and (E)-phytol (0.2%) were found only in SS sample. Various hemiterpene esters and analogs usually found in *Cladanthus* species [31] (up to 1.5%) were identified: isobutyl isobutyrate, isobutyl 2-methylbutyrate, isobutyl isovalerate, isopentyl isobutyrate, 2-methylbutyl isobutyrate, isobutyl angelate, 2-methylbutyl 2-methyl butyrate, 3-methylpentyl isobutyrate, and (Z)-2-hexenyl hexanoate. Lastly, it could be pointed out that the content of 3,6,6,9-bis-epoxyfarnesa-1,7(14),10-triene (**44**) and its three epimers (**45**, **46**, and **47**) were similar in both oil samples (8.3% vs. 9.7%), while the content of 6,9-epoxy-farnesa-1,7(14),10-trien-3-ol (**64**) and its epimers (**65**, **67**, and **68**) was substantially higher in sample SS than in sample SH (15.3% vs. 5.9%).

5. Conclusions

Although oil samples whose chemical composition was dominated by (i) α -pinene and/or 1,8-cineole, (ii) (E)-nerolidol, or (iii) (E)- β -farnesene have been reported, the occurrence of oxygenated farnesane derivatives at appreciable contents (up to 25%) brings originality to the investigated *C. mixtus* oil samples. Our results confirm the tremendous chemical variability of Moroccan *C. mixtus* essential oil and the potential of ^{13}C NMR analysis, in combination with GC(RI), for the identification of uncommon oxygenated sesquiterpenes.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/compounds3020028/s1>. Table S1: ^{13}C NMR chemical shifts of compounds **44–47**, **64**, **65**, **67**, and **68**.

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References

1. Bellakhdar, J. *Médecine Arabe Ancienne et Savoirs Populaires, la Pharmacopée Marocaine Traditionnelle*; Ibis Press: Paris, France, 1997.
2. Aafi, A.; Achhal, A.K.; Benabid, A.; Rouchdi, M. Richesse et diversité floristique de l'écosystème de chêne-liège de la forêt de la Mamora. *Acta Bot. Malacit.* **2005**, *30*, 127–138. [[CrossRef](#)]
3. Benjilali, B.; Zrira, S. *Plantes Aromatiques et Médicinales, Atouts du Secteur et Exigences Pour Une Valorisation Durable*; Actes Editions Institut Agronomique et Vétérinaire Hassan-II: Rabat, Morocco, 2005.
4. Haddad, P.S.; Depot, M.; Settaf, A.; Chabli, A.; Cherrah, Y. Comparative study on the medicinal plants most recommended by traditional practitioners in Morocco and Canada. *J. Herbs. Spices Med. Plants* **2003**, *10*, 25–45. [[CrossRef](#)]
5. Elouaddari, A.; El Amrani, A.; Cayuela Sánchez, J.A.; Ould Bellahcen, T.; Zouiten, A.; Jamal Eddine, J. Chemical Composition and Biological Activities of the *Cladanthus mixtus* Essential Oil: A Review. *Anal. Chem. Lett.* **2019**, *9*, 649–663. [[CrossRef](#)]
6. Toulemonde, B.; Beauverd, D. Contribution à l'étude d'une camomille sauvage du Maroc: L'huile essentielle d'*Ormenis mixta* L. *Parfum. Cosmétiques Arômes* **1984**, *68*, 65–67.
7. Satrani, B.; Ghanmi, M.; Farah, A.; Aafi, A.; Fougrach, H.; Bourkhiss, B.; Bousta, D.; Talbi, M. Composition chimique et activité antimicrobienne de l'huile essentielle de *Cladanthus mixtus*. *Bull. Soc. Pharm. Bordx.* **2007**, *146*, 85–96.
8. Zrira, S.; Menut, C.; Bessiere, J.M.; Benjilali, B. Chemical composition of the essential oils of Moroccan *Ormenis mixta* (L.) Dumort. ssp. *Multicaulis*. *J. Essent. Oil Bear. Plants* **2007**, *10*, 378–385. [[CrossRef](#)]
9. Hajjaj, G.; Bahlouli, A.; Tajani, M.; Alaoui, K.; Cherrah, Y.; Zellou, A. Profil neuropharmacologique et analyse chimique d'*Ormenis mixta* (L.) marocain. *Phytothérapie* **2018**, *16*, S55–S64. [[CrossRef](#)]
10. Zeroual, A.; Sakar, E.H.; Eloutassi, N.; Mahjoubi, F.; Chaouch, M.; Chaqroune, A. Wild Chamomile [*Cladanthus mixtus* (L.) Chevall.] Collected from Central-Northern Morocco: Phytochemical Profiling, Antioxidant, and Antimicrobial Activities. *Biointerface Res. Appl. Chem.* **2021**, *11*, 11440–11457.
11. Chraïbi, M.; Fadil, M.; Farah, A.; Lebrazi, S.; Fikri-Benbrahim, K. Antimicrobial combined action of *Mentha pulegium*, *Ormenis mixta* and *Mentha piperita* essential oils against *S. aureus*, *E. coli* and *C. tropicalis*: Application of mixture design methodology. *LWT—Food Sci. Technol.* **2021**, *145*, 111352. [[CrossRef](#)]
12. Elouaddari, A.; El Amrani, A.; Jamal Eddine, J.; Correia, A.I.D.; Barroso, J.G.; Pedro, L.G.; Figueiredo, A.C. Yield and chemical composition of the essential oil of Moroccan chamomile [*Cladanthus mixtus* (L.) Chevall.] growing wild at different sites in Morocco. *Flavour Fragr. J.* **2013**, *28*, 360–363. [[CrossRef](#)]
13. Elouaddari, A.; El Amrani, A.; Jamal Eddine, J. Effect of the Parts of Plant Material (Flowers and Leaves) on Essential Oil Chemical Composition of *Ormenis mixta* from Morocco. *J. Essent. Oil Bear. Plants* **2015**, *18*, 398–408. [[CrossRef](#)]
14. Ainane, T.; Elkouali, M.; Ainane, A.; Talbi, M. Moroccan traditional fragrance based essential oils: Preparation, composition and chemical identification. *Der Pharma Chem.* **2014**, *26*, 84–89.
15. Elouaddari, A.; El Amrani, A.; Jamal Eddine, J.; Barroso, J.G.; Pedro, L.G.; Figueiredo, A.C. Intraspecific variability of the essential oil of *Cladanthus mixtus* from Morocco. *Nat. Prod. Commun.* **2014**, *9*, 133–136. [[CrossRef](#)] [[PubMed](#)]
16. Elouaddari, A.; El Amrani, A.; Moutia, M.; Oubrim, N.; Habti, N.; Jamal Eddine, J. Chemical composition and evaluation of antioxidant, antimicrobial and cytotoxic activities of Moroccan *Cladanthus mixtus* essential oil and extracts. *J. Essent. Oil Bear. Plants* **2019**, *22*, 1450–1466. [[CrossRef](#)]
17. Wanner, J.; Schmidt, E.; Bail, S.; Jirovetz, L.; Buchbauer, G.; Gochev, V.; Girova, T.; Atanasova, T.; Stoyanova, A. Chemical composition, olfactory evaluation and antimicrobial activity of selected essential oils and absolutes from Morocco. *Nat. Prod. Commun.* **2010**, *5*, 1349–1354. [[CrossRef](#)] [[PubMed](#)]
18. Ouedrhiri, W.; Balouiri, M.; Bouhdid, S.; El Harki, H.; Moja, S.; Greche, H. Antioxidant and antibacterial activities of *Pelargonium asperum* and *Ormenis mixta* essential oils and their synergistic antibacterial effect. *Environ. Sci. Pollut. Res.* **2018**, *25*, 29860–29867. [[CrossRef](#)]
19. Babushok, V.I.; Linstrom, P.J.; Zenkevich, I.G. Retention indices for frequently reported compounds of plant essential oils. *J. Phys. Chem. Ref. Data* **2011**, *40*, 043101–043147. [[CrossRef](#)]
20. Terpenoids Library Website. Available online: https://massfinder.com/wiki/Terpenoids_Library_List (accessed on 14 April 2022).
21. König, W.A.; Hochmuth, D.H.; Joulain, D. *Terpenoids and Related Constituents of Essential Oils*; Library of MassFinder 2.1; Institute of Organic Chemistry: Hamburg, Germany, 2001.
22. National Institute of Standards and Technology. *PC Version of the Mass Spectral Library*; Norwalk: Connecticut, CT, USA, 2014.
23. Adams, R.P. *Identification of Essential Oils Components by Gas Chromatography/Mass Spectroscopy*, 4th ed.; Allured: Carol Stream, IL, USA, 2007; p. 455.
24. Tomi, F.; Bradesi, P.; Bighelli, A.; Casanova, J. Computer-aided identification of individual components of essential oil using carbon-13 NMR spectroscopy. *J. Magn. Reson. Anal.* **1995**, *1*, 25–34.

25. Tomi, F.; Casanova, J. ^{13}C -NMR as a tool for identification of individual components of essential oils from Labiatae—A review. *Acta Hort.* **2006**, *723*, 185–192. [[CrossRef](#)]
26. Ouattara, Z.A.; Boti, J.B.; Ahibo, A.C.; Sutour, S.; Casanova, J.; Tomi, F.; Bighelli, A. The key role of ^{13}C NMR analysis in the identification of individual components of *Polyalthia longifolia* leaf oil. *Flavour Fragr. J.* **2014**, *29*, 371–379. [[CrossRef](#)]
27. Bazzali, O.; Tran Huy, T.; Tran Minh, H.; Nguyen Sinh, K.; Nguyen Thi, H.; Casanova, J.; Bighelli, A.; Tomi, F. Wood oil from *Xanthocyparis vietnamensis* Farjon et Hiep. Integrated analysis by chromatographic and spectroscopic techniques. *Molecules* **2016**, *21*, 840. [[CrossRef](#)] [[PubMed](#)]
28. Tomi, F.; Casanova, J. Contribution de la RMN du carbone-13 à l'analyse des huiles essentielles. *Ann. Fals. Exp. Chim.* **2000**, *952*, 313–330.
29. Cavalli, J.F.; Tomi, F.; Bernardini, A.F.; Casanova, J. Composition and chemical variability of the bark oil of *Cedrelopsis grevei* H. Baillon from Madagascar. *Flavour Fragr. J.* **2003**, *18*, 532–538. [[CrossRef](#)]
30. Binder, R.G.; Turner, C.E.; Flath, R.A. Comparison of yellow star thistle volatiles from different plant parts. *J. Agric. Food Chem.* **1990**, *38*, 764–767. [[CrossRef](#)]
31. El Hafidi, S.; Bakhy, K.; Ouhssine, M.; Casanova, J.; Tomi, F.; Paoli, M. Essential oil composition of *Cladanthus eriolepis* (Coss. ex Maire) Oberpr. & Vogt, an endemic species to Morocco. *J. Essent. Oil Res.* **2021**, *33*, 369–375. [[CrossRef](#)]
32. Blanc, M.-C.; Muselli, A.; Bradesi, P.; Casanova, J. Chemical composition and variability of the essential oil of *Inula graveolens* from Corsica. *Flavour Fragr. J.* **2004**, *19*, 314–319. [[CrossRef](#)]
33. Binder, R.G.; Flath, R.A. Volatile components of pineapple guava. *J. Agric. Food Chem.* **1989**, *37*, 734–736. [[CrossRef](#)]
34. Ashes, J.R.; Haken, J.K. Gas chromatography of homologous esters. IX. Structure-retention increments of unsaturated esters. *J. Chromatogr.* **1975**, *111*, 171–187. [[CrossRef](#)]
35. Fröhlich, O.; Duque, C.; Schreier, P. Volatile constituents of curuba (*Passiflora mollissima*) fruit. *J. Agric. Food Chem.* **1989**, *37*, 421–425. [[CrossRef](#)]
36. Paolini, J.; Muselli, A.; Bernardini, A.-F.; Bighelli, A.; Casanova, J.; Costa, J. Thymol derivatives from essential oil of *Doronicum corsicum* L. *Flavour Fragr. J.* **2007**, *22*, 479–487. [[CrossRef](#)]
37. Paolini, J.; Costa, J.; Bernardini, A.F. Analysis of the essential oil from aerial parts of *Eupatorium cannabinum* subsp. *corsicum* (L.) by gas chromatography with electron impact and chemical ionization mass spectrometry. *J. Chromatogr. A* **2005**, *1076*, 170–178. [[CrossRef](#)] [[PubMed](#)]
38. Blanc, M.C.; Bradesi, P.; Gonçalves, M.J.; Salgueiro, L.; Casanova, J. Essential oil of *Dittrichia viscosa* ssp. *viscosa*: Analysis by ^{13}C -NMR and antimicrobial activity. *Flavour Fragr. J.* **2006**, *21*, 324–332. [[CrossRef](#)]
39. Ferrari, B.; Tomi, F.; Casanova, J. Composition and chemical variability of *Ferula communis* essential oil from Corsica. *Flavour Fragr. J.* **2005**, *20*, 180–185. [[CrossRef](#)]
40. Lesueur, D.; Ninh Khac, B.; Bighelli, A.; Muselli, A.; Casanova, J. Analysis of the root oil of *Fokienia hodginsii* (Dunn) Henry et Thomas (Cupressaceae) by GC, GC-MS and ^{13}C -NMR. *Flavour Fragr. J.* **2006**, *21*, 171–174. [[CrossRef](#)]
41. Garcia, G.; Garcia, A.; Gibernau, M.; Bighelli, A.; Tomi, F. Chemical compositions of essential oils of five introduced conifers in Corsica. *Nat. Prod. Res.* **2017**, *31*, 1697–1703. [[CrossRef](#)]
42. Pino, J.A.; Marbot, R.; Vázquez, C. Characterization of volatile in Costa Rican Guava [*Psidium friedrichsthalianum* (Berg) Niedenzu] fruit. *J. Agric. Food Chem.* **2002**, *50*, 6023–6026. [[CrossRef](#)]
43. Werka, J.S.; Boehme, A.K.; Setzer, W.N. Biological activities of essential oils from Monteverde, Costa Rica. *Nat. Prod. Commun.* **2007**, *2*, 1215–1219. [[CrossRef](#)]
44. Baser, K.H.C.; Demirci, B.; Kirimer, N.; Satil, F.; Tumen, G. The essential oils of *Thymus migricus* and *T. fedtschenkoi* var. *handellii* from Turkey. *Flavour Fragr. J.* **2002**, *17*, 41–45. [[CrossRef](#)]
45. Bicchi, C.; Fresia, M.; Rubiolo, P.; Monti, D.; Franz, C.; Goehler, I. Constituents of *Tagetes lucida* Cav. ssp. *lucida* essential oil. *Flavour Fragr. J.* **1997**, *12*, 47–52. [[CrossRef](#)]
46. Weyerstahl, P.; Marshall, H.; Thefeld, K.; Rustaiyan, A. Constituents of the essential oil of *Tanacetum* (syn. *Chrysanthemum*) *fruticulosum* Ledeb. from Iran. *Flavour Fragr. J.* **1999**, *14*, 112–120. [[CrossRef](#)]

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