GC, GC-MS and NMR spectroscopy Integrated Analysis of The Tunisian *Pulicaria laciniata* essential oil

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(Reçu le 10 Mai 2011, accepté le 10 Septembre 2011)

ABSTRACT: The essential oils from aerial parts and roots of *Pulicaria laciniata* were obtained by hydro distillation and analyzed by GC and GC-MS. The chemical composition of the root oil of this species is reported here for the first time. Thirty-eight components, accounting for 98.25% and 98.79% of the aerial part and root oils, respectively, were identified. The two oils were characterized by high proportion of oxygenated monoterpenes (68.46 and 85.91%) and belonged to the geranyl isobutyrate (55.91 and 77.92%) chemotype. The phenolic derivatives represented the second major fraction (17.22 and 4.85%) among which cis-isoelemicin (11.04 and 4.85%) was the predominant compound. The major constituent was isolated and identified as (*Z*)-geranyl isobutyrate **1**, by analysis of spectroscopic data (^{1}H-NMR, ^{13}C-NMR, DEPT 135, CHcorr, HMBC, ^{1}H-^{1}H COSY and NOESY experiments).

Keywords: Essential oil, *Pulicaria laciniata*, Asteraceae, (*Z*)-geranyl isobutyrate, 1 and 2D-NMR.

INTRODUCTION:

Pulicaria genus belongs to the family of the Asteraceae, which contains 100 species [1]. The chemical investigation of the genus showed the occurrence of molecules such as diterprenes [2, 3], sesquiterpenes [4-7], caryophyllene derivatives [8-11] and flavonoids [12-14].

Various biological activities have been reported for some species of *Pulicaria*, such as cytotoxic, antibacterial, spasmodic and histaminic effects [15-18].

Different species of *Pulicaria* have been extensively studied to establish the chemical composition of their essential oils [19-21]. However, *Pulicaria odora* L. essential oil was characterized by high mean percentage of oxygenated monoterpenes such as thymol (47.83%) and thymol isobutyrate (30.05%) and the essential oil of *P. laciniata* flowers was rich in monoterpenes such as α-pinene (36.91%) and terpinen-4-ol (31.08%) [21].

As an extension of the chemical investigation of *P. laciniata*, we report here the chemical composition of the essential oils isolated from its aerial part and root. Conventional techniques (GC and GC-MS) were used in this study. In order to identify with certainty the stereochemistry of the major compound **1** of these oils, the aerial part one was subjected to silica gel column chromatography having allowed its isolation and its identification as being a (*Z*)-geranyl isobutyrate **1** by analysis of its spectroscopic data (MS, ^{1}H-NMR, ^{13}C-NMR, DEPT 135, ^{1}H-^{1}H COSY, HMBC and NOESY experiments).

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RESULTS AND DISCUSSION:

1- Chemical composition:

The hydrodistillation of *P. laciniata* aerial part and root yielded yellow oils. The essential-oil yields (% w/w), calculated from fresh material, were 0.053 and 0.023 %, for aerial part and root, respectively. The composition of the oils was determined by GC, GC-MS and NMR. The percentage composition, together with Retention Indices (RIs) calculated for each compound, and the identification methods are reported in table I.

A total of 38 constituents, accounting for 98.25% and 98.79% respectively, were identified. The essential oils consisted chiefly of monoterpenes. Indeed, geranyl isobutyrate was by far, the major component in the *P. laciniata* oils. This compound was identified by spectroscopic means after its isolation by CC- silica gel using ^{1}H, ^{13}C, DEPT 135, ^{1}H-^{1}H COSY, CHcorr, HMBC and NOESY as well as MS. Its spectral data are summarized in table II.

Analysis of the aerial part oil led to the identification of 29 components (seven monoterpenes and derivatives, eight sesquiterpenes, four phenols, two diterpenes and eight non terpenic acyclic aldehydes, ester and alcohols) representing 98.25 % of the total oil (Table I). The essential oil consisted chiefly of oxygenated monoterpenes and derivatives (63.91% of the total oil), obviously belonging to the geranyl isobutyrate (**18**; 55.91%) chemotype. neryl acetone (**16**; 5.6%), nerol (**8**; 1.2%) were also found among the major components. Monoterpene hydrocarbons were mostly represented by terpinolene (4; 0.6%). The sesquiterpene and derivatives, which constituted 8.66% of the identified components, consisted mainly of the bisabolone (**27**; 1.9%) and farnesyl acetate (**31**; 1.55%). The phenolic components were mostly represented by cis-isoelemicin (**20**; 11.04%) and dillapiole (**22**; 4.3%). Diterpene hydrocarbons (0.67%) and non terpenic acyclic compounds (7.19%) were present at low contents. The essential oil roots are rich in oxygenated monoterpenes, which accounted for 84.98% of the oil represented essentially by geranyl isobutyrate 77.92%, however it was characterized by a low level of oxygenated sesquiterpenes (5.61%). The high content of oxygenated identified compounds might explain the characteristic and fragrant odor of the oil.

We remind that our anterior work related to the study of the chemical composition of the flower essential oil of the same species showed α-pinene (36.91%) and terpinen-4-ol (31.08%) are identified as being the major compounds of this oil [21]. Our actual results showed the total absence of these two predominant compounds in the aerial part and root essential oils. This observation was already met in several cases cited in the literature which show sometimes a total difference of the chemical composition of essential oils of certain organs of the same plant [22].

Table I: Chemical composition of the aerial part and root oils of *P. laciniata*.

N°	Compounds	Retention Index(*RI*)	Retention Index($RI_{litt.}$)	Composition [%] A. part	Root	Identification
1	Isopropyl butyrate	842	834	0.2	-	GC-MS, RI
2	*p*-Cymene	1025	1028	-	0.13	GC-MS, RI
3	Linalool	1102	1112	-	0.65	GC-MS, RI
4	Terpinolene	1103	1096	0.6	-	GC-MS, RI
5	Benzyl acetate	1163	1164	0.63	-	GC-MS, RI
6	Nerol Oxide	1164	1158	-	0.37	GC-MS, RI
7	α-Terpineol	1207	1192	0.5	1.1	GC-MS, RI
8	Nerol	1243	1245	1.2	1.42	GC-MS, RI
9	*p*- Mentha-1,3-dien-7-al	1281	1282	0.17	-	GC-MS, RI
10	3-Phenyl-2-propene-1-ol	1304	1310	-	0.92	GC-MS, RI
11	Carvacrol	1310	1317	1.62	-	GC-MS, RI
12	2-Phenyl propionate	1349	1352	0.25	-	GC-MS, RI

Table I (Continued**)**

13	Neryl acetate	1375	1371	0.53	0.73	GC-MS, RI
14	Longifolene	1403	1418	-	0.36	GC-MS, RI
15	Caryophyllene	1412	1427	0.71	-	GC-MS, RI
16	Neryl acetone	1433	1427	**5.6**	0.93	GC-MS, RI
17	β-Santalene	1462	1468	-	0.23	GC-MS, RI
18	(*Z*)-Geranyl isobutyrate	1505	1514	**55.91**	**77.92**	GC-MS, RI NMR
19	α- Cadinene	1536	1538	0.57	0.22	GC-MS, RI
20	*Cis*- Isoelemicin	1574	1572	**11.04**	**4.85**	GC-MS, RI
21	Ledol	1605	1608	-	0.63	GC-MS, RI
22	Dillapiole	1624	1622	**4.3**	-	GC-MS, RI
23	T-Cadinol	1637	1640	-	1.86	GC-MS, RI
24	α- Eudesmol	1651	1647	-	0.38	GC-MS, RI
25	ar- Turmerone	1664	1665	1.03	-	GC-MS, RI
26	*Trans*- Stilbene	1707	1708	2.04	0.58	GC-MS, RI
27	Bisabolone	1723	1746	1.9	0.25	GC-MS, RI
28	(*E*)- Nuciferol	1755	1748	0.7	-	GC-MS, RI
29	Bisabolenols	1785	1788	0.8	0.19	GC-MS, RI
30	Acorone	1805	1816	1.4	-	GC-MS, RI
31	Farnesyl acetate	1843	1856	**1.55**	**4.16**	GC-MS, RI
32	1-Hexadecanal	1872	1873	1.82	0.59	GC-MS, RI
33	Nonadecane	1905	1900	0.97	0.14	GC-MS, RI
34	Beyerene	1931	1924	0.42	-	GC-MS, RI
35	Sclarene	1967	1962	0.25	-	GC-MS, RI
36	Eicosane	2000	2000	1.1	0.18	GC-MS, RI
37	Bergaptene	2044	2048	0.26	-	GC-MS, RI
38	Heneicosane	2100	2100	0.18	-	GC-MS, RI
	Total			98.25	98.79	
	Monoterpene hydrocarbons			0.6	0.13	
	Oxygenated Monoterpenes and derivatives			63.91	84.98	
	Sesquiterpene hydrocarbons			1.28	0.81	
	Oxygenated sesquiterpenes			7.38	5.61	
	Diterpene hydrocarbons			0.67	-	
	Phenolic derivatives			17.22	4.85	
	Others			7.19	2.41	

Order of elution and percentages of individual components are given on a HP-5 capillary column. Identification was made on the basis of their RI [23] and MS (GC-MS) [25,26]. Bold type indicated major components. t = trace (<0.10%).

2- Structural determination:

Compound **18** was obtained as colorless oil with a molecular ion signal at *m/z* 224 (EI-MS) corresponding to the molecular formula $C_{14}H_{24}O_2$.

The ^{13}C-NMR displayed 14 carbon resonances, which were assigned by CHcorr and DEPT 135 experiments to three methines, three methylenes, three quaternary and five methyl carbon atoms consistent with the molecular formula.

The presence of an isopropyl group (δ 2.43, 1H, hept, J= 7.2Hz; δ 1.06, 6H, d, J= 7.2 Hz) was easily determined by ^{1}H-NMR, and was reinforced by the ^{1}H-^{1}H COSY spectrum which showed a correlation between H_2 and $H_{3,4}$ (Table 2).

The position of the isopropyl group was determined from the HMBC spectrum showing the ^{2}J (H_2-C_1) and ^{3}J (H_3-C_1) correlations (Table 2).
The ^{13}C-NMR resonance at δ 176.8 suggested that a carbonyl moiety existed in the structure.
The position of the methyl groups was determined with the help of the HMBC spectrum (Table 2) which showed correlations between $H_{10'}$ and C(4'), C(2') and C(3') as well as correlations between $H_{8',9'}$ and C(7') and C(6') indicating that the methyl group CH_3(10'), CH_3(8') and CH_3'9') were attached to C(3') and C(7'), respectively (Table 2).
The position of the C=C double bond in the molecule were evident through the ^{1}H-^{1}H COSY and HMBC experiment (Table 2).
The configuration of the $\Delta^{2'}$ was identified as being *Z* by the observation of the *nOes* $H_{1'}$-$H_{4'}$ and $H_{2'}$-$H_{10'}$ (Figure 1).

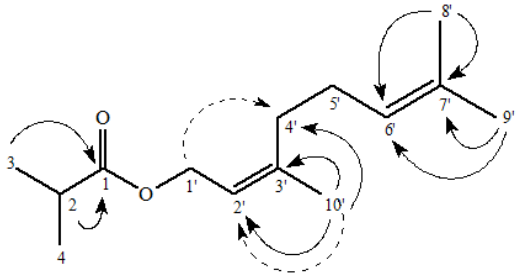
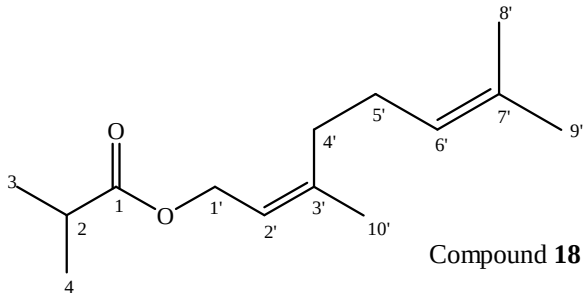


Figure 1: Correlations H⟶ C (HMBC) and H - - → H (NOESY) of compound **18**



Compound **18**

Table II: ^{1}H, ^{13}C, HMBC and ^{1}H-^{1}H COSY spectroscopic data of compound **18**.

Position	^{1}H	^{13}C	HMBC	^{1}H-^{1}H COSY
1	-	176.8	H_3, H_4, H_2, $H_{1'}$	
2	2.43 hept (7.2)	33.9	H_3, H_4	H_3, H_4
3	1.06 d (7.2)	18.9		
4	1.06 d (7.2)	18.9		
1'	4.46 d (7.2)	60.8		$H_{2'}$
2'	5.26 t (7.2)	119.5	$H_{1'}$, $H_{10'}$	$H_{1'}$, $H_{10'}$
3'	-	141.9	$H_{1'}$, $H_{4'}$, $H_{10'}$	
4'	2.03 m	32.0	$H_{2'}$, $H_{5'}$, $H_{10'}$	$H_{5'}$
5'	1.98 m	26.6	$H_{4'}$, $H_{6'}$, $H_{8'}$	$H_{6'}$
6'	5.01 m	123.5	$H_{4'}$, $H_{5'}$, $H_{8'}$, $H_{9'}$	$H_{8'}$, $H_{9'}$
7'	-	131.8	$H_{8'}$, $H_{9'}$	
8'	1.51 s	17.5	$H_{9'}$	$H_{5'}$, $H_{6'}$, $H_{9'}$
9'	1.58 s	25.5		
10'	1.67 s	23.3		

EXPERIMENTAL SECTION:

1- Plant material:

Pulicaria laciniata (coss. et kral.) Tell. [24] was collected in the region of EL Hwareb (Kairouan, Tunisia) in april 2008. Identification was performed at the Laboratoire de Biologie Végétale et Botanique, Institut Supérieur Agronomique de Chott meriem, Ministère de l'Agriculture and Université de Sousse, Sousse, Tunisia. Voucher specimen (PL-08) was deposited in the herbarium of the above laboratory.

2- Extraction of essential oils:

Extraction was carried out by hydrodistillation on a *Clevenger*-type apparatus during 4h. The essential oils were collected by decantation, dried with Na_2SO_4, weighed and stored in sealed glass vials at 4-5°C prior to analysis.

3- Fractionation of the essential oil of *Pulicaria laciniata*:

The essential oil of the aerial part of *P. laciniata* (500 mg) was subjected to column chromatography over silica gel eluted with petroleum ether/CH_2Cl_2 to yield 10 fractions. The 5th of which was purified by column chromatography over silica gel using CH_2Cl_2/petroleum ether (10:90) as eluent to afford **18** (319.5 mg).

4- General methods:

^1H (300 *MHz*), ^{13}C (75 *MHz*) and 2D NMR spectra of compound **18** were recorded in $CDCl_3$ with a Bruker NMR-300 spectrometer. The residual solvent resonance was used as internal reference. Coupling constants are given in Hertz. The mass spectrum of **18** was obtained with an *Autospec* GC-MS system.

GC analysis:

The composition of the oil was investigated by GC and GC/MS. The analytical GC was carried out on an HP5890-series II gas chromatograph equipped with Flame ionization detectors (FID) under the following conditions: the fused silica capillary column HP-5 **(**30m x 0.25mm ID, film thickness 0.25µm). The oven temperature was held at 50°C for 1 min then programmed at 5°C/min to 240°C at rate of and held isothermal for 4 min. The carrier gas was nitrogen at a flow rate (1.2ml/min); injector and detector temperature: 250°C and 280°C, respectively; the volume injected: 0.1µl of 1 % solution (diluted in hexane). The relative proportions of the essential oil constituents were expressed as percentages, calculated using the *software HP chemstation*, which allows assimilating the percentages of the peak areas with the percentages of the various constituents. Retention indices (*RI*) were obtained by running a series of aliphatic hydrocarbons (C9-C28) in increasing the number of carbon atoms on the HP-5 GC column.

GC-MS analysis:

The analysis of the volatiles was performed on an *Autospec* GC-MS system. The fused-silica *HP5-MS* 5% methylphenylsiloxane capillary column (30 m x 0.25mm i.d., film thickness 0.25µm) was directly coupled to the MS. Heluim was used as the carrier gas, with a flow rate of 1.2 ml/min. The mass spectrometer operating conditions were: ionisation voltage 70 eV; ion source 230°C. The GC-MS parameters were identical to those for the GC analysis.

Compound identification:

The identification of compounds by GC (*RI*) and GC-MS was based on comparison of their retention indices with those of authentic samples, and by comparison of their mass spectra with those of pure compounds compiled in computerized libraries by means of NBS75K.L. and Wiley 275 databases and with the literature data [25, 26].

CONCLUSION:

In this work, we were interested in the study, for the first time, of the chemical composition of essential oils from the aerial part and the roots of the Tunisian plant *Pulicaria laciniata*. The results showed that the analyzed essential oils consisted chiefly of oxygenated monoterpenes and derivatives (63.91-84.98%). (*Z*)-Geranyl isobutyrate was to be found the major in the two *P. laciniata* essential oils, it represented 55.91% and 77.92% in the total aerial part and roots essential oils, respectively. It was isolated by silica gel column chromatography and its structure and stereochemistry were confirmed on the basis of its spectroscopic data (MS, ^1H-NMR, ^{13}C-NMR, DEPT 135, ^1H-^1H COSY, HMBC and NOESY experiments).

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