

# CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITIES OF *Pulicaria laciniata* OILS ISOLATION AND STRUCTURE ELUCIDATION OF A BIOACTIVE SESQUITERPENE LACTONE

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**ABSTRACT:** This study deals with the valorisation of medicinal and aromatic plants of the Tunisian flora, in order to find new bioactive natural products. The essential oil constituents of *Pulicaria laciniata* (Asteraceae) flowers were extracted by hydrodistillation and analysed by GC-MS. 29 constituents were identified. The most representative compounds were  $\alpha$ -pinene (36.9 %), terpinen-4-ol (31.1 %), (*E*)-caryophyllene (7.7 %) and 4,6,9-trimethyldec-8-en-3,5-dione (6.3 %). As a part of our continuing research on the isolation of bioactive compounds from *Pulicaria laciniata*, the fractionation of the aerial part essential oil led to the isolation of a sesquiterpene named alantolacton. The structure was established by spectroscopic procedures.

**Key words:** *Pulicaria laciniata*, Asteraceae, essential oils, sesquiterpene lactone, 2D NMR, antibacterial activity.

## 1. INTRODUCTION

In the last decades, the essential oils and various extracts of plants have been of great interest as they have been the sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases and the preservation of the foods from the toxic effects of the oxidants. Particularly, the antimicrobial activities of plant oils and extracts have formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [1], we have now studied the plant *Pulicaria laciniata* (L.) (Asteraceae). This Mediterranean plant, widely distributed in Tunisia, is also regarded as being an Algero-Tunisian endemic species [2]. The genus *Pulicaria* comprises about 21 species predominately distributed around the Mediterranean. In Tunisia, beside *P. laciniata* 6 other species have been found. It is known that the genus *Pulicaria* contains a variety of sesquiterpene lactones [3] and various other compounds such as flavonoids [4-6]. Recently, earlier chemical investigations on the later gathered in Algeria led to the isolation of a guaianolide epoxid and a pseudoguaianolide [7].

To our sources, it has never been used in folk medicine in Tunisia, The volatile constituents of its essential oil has never been carried out, consequently, we report here on the results of the essential oil composition analysis of *Pulicaria laciniata* flowers as well as the isolation and structure elucidation of a sesquiterpene deriving from the volatile aerial parts fraction of *P. laciniata*. Separation of this fraction by normal-phase silica gel chromatography furnished the alantolactone.

## 2. RESULTS AND DISCUSSION

Two essential oils were extracted by hydrodistillation from *P. laciniata* and investigated. The yields were 0.02% for flowers and 0.012% for aerial parts.

### 2.1. Flowers essential oil Chemical composition:

The detailed analysis of a sample of *P. laciniata* fresh flowers essential oil by GC-RI, led to the

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identification of 29 components amounting to 97,45% of the oil (Table 1). The chemical composition of the oil was dominated by Monoterpenes (77.7%) including the major component  $\alpha$ -pinene (36.9 %), followed by terpinen-4-ol (31.1 %), (*E*)-caryophyllene (7.7 %) and linalool (4.9 %). The main group of diverse compounds contained hydrocarbons (47.8 %), alcohols (38.1 %), phenols (1.7 %), ether (1.3 %), ketone (7.8 %), esters (0.4 %) and carboxylic acid (0.4 %) (Table 1).

**Table 1.** Volatile constituents of *Pulicaria laciniata* flowers essential oil. Identification based on retention index followed by comparison of mass spectra.

N°	Component	Weight (%)	RI (BP 20)	Identification
1	<b><math>\alpha</math>-Pinene</b>	<b>36.91</b>	1021	MS. RI
2	Camphene	0.16	1067	MS. RI
3	4-methylhexan-3-one	0.41	1077	MS. RI
4	$\beta$ -Pinene	0.16	1115	MS. RI
5	Limonene	0.92	1203	MS. RI
6	<b>1,8-Cineole</b>	<b>1.24</b>	1227	MS. RI
7	( <i>Z</i> )-hex-3-en-1-ol	0.12	1353	MS. RI
8	2,6-dimethyl-octa-2,7-dien-1,6-diol	0.14	2316	MS. RI
9	Terpinolene	0.30	1369	MS. RI
10	$\alpha$ -Copaene	0.30	1492	MS. RI
11	Camphre	0.92	1496	MS. RI
12	<b>Linalol</b>	<b>4.85</b>	1507	MS. RI
13	$\alpha$ -Bergamotene	0.86	1569	MS. RI
14	<b>Terpinen-4-ol</b>	<b>31.08</b>	1592	MS. RI
15	( <i>E</i> )-Caryophyllene	7.69	1596	MS. RI
16	<i>allo</i> -Aromadendrene	0.28	1638	MS. RI
17	<i>Trans</i> -pinocarveol	0.48	1650	MS. RI
18	<b><math>\alpha</math>-Terpineol</b>	<b>0.17</b>	1685	MS. RI
19	$\gamma$ -Cadinene	0.20	1764	MS. RI
20	Propionate de neryle	0.43	1778	MS. RI
21	Myrtenol	0.34	1792	MS. RI
22	Undecanol	0.14	1855	MS. RI
23	4,6,9-Trimethyldec-8-en-3,5-dione	6.32	1876	MS. RI
24	2-phenylethanol	0.61	1903	MS. RI
25	$\beta$ -( <i>E</i> )-Ionone	0.14	1940	MS. RI
26	1- <i>epi</i> -cubenol	0.14	2023	MS. RI
27	<b>Thymol</b>	<b>1.40</b>	2167	MS. RI
28	Vanilline methyl ether	0.26	2576	MS. RI
29	Hexadecanoïque acid	0.37	2834	MS. RI
<b>Chemical classes of the constituents</b>				
	Monoterpene hydrocarbons	38.47		
	Oxygenated monoterpenes	39.27		
	Sesquiterpene hydrocarbons	9.34		
	Oxygenated sesquiterpenes	0.14		
	Other compounds	10.23		

## 2.2. Isolation and Identification of sesquiterpene *Pulicaria laciniata* aerial parts:

Normal-phase silica gel chromatography of the volatile aerial parts of *P. laciniata* eluted with a gradient of EtOAc in petroleum ether, afforded 24 fractions. Silica gel column chromatography of the fractions (5 and 6) eluted with E.P./AcOEt (9:1) afforded the alantolactone 1.

The molecular formula of compound 1, a white crystalline solid, was deduced as C<sub>15</sub>H<sub>20</sub>O<sub>2</sub>, by EIMS analysis in combination with IR, <sup>1</sup>H NMR and <sup>13</sup>C NMR data (Table 2). The IR spectra suggested the presence of lactone moiety (1742 cm<sup>-1</sup>).

Analysis of the  $^1\text{H}$  NMR spectrum of 1 revealed characteristic resonances of three olefinic protons appearing at  $\delta$  5.08;  $\delta$  5.56 and 6.13 ppm, two of which having chemical shift values and coupling constant data of an ethylenic methylene group ( $\delta$  5.56 and 6.13, d,  $J=1.6$ ,  $\text{H}_{13}^{\text{a/b}}$ ) and indicating the presence of two double bonds in the structure. Furthermore, the same spectrum pointed the presence of an oxygenated methine group on the basis of the characteristic resonance of proton  $\text{H}_9$  (brs) at  $\delta$  4.76. The  $^{13}\text{C}$  NMR spectrum displayed 15 signals suggesting that compound 1 is a sesquiterpene lactone. Comparison of  $^1\text{H}$  and  $^{13}\text{C}$  NMR data with those of the literature linked to sesquiterpene [14-15] allowed us to suggest that compound 1 is alantolactone.

The use of some 2D NMR experiments has permitted the confirmation of the proposed structure. In fact, the presence of a cyclohexene ring was established on the basis of long range correlations between proton  $\text{H}_{10\beta}$  ( $\delta$  2.10) and carbons  $\text{C}_8$  ( $\delta$  39.5),  $\text{C}_9$  ( $\delta$  76.4),  $\text{C}_1$  ( $\delta$  32.7),  $\text{C}_6$  ( $\delta$  149.1) and  $\text{C}_{14}$  ( $\delta$  28.5) as well as significant connectivities between the ethylenic proton  $\text{H}_7$  with  $\text{C}_9$ ,  $\text{C}_1$  and  $\text{C}_5$  ( $\delta$  37.6). On the other hand, the  $^1\text{H}$ - $^1\text{H}$  COSY NMR spectrum showed connectivities between the proton  $\text{H}_{15}$  with  $\text{H}_5$  which in turn had a cross peak with  $\text{H}_3$ . The relative stereochemistry of alantolactone was determined on the basis of significant NOE  $\text{H}_8$ - $\text{H}_9$  indicating that should be *cis*.

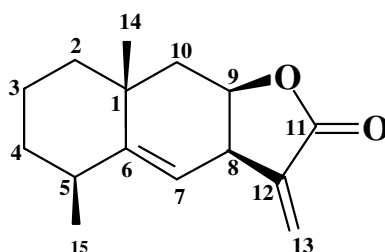


Figure 1: Alantolactone

Table 2. NMR spectral data of Alantolactone;  $^1\text{H}$  NMR (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) in  $\text{CDCl}_3$

Atom no.	$\delta_{\text{H}}$ (mult., $J$ in Hz)	$\delta_{\text{C}}$	$^1\text{H}$ - $^1\text{H}$ COSY	HMBC
1		32.7		
2	1.61 1.15(m)	41.7	11	
3	1.81 1.43(m)	16.7	10-12	
4		32.7	9-11	
5 $\alpha$	2.45	37.6	10-15	
6		149.1		
7	5.08(d, 3.9)	118.7	9	4-6-9
8 $\alpha$	3.60(brs)	39.5	4-8-13	
9 $\alpha$	4.76(brs)	76.4	5	
10 $\alpha$	1.52(m)	42.6		6-14
10 $\beta$	2.10(dd, 15, 2)			3-4-6-7-14
11		170.3		
12		139.8		
13a	6.13(d, 1.6)	121.5	3	1-2-3
13b	5.56(d, 1.6)			1-3
14	1.19(s)	28.5		5-6-7-12
15	1.09(d, 1.5)	22.5	9	7-9-10

### 2.3. Antibacterial activity:

The antibacterial activities of alantolactone were determined against seven bacterial strains. It showed best activity against *Staphylococcus aureus* ATCC 29213 and *E. faecalis* ATCC 29212. Nevertheless, alantolactone presented minor activity against negative Gram rods *E. coli* ATCC35218 and *Se. marcescens*.

**Table 3:** Antibacterial effects of Alantolactone (40µg/disk).

<i>E. coli</i>	<i>St.</i>	<i>P.</i>	<i>S.</i>	<i>E.</i>	<i>Se.</i>	<i>L.</i>
ATCC 35218	<i>aureus</i> ATCC 29213	<i>aeruginosa</i> ATCC 27853	<i>typhimurium</i> (-)	<i>faecalis</i> ATCC 29212	<i>marcescens</i> (7)	<i>monocytogenes</i> (-)
(7)	(9)	(-)	(-)	(8)	(7)	(-)

*E.*: *Enterococcus* ; *E.*: *Escherichia* ; *L.* : *Listeria* ; *P.*: *Pseudomonas*; *Se.* *Serratia*; *S.*: *Salmonella*; *St.*: *Staphylococcus*.  
ATCC: American Type Culture Collection.

(-): No activity detected; (+): Positive activity measured in mm showed by inhibited clear area.

### 3. EXPERIMENTAL SECTION

**3.1. Plant material:** *Pulicaria laciniata* (Asteraceae) collected in May 2004 from Eljem (Tunisia). The plant was identified in the Laboratoire de Biologie végétale et Botanique, Institut Supérieur Agronomique, Université de Sousse (Sousse, Tunisia). A voucher specimen (PL-06) is deposited at the indicated Laboratory.

**3.2. Extraction of essential oils:** 850 g of *Pulicaria laciniata* fresh flowers were separated from the aerial parts before being subjected to water distillation. The aqueous phase thus obtained was successively extracted with chloroform and dried over anhydrous sodium sulphate yielding after solvent evaporation  $2.06 \cdot 10^{-2}$  (w/w) of pale yellow oil.

**3.3. Extraction of alantolactone:** 2150 g of aerial parts of *P. laciniata* were subjected to water distillation. The aqueous phase thus obtained was successively extracted with chloroform and dried over anhydrous sodium sulphate yielding after solvent evaporation  $12.3 \cdot 10^{-3}$  (w/w) of pale yellow oil (230 mg) and then subjected to silica gel column chromatography eluted with petroleum ether-AcOEt to yield 24 fractions.

The mixture of fractions 5 and 6 (20 mg) was chromatographed on silica gel CC eluted with petroleum ether-AcOEt (9:1) to give the alantolactone (9 mg).

#### 3.4. Analysis of the essential oil:

**Gas Chromatography:** HP 5890-series II equipped with: Flame ionisation detectors (FID), a HP Innowax (BP-20) (polyethylenglycol) 30 m x 0.25 mm ID, 0.25 µm film thickness fused capillary column. The carrier gas was nitrogen ( $1.2 \text{ mL}\cdot\text{min}^{-1}$ ). The over temperature program was 1 min. isothermal at 50°C, then 50-250°C at rate of 5°C/min and held isothermal for 1 min, detector 280°C [8,9]. Volume injected: 1µL of 1 % solution (diluted in hexane). Percentages of the constituents were calculated by electronic integration of FID peak areas without the use of the response factor correction.

**GC/MS:** The analysis of the volatile constituents were run on a Hewlett-Packard GC-MS system (GC: 5890 series II; MSD 5972). The HP Innowax polar column (30 m x 0.25 mm ID, film thickness of 0.25 µm) was directly coupled to the MS. The carrier gas was helium, with a flow rate of  $1.2 \text{ mL}\cdot\text{min}^{-1}$ . Oven temperature was programmed (50°C for 1 min, then 50-250°C at 5°C/min) and subsequently, held isothermal for 20 min. Injector port: 250 °C, detector: 280 °C, split ratio 1:50. Volume injected: 1µL of 1 % solution (diluted in hexane).

**Mass spectrometer:** HP 5972 recording at 70 eV; scan time 1.5 sec; mass range 40-300 amu. Software adopted to handle mass spectra and chromatograms was a ChemStation.

**Analysis of the composition:** The oil components were identified by comparison of their retention index [relative to C<sub>9</sub>-C<sub>28</sub> alkanes] and mass spectra with those of authentic standards of library searches of the oil library LIBR Tped using the Finnigan library search routine based on fit and purity of mass spectra [10-11].

**3.5. Antibacterial activity:** The antibacterial activity of alantolactone was evaluated by agar-diffusion method.

#### Bacterial strains

The antibacterial activity was studied against the following seven bacteria, including Gram positive

bacteria: *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *Listeria monocytogenes* and Gram-negative bacteria: *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* and *Serratia marcescens*.

The nutrient broth (bio-Rad) and Mueller-Hinton Agar (bio-Rad) were used, respectively, for growing and diluting the microorganism suspensions for the antimicrobial assays.

#### **Agar diffusion method:**

Antibacterial activities were assessed using the paper disk agar diffusion method [12] inoculates grown in the nutrient broth at 37 °C for 24 h were diluted to approximately  $5 \times 10^6$ -  $10^7$  CFU/mL. The alantolactone was dissolved in chloroform in order to have the concentration of 40µg/disk. So, the Whatman disk (No. 3) with 6 mm diameter was impregnated with 20 µl of the prepared solution, after evaporation of the solvent, disk was placed on the surface of inoculated plates (90 mm) and incubated at 37 °C for 18-24 h. Negative control was prepared using a disk impregnated with the same solvent as that used to dissolve the compound. Antibacterial activity was assessed by measuring the clear inhibition zone. This was the zone that visibly showing absence of growth, including the diameter of disk.

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