SHORT REPORT

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Phytochemical Studies on *Ptilostemon greuteri* Raimondo & Domina (Compositae)

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Abstract: *Ptilostemon greuteri* Raimondo & Domina is described as a new species and its growth is limited to the area of the province of Trapani. Essential oils of aerial parts of *P. greuteri* were analized by gas chromatography-mass spectrometry (GC-MS). The analysis of acetonic extract of aerial parts led to identification of triterpenes components: α-amyrin, β-amyrin, α-amyrin acetate, β-amyrin acetate, lupeol, lupeol acetate and taraxasterol. CC and preparative TLC of acetonic extracts has yielded lignan lactone and a sesquiterpene lactone that have been isolated previously from other *Ptilostemon* species.

Keywords: *Ptilostemon greuteri*; Compositae; sesquiterpene lactone; lignan lactone; pentacyclic triterpenes; essential oil.

1. Plant source

The genus *Ptilostemon* (Compositae) is one of the ten genera that comprise one of the largest informal groups defined in the subtribe Carduinae or 'thistles'[1]. *Ptilostemon* is placed among the 'thistles' on both morphological and molecular grounds [1-3]. Point of interest of *Ptilostemon* genus is its geographic distribution, which is restricted to the Mediterranean area, from Crimea and Turkey to the Iberian Peninsula and Morocco. The genus *Ptilostemon* includes 14 species; in Sicily the genus is represented by *P. niveus*, a hemicryptophyte that grows strictly in the Madonie mountains and *P. stellatus*, a therophyte that grows in the Madonie, Nebrodi and Peloritani mountains.

Ptilostemon greuteri Raimondo & Domina, is an endemic species widespread in North East of Sicily and it was been recently described as a new species [4]. *P. greuteri* grows in a limited area of the province of Trapani at 250-500 m altitude on the north facing slope of a limestone mountain. The locality is characterized by a thermo-Mediterranean climate with an annual mean temperature of 18 °C and an annual mean precipitation of 600 mm.

2. Previous study

The main chemical constituents of *Ptilostemon* species are acetylenes, triterpenes, sesquiterpenes and lignans [5-7]. There is no any literature data concerning chemical composition of *Ptilostemon greuteri* Raimondo & Domina.

3. Present study

Aerial parts of *P. greuteri* Raimondo & Domina were collected during the flowering season (May-June 2010) on the northern slope of Inici mountain near Castellammare del Golfo (Trapani). Voucher specimens are in Botanical Garden of Palermo (GU907756, GU907734).

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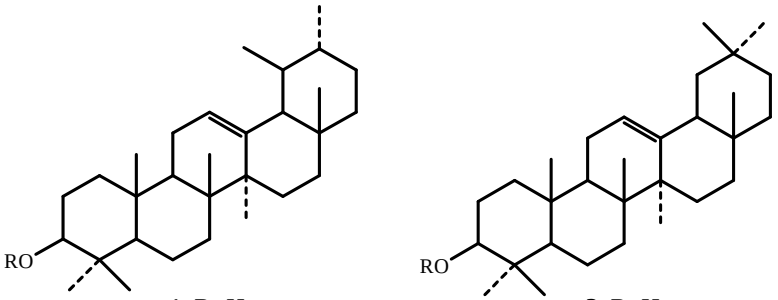
A sample of fresh aerial parts (100 g) was chopped and hydrodistilled for 2h in a Claevenger type apparatus, to obtain essential oil. Essential oil was analyzed by GC-MS using a Hewlett-Packard GC, model 6890. It was equipped with a AT-5MS capillary column: 5% phenyl-methylpolysiloxane, film thickness 0.25 μm, a length of 30 m, 0.25 mm (i.d.). The working conditions were: injector temperature 280 °C; 60°C initial temperature, hold for 5 min; increased at 5 °C/min to 300 °C, followed by 5 min. under isothermal conditions. Helium was the carrier gas at 1.0 mL/min; the sample oil was diluted in $CHCl_3$ to give a 1% w/v solution; 1 μL was injected using split mode (1:50). The GC was interfaced with a Micromass AutoSpec Ultima-OToF double-focusing magnetic sector mass spectrometer. Identification of the individual components was based on matching with NIST 2002 mass spectra library and comparison with spectra of authentic samples and literature data. At the same time, the compounds were identified by their retention indices (RI determined with reference to a homologous series of *n*-alkanes). The chemical profile of the *P. greuteri* essential oil showed δ-cadinene (35.7%), β-cubebene (25.6%) and farnesol (10%) as main components.

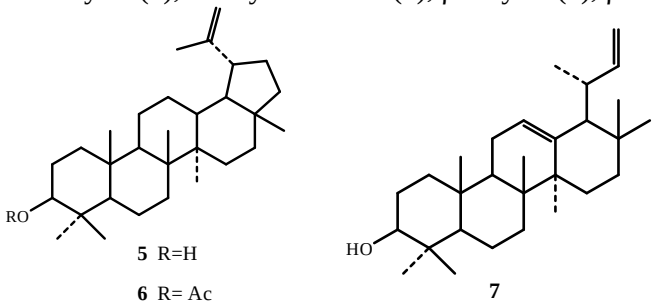
Afterwards, air-dried and powdered aerial parts of *Ptilostemon greuteri* (215 g) were extracted with Me_2CO (1000 mL) at room temperature for a week. After removal of the solvent at reduced pressure, the brown extract residue (14.1 g) was adsorbed on Si gel and was subjected to column chromatography. By elution with gradients of petroleum ether and EtOAc were obtained totally 7 fractions. Fraction 1 has been previously subjected to CC and after to GC-MS. Mixtures analyzed allowed the identification of seven known triterpenes (**1-7)**. Fractions 3-6 were combined and fractionated by CC and preparative TLC to isolate compound **8** and **9**. Structures of these compounds were elucidated mainly on the basis of IR, UV, MS and NMR (^1H and ^{13}C) spectral analyses as well as by comparison with respective published data [6, 8-10].

GC-MS analysis of fraction 1 was carried out using GC-MS method above cited, and allowed the identification of known triterpenes as α-amyrin (**1**), α-amyrin acetate (**2**), β-amyrin (**3**), β-amyrin acetate (**4**) (Figure 1), lupeol (**5**), lupeol acetate (**6**) and taraxasterol (**7**) (Figure 2), by direct comparison with authentic compounds isolated from different plant material in our laboratory and by comparison of their mass spectral fragmentation patterns with those reported in the literature and in NIST 2002 library [11].



1 R=H
2 R= Ac
3 R=H
4 R= Ac

Figure 1. α-Amyrin (**1**), α-amyrin acetate (**2**), β-amyrin (**3**), β-amyrin acetate (**4**)



5 R=H
6 R= Ac
7

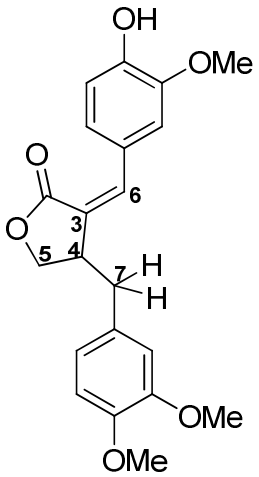
Figure 2. Lupeol (**5**), lupeol acetate (**6**) and taraxasterol (**7**)

4,5-Dihydro-4-(3″,4″-dimethoxybenzyl)-3-(4′-hydroxy-3′-methoxybenzylidene)furan-2(3H)-one (**8**): colorless oil, UV λ_{max} (MeOH): 284, 330 nm; IR υ_{max}, cm^{-1}: 3560 (OH), 1750 (lactone C=O); HRMS *m/z*: 400,4648 ($C_{23}H_{28}O_6$), 370, 353, 247, 219; ^{1}H-NMR ($CDCl_3$, 300MHz) δ: Table 1; ^{13}C-NMR ($CDCl_3$, 75MHz) δ: Table 1.

Table 1. ^{1}H NMR and ^{13}C NMR spectral data of compound **8 (**in $CDCl_3$)

No.	δ_C	δ_H	No.	δ_C	δ_H
1	-	-	3′	149.7	-
2	172.0	-	4′	147.9	-
3	126.1	-	5′	111.7	6.99 (1H, d, J=7.9 Hz),
4	45.1	3.83 (1H, m)	6′	122.5	7.21 (1H, dd, J=7.9, 1.9 Hz),
5a	69.5	4.28 (1H, d, J=4)	1″	131.7	-
5b			2″	113.6	6.68 (1H, d, J=2.1 Hz)
6	138.5	7.52 (1H,d, J=2 Hz).	3″	147.6	-
7a	37.5	3.08 (1H, dd, J=4, 14.5 Hz)	4″	146.0.0	-
7b		2.65 (1H, dd, J=10, 14.5 Hz)	5″	115.7	6.81 (1H, d, J=8.1 Hz)
1′	127.1	-	6″	122.9	6.74(1H, dd, J=8.1, 2.1 Hz)
2′	111.5	7.04 (1H, d, J=1.9 Hz)	3OCH_3	55.3, 56.7; 57.1	3.92; 3.86

δ in ppm from TMS. ^{1}H NMR spectral data (300 MHz). ^{13}C NMR spectral data (75 MHz)



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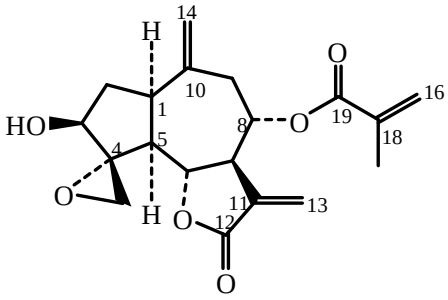

9

Figure 3: 4,5-Dihydro-4-(3″,4″-dimethoxybenzyl)-3-(4′-hydroxy-3′-methoxybenzylidene)furan-2(3*H*)-one **(8)** and Desoxyrepin (**9**)

Desoxyrepin (**9**): amorphous solid, UV λ_{max} (MeOH): 213 nm; IR ($CHCl_3$) υ_{max}, cm^{-1}: 3600, 1740 (lactone C=O), 1660; HRMS *m/z*: 346,1416 ($C_{19}H_{22}O_6$), 260, 242, 57; ^{1}H-NMR ($CDCl_3$, 300MHz) δ: Table 2; ^{13}C-NMR ($CDCl_3$, 75MHz) δ: Table 2.

The isolation and identification of compound **8** was reported for the first time from *Ptilostemon diacantum* (Labill.) Greuter, including the sesquiterpene lactone desoxyrepin **9**. All these data were in good agreement with the respective literature data [6, 8-10, 12]

Table 2. ^{1}H NMR and ^{13}C NMR spectral data of compound **9** (in $CDCl_3$)

No.	δ_C	δ_H	No.	δ_C	δ_H
1	46.0	3.35 (1H, m)	12	169.0	-
2a	38.9	1.82 (1H, ddd J=9.8, 14.6, 4.3 Hz)	13a	121.1	5.57 (1H, d J=3.5 Hz)
2b		2.49 (1H, ddd J=9.4, 14.6, 6.5 Hz)	13b		6.21 (1H, d J=3.1 Hz)
3	75.5	3.99 (1H, dd J=4.3, 6.5 Hz)	14a	118.0	4.99 (1H, br s)
4	69.2	-	14b		5.20 (1H, br s)
5	53.1	2.06 (1H, dd J=8.6, 11 Hz)	15a	48.6	3.33 (1H, d J=4.2 Hz)
6	77.5	4.63 (1H,dd, J=11, 9.2 Hz).	15b		3.06 (1H, d J=4.2 Hz)
7	47.8	3.07 (1H, m)	16a	126.0	6.15 (1H, dd, J=7.8, 1.9 Hz)
8	75.3	5.14(1H,ddd, J=5, 9.3, 3.1 Hz)	16b		5.65 (1H, dd, J=7.8, 1.9 Hz)
9a	36.4	2.38 (1H, dd, J=3.1, 14.9 Hz)	17	21.3	1.97 (3H, s)
9b		2.73 (1H, dd, J=5, 14.9 Hz)	18	125.0	-
10	142.7	-	19	166.0	-
11	138.6	-			

δ in ppm from TMS. ^{1}H NMR spectral data (300 MHz). ^{13}C NMR spectral data (75 MHz)

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