



RETRACTED: Isoflavone and triterpenoid isolated from an endemic plant *Genista microcephala* Coss et Dur.

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This article has been retracted at the request of the Co-Authors with the Editor-in-Chief approval.

The Journal of Pharmacy & Pharmacognosy Research (JPPRes) retracts the article “Isoflavone and triterpenoid isolated from an endemic plant *Genista microcephala* Coss et Dur.” by lhem Bouakaz et al., which was published in 2015.

Against the policy agreed to by the JPPRes in relation to corresponding author, this paper was submitted for publication without the consent of two of the authors (G. Massiot and H. Haba). One of the conditions of submission of a paper for publication in JPPRes is that as corresponding author declares explicitly that “all authors are aware that study has been submitted to Journal of Pharmacy & Pharmacognosy Research (JPPRes)”. This article has been retracted as its publication violates this policy.

As such this article represents a severe abuse of the scientific publishing system. The scientific community takes a very strong view on this matter and apologies are offered to readers of this journal because this inconvenient was not detected during the submission process.



Isoflavone and triterpenoid isolated from an endemic plant *Genista microcephala* Coss et Dur

[Isoflavona y triterpenoide aislados de una planta endémica *Genista microcephala* Coss et Dur]

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Abstract

Context: *Genista microcephala* Coss et Dur is a plant that grows in northern of Algeria. The *Genista* species show interesting biological properties.

Aims: To study phytochemically of the aerial parts of *G. microcephala* and search new compounds with pharmacological interest.

Methods: Aerial parts of *G. microcephala* were extracted using chloroform. The extract was purified by several chromatography processes. Isolated compounds were analyzed by the usual spectroscopic methods and 2D NMR technics.

Results: From the chloroformic extract of the air parts of *Genista microcephala* (Leguminosae) two known components were obtained. The structures of these compounds were oleanolic acid (**1**), alpinum isoflavone (**2**) elucidated by the usual spectroscopic methods and 2D NMR technics and by comparison with literature data.

Conclusions: Two known components were obtained from the chloroformic extract of the air parts of *Genista microcephala* but they have not previously been reported from this species.

Keywords: *Genista microcephala*; isoflavone; Leguminosae; triterpenoid.

Resumen

Contexto: *Genista microcephala* Coss et Dur, es una planta que crece en el norte de Argelia. Las especies de *Genista* muestran propiedades biológicas interesantes.

Objetivos: Estudiar desde el punto de vista fitoquímico las partes aéreas de *G. microcephala* y buscar nuevos compuestos que tengan interés farmacológico.

Métodos: Las partes aéreas de *G. microcephala* fueron extraídas con cloroformo. El extracto fue purificado mediante varios procesos cromatográficos. Los compuestos aislados fueron analizados por métodos espectroscópicos usuales y técnicas de 2D RMN.

Resultados: Se obtuvieron dos compuestos conocidos del extracto cloroformico de las partes aéreas de *Genista microcephala* (Leguminosae). Las estructuras de estos compuestos fueron ácido oleanólico (**1**) y alpinum isoflavona (**2**) elucidadas por métodos espectroscópicos usuales y técnicas de 2D RMN y la comparación con datos de la literatura.

Conclusiones: Se obtuvieron dos compuestos conocidos en el extracto cloroformico de las partes aéreas de *G. microcephala* pero estos son reportados por primera vez para esta especie.

Palabras Clave: *Genista microcephala*; isoflavona; Leguminosae; triterpenoide.

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INTRODUCTION

The species *Genista microcephala* Coss et Dur. belongs to the genus *Genista* of the family Fabaceae, also called Leguminosae. The genus *Genista* consists of 100 species predominantly distributed in the Mediterranean area and Western Asia (Noccioli et al., 2011). Among these species, 23 grow in Algeria including 11 endemics distributed particularly in the Northern parts of the country (Quezel and Santa, 1963). Previous phytochemical investigations on the *Genista* genus indicated the presence of alkaloids (Kirch et al., 1995; Pistelli et al., 2001; Martins et al., 2005; Zellagui et al., 2005); flavonoids, isoflavonoids (Pistelli et al., 1998; 2000; Giachi et al., 2002; Mekkiou et al., 2005; Tosun et al., 2009), which are chemotaxonomic markers of the genus (Harborne, 1994) and recently saponins (Boutaghane et al., 2013).

The genus *Genista* have recently attracted the attention of various authors for their phytochemical and biological properties, particularly antidiabetic (Rauter et al., 2005; 2009), anti-inflammatory, antiulcer (Ilarionov et al., 1979; Rainova et al., 1988) spasmolytic, antioxidant, phytoestrogenic (Luczkiewicz and Glod, 2003; Garritano et al., 2005) and cytotoxic activity against different human cancer cell lines (Scarpato et al., 2008; Rigano et al., 2009; 2010), and effects on the thyroid gland (Korpachov et al., 1995).

As a part of our ongoing research on new bioactive compounds from endemic plants of Algeria, we have studied the aerial parts of *Genista microcephala*. In the present work, we describe the isolation and characterization of two known components oleanolic acid (**1**) and alpinum isoflavone (**2**) (Fig. 1). All compounds were identified by extensive spectroscopic methods including 1D- (¹H and ¹³C) and 2D-NMR (COSY, HSQC and HMBC) and comparison of their spectral data with the literature. To our best knowledge, this is the first report of oleanolic acid (**1**) and alpinum isoflavone (**2**) in *Genista microcephala*.

MATERIAL AND METHODS

General method

Silica gel 60 (230–400 mesh, Merck Kieselgel) were used for flash and column chromatography, respectively. Analytical TLC were performed on silica gel (Merck Kieselgel 60 F254) and visualized under UV light at 254 and 365 nm and/or sprayed with sulphuric acid-vanillin. ¹H and ¹³C NMR spectra (samples solved in CD₃Cl) were recorded on a BrukerAvance DRX 500 NMR spectrometer (¹H at 500 MHz and ¹³C at 125 MHz). ESI spectra were recorded on Bruker LC-MS/MS type esquire-LC.

Plant material

The aerial parts of *Genista microcephala* was collected in May 2003 in the vicinity of Biskra (Elkantara, North-Eastern Algeria). The plant was identified by Prof. Oudjehih Bachir, Agronomic Departement of the University of Batna, where a voucher specimen has been deposited under N° 188.

Extraction and separation

Dried and powdered aerial parts of *Genista microcephala* (2.5 kg) were extracted in a Soxhlet apparatus with petroleum ether, chloroform and butanol in turn, to obtain the etheropetrolic extract (8 g), the chloroformic extract (16 g) and the butanolic extract (50 g), respectively. Chloroformic extract was subjected to silica gel column chromatography using a mixture of petroleum ether/ethyl acetate (EP/AcOEt) of increasing solvent polarity as eluent. The fractions 95-96 (80 mg) were subjected to a precipitation with ethyl acetate give compound (**1**) (10 mg). The fractions 133-135 (90 mg) were subjected to thin layer chromatography with eluent petroleum ether/ethyl acetate (85/15) give compound (**2**) (20 mg).

RESULTS AND DISCUSSION

Column chromatography of the chloroformic extract led to the isolation of two known constituents (Fig. 1). They were identified by using spectroscopic methods including ESI-MS, 1D and 2D NMR analysis and also by comparing experimental data with those described in the literature as oleanolic acid (1) and alpinum isoflavone (2).

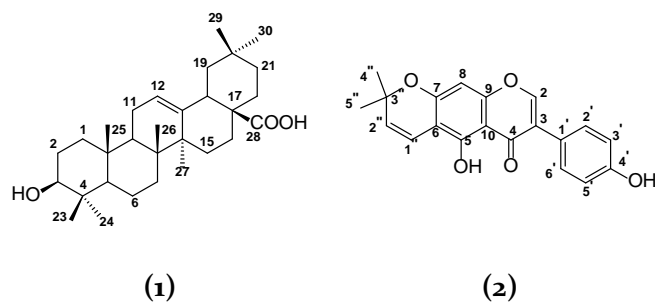


Figure 1. Chemical structures of oleanolic acid (1) and alpinum isoflavone (2).

Compound (1) gave positive, showed a molecular ion at m/z 456 corresponding to $C_{30}H_{48}O_3$. The 1H -NMR spectrum showed seven tertiary methyl groups at δ_H 0.76, 0.78, 0.91, 0.92, 0.93, 0.99 and 1.14 on an oleanane skeleton. ^{13}C NMR (Table 1) showed seven signals between δ_C 15.2 and 33.2, which confirmed the structure of the skeleton. A doublet-doublet of one proton at δ_H 2.82 and a triplet of one vinyl proton at δ_H 5.28 were assigned to H-18 and H-12, respectively, suggesting an olea-12-ene skeleton. One methine proton at δ_H 3.22 (dd, $J=11.7$ and 4.75 Hz) showed that compound (1) has at least one hydroxyl group. In ^{13}C -NMR spectrum, the signal corresponding to the carboxyl C-28 appeared at δ_C 181.0. The spectral data were similar to the ones reported for oleanolic acid (Hung and Yen, 2001).

Compound (2) was obtained as needle-shaped crystal. It gave a positive showed a molecular ion at m/z 336 corresponding to $C_{20}H_{16}O_5$. It melted at $213^\circ C$, which was identical to that reported for alpinum isoflavone. It was found to be soluble in ethyl acetate, chloroform, acetone and methanol.

The 1H NMR spectrum (Table 2) of compound (2) revealed well-resolved signals typical of an isoflavone nucleus having a pyran ring. Thus the 1H NMR spectrum showed a pair of doublets ($J=10.0$ Hz) centered at δ_H 5.52 and 6.72 and a sharp singlet of six proton intensity at δ_H 1.47 these were assigned to a 2,2-dimethylchromene ring system.

Table 1. ^{13}C and 1H NMR data of compound 1 in $CDCl_3$.

Position	δ_C (ppm)	Type C	δ_H (ppm) J (Hz)
1	38.4	CH ₂	
2	27.2	CH ₂	
3	78.9	CH	3.22 (dd, $J=11.7, 4.75$ Hz)
4	38.7	C	
5	55.2	CH	6.73 (dd, $J=11.8, 1.7$ Hz)
6	18.3	CH ₂	
7	33.1	CH ₂	
8	41.7	C	
9	47.6	CH	
10	37.1	C	
11	23.0	CH ₂	
12	122.4	CH	5.28 (t, $J=3.5$ Hz, H-12)
13	143.8	C	
14	39.3	C	
15	27.7	CH ₂	
16	23.4	CH ₂	
17	46.0	C	
18	41.1	CH	2.82 (dd, $J=13.6, 4.14$ Hz)
19	46.1	CH ₂	
20	30.7	C	
21	33.9	CH ₂	
22	32.4	CH ₂	
23	28.1	CH ₃	0.99 s
24	15.5	CH ₃	0.76 s
25	15.3	CH ₃	0.92 s
26	17.1	CH ₃	0.78 s
27	25.9	CH ₃	1.14 s
28	181.0	COOH	
29	33.1	CH ₃	0.91 s
30	23.6	CH ₃	0.93 s

The characteristic C-2 proton of the isoflavone skeleton was evident as a singlet at δ_H 7.81 (1H). The 1H NMR spectrum also displayed a pair of

doublets ($J=8.62$ Hz), each integrating for two protons, at δ_H 6.91 and 7.36, which were assigned to the H-3' & H-5' and H-2' & H-6' of the para-disubstituted aromatic nucleus. The relatively upfield resonance δ_H 6.32 of H-3' and H-5' suggested the presence of an oxygenated substituent at C-4'. This was substantiated by the presence of a broad singlet at δ_H 5.62 (1H), due to a hydroxyl group proton. The remaining two signals at δ_H 13.18 and 6.34 (each 1H) could be attributed to the chelated hydroxyl group proton at C-5 and H-8, respectively. This hypothesis is confirmed by HMBC, which show that proton at δ_H 6.32 correlated with three carbons at δ_C 159.45, 157.31, 106.12 attributed respectively to C₇, C₉, C₁₀.

On the basis also of the above spectral data and by comparison of these values with those reported for alpinum isoflavone (Martinez et al., 1982;

Hernandez et al., 2000; Rahman et al., 2007) the identity of the compound (**2**) was confirmed as alpinum isoflavone, and this is the first report of its isolation from *Genista microcephala*.

CONCLUSIONS

The chemical study of the aerial parts of *Genista microcephala* afforded oleanolic acid (**1**) and alpinum isoflavone (**2**). The structures of these compounds were established by extensive spectroscopic studies and comparison with published data but these two known constituents have not previously been reported from this species.

Other studies for extracting new compounds from this species are now under investigations.

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

Position	δ_C (ppm)	δ_H (ppm) J (Hz)	HMBC (H to C)	COSY
2	152.42	7.81 s	C-3, C-4, C-9, C-10, C-1'	
3	122.21	-		
4	180.99	-		
5-OH	156.92	13.18 s	C-4, C-5, C-6, C-7, C-10	
6	105.51	-		
7	159.45	-		
8	94.81	7.37 s	C-7, C-9, C-10	
9	157.21	-		
10	106.12	-		
1'	123.68	-		
2'	130.17	7.36 d 8.62	C-3, C-4', C-6'	H-3'
3'	115.68	6.91 d 8.62	C-1', C-4', C-5'	H-2'
4'-OH	156.92	6.32 s	C-4, C-6, C-7', C-9, C-10, C-4''	
5'	115.68	6.95 d 8.62	C-1', C-4', C-5'	H-6'
6'	130.17	6.37 d 8.62	C-3, C-2', C-4'	H-5'
2''	77.99	-		
3''	128.11	5.62 d 10.0	C-6, C-7, C-2'', C-5'', C-6''	H-4''
4''	115.49	6.72 d 10.0	C-6, C-6, C-2'', C-5'', C-6''	H-3''
5''	28.28	1.47 s	C-7, C-2'', C-3'', C-4'', C-6''	
6''	28.28	1.47	C-7, C-2'', C-3'', C-4'', C-5''	

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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