Flavonoids from *Cotula cinerea* Del.

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**Article History:** Received 7th October 2012, Revised 27th December 2012, Accepted 28th December 2012.

Abstract: The phytochemical investigation of *Cotula cinerea* Del. afforded eighteen compounds: a germacranolide, Tatridin A, 1, and seventeen flavonoids 2-18. Compounds 1, 3, 4, 5, 6, 7, 8, 11, 12, 13, 14, 17 are new for the genus *Cotula*.

Keywords: *Cotula cinerea* Del.; Asteraceae; Flavonoids; Germacranolide.

Introduction

The genus *Cotula* (Asteraceae) comprising 80 species is widespread in the southern hemisphere (Heywood et al. 1977) and is represented by three species in Algeria among which *Cotula cinerea* Del. (syn. *Brocchia cinerea*) (Ozenda 1963).

It is used in traditional medicine for its anti-inflammatory, analgesic and antiseptic properties (Jana 1996) and as aromatic and digestiv substance in tea (Bellakhdar 1997). It has also been reported as analgesic, antipyretic (Larhsini et al. 2002), bacteriostatic (Jana et al. 1992) and antiprotozoar (Markouk et al. 1999). Previous phytochemical investigation of *Cotula cinerea* led to the isolation of several flavonoids including aglycones such as kaempferitin, quercetrin, quercetin, luteolin, apigenin and kaempferol, and heterosides such as luteolin 7-O-glucoside, 7-O-diglucosides, rhamnosyl and glucosyl apigenin derivatives (Ahmed et al. 1987; Mahran et al., 1976). Eudesmanolides, guaianolides, glaucolides (Jakupovitch et al. 1988; Metwalli et al., 1986) and coumarins (Greger et al. 1985) were also described from this species. The chemical composition of the essential oil of this species was also investigated (Aboutabl et al., 1990; Fournier et al., 1989). As a part of our study on Algerian Saharian plants (Benayache et al. 2012; 2011), we have investigated the aerial parts of *Cotula cinerea* Del. The present work describes the isolation and structural identification of eighteen compounds; twelve are described for the first time for the genus *Cotula*.

Material and methods

Plant material

*Cotula cinerea* Del. was collected during the flowering period (may 2010) from Merrara near Touggourt in Southern Algeria. A voucher specimen (CC 16/05/10) has been deposited in the Herbarium of the VRNMB research unity (University Mentouri of Constantine).

Results and discussion

We report herein the isolation of seventeen flavonoids and a germacranolide (Figure 1) from the ethanolic-aqueous extract of the aerial parts of *Cotula cinerea*: 1α,6α-dihydroxy-germacra-4E,9Z,11(13)-trien-12,8α-olide 1
Flavonoids from Cotula cinerea Del.


Figure 1: Cotula cinerea Del.

Figure 2: HPLC Chromatographic profile of extract of aerial parts of Cotula cinerea Nucleosyl column (250x4.6 mm) with gradient of two solvents: A = (H2O/CH3CN/AcOH, 9:1:0.2) B = (H2O/CH3CN/AcOH, 2:8:0.2)

Aerial parts (277 g) were extracted with 70% EtOH for 48 h three times. The EtOH extract was concentrated and suspended in H2O (300 ml). The solution was extracted successively with petroleum ether, ethyl acetate and n-BuOH. The organic phases were dried with Na2SO4, filtered using common filter paper and concentrated under vacuum to obtain the following extract: CHCl3 (1.9 g), ethyl acetate (2.7 g) and n-BuOH (7.9 g). The chloroform extract was applied to a silicagel column chromatography, eluted with petroleum ether/ethyl acetate with increasing polarity to give fifteen fractions. Fraction 7 (85:15) was rechromatographed on silicagel column, flash chromatography using a mixture of Hexane/CHCl3/EtOAc (1:1:1) to give 10 subfractions. Subfraction 5...
was purified by silicagel TLC (Hex-
ane/CHCl₃/EtOAc 1:1:1) to give compound 1. 
Fraction 9 (70:30) was purified by repeated 
preparative silicagel TLC (Hexane/CHCl₃/
AcOEt 1:1:3) to give compounds 2 and 3. Frac-
tion 10 (65:35) was rechromatographed on si-
ilicagel column eluted with a gradient of Hex-
ane/EtOAc to give 7 subfractions. Subfraction 3 
(70:30) afforded compound 4 as a precipitate 
which was washed with methanol.

The n-BuOH and AcOEt extracts were 
joined on the basis of their TLC profiles. The 
mixture (10.6g) was applied to a column of 
polyamide MN SC6 eluted with a gradient of 
toluene-MeOH. Fifteen flavonoids 5-18 con-
tained in four main fractions were isolated. 
Fraction 2 (75:25) was purified by preparative 
TLC on polyamide DC6 
(H₂O/MeCOEt/MeOH/Ac₂CH₂ 13:3:3:1) to 
give compounds 5, 6 and 9. Fraction 3 (70:30) 
was purified by PC on Whatman 3 MM using 
15% AcOH to give compound 10 and a mixture 
which was rechromatographed by preparative 
TLC on polyamide DC6 (H₂O/MeCOEt/
MeOH/Ac₂CH₂ 13:3:3:1) to give compounds 
12 and 17. Fraction 4 (65:35) and (55:45) was 
purified by preparative TLC on polyamide DC6 
(H₂O/ MeCOEt/MeOH/Ac₂CH₂ 13:3:3:1) to give compound 14. Fraction 5 (60:40 to 80:20) 
was purified by repeated PC on Whatman 3 
MM using 15% AcOH and (n-BuOH/
AcOH/H₂O, 4:1:5) to give compound 13 and 
two mixtures which were rechromato-graphed 
by repeated PC on Whatman 3 MM using (n-
BuOH/AcOH/H₂O, 4:1:5) to give compounds 
7,8,11,13, 15, 16 and 18.

**Table 1:** NMR ¹H (400 MHz) and ¹³C (100 
MHz) data of compound 1 in CDCl₃.

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<th>δH (ppm)</th>
<th>δC (ppm)</th>
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<tbody>
<tr>
<td>1</td>
<td>4.3</td>
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<tr>
<td>2</td>
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<td></td>
<td>1.69</td>
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<td>3</td>
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<td></td>
<td>1.88</td>
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<td>4</td>
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<tr>
<td>6</td>
<td>4.46</td>
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<td>8</td>
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<td>15</td>
<td>1.70</td>
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**Figure 4:** NMR ¹H (400 MHz) spectrum of compound 1 in CDCl₃.
**Figure 5**: HSQC spectrum of compound 1.

**Figure 6**: COSY spectrum of compound 1.
Figure 7: HMBC spectrum of compound 1.

Purification of each compound for spectral analysis was carried out using MeOH over Sephadex LH-20 column, eluted with MeOH (Mabry et al.1970; Markham, 1982). Hydrolysis of the glycosides (HCl 0.1 N, 2h) yielded the sugar residues and the aglycones. All of which were co-chromatographed with authentic sam-
ples. $^1$H and $^{13}$C and 2D NMR spectra were recorded in CDCl$_3$ and CD$_3$OD at 400 MHz and 100 MHz (for $^{13}$C). All these results were in good agreement with literature data.

This work led to the isolation of seventeen flavonoids among which seven flavones including four aglycones and three glucosides and ten flavonols including seven aglycones and three glucosides from Cotula cinerea Del. For all the heterosides, O-glycosylation occurs at C-7 position and the sugar moiety was glucose in all cases. The flavonol aglycones isolated were highly methylated. Twelve compounds: 1, 3, 4, 5, 6, 7 ,8 ,11 ,12 ,13, 14, 17 were described for the first time for the species and the genus Cotula.

We can notice the absence of 5 substitution which was reported for other Cotula species. The observed substitution pattern in 6 and 7 positions and the high methylation of flavonols were also found in the genus Artemisia. It is interesting to notice also that Tatridein A 1, and compounds 3, 4, 5, 6, 7, 8, 11, 12, 13, 14 and 17 which are described for the first time for Cotula cinerea were also found in several Artemisia species. On the basis of this data, the species Cotula cinerea Del. seems to be close to the genus Artemisia. These observations may be of chemotaxonomic importance.

Conclusion

The phytochemical investigation of the aqueous-EtOH extract of the aerial parts of Cotula cinerea afforded a germacranolide described for the first time for the species and seventeen flavonoids among which twelve compounds were not previously reported for the genus Cotula.

Acknowledgements: Thanks are due to DGRSDT for financial support.
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