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Chemical Constituents from *Centaurea parviflora* Desf.

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ABSTRACT

Phytochemical investigation of the aerial parts of *Centaurea parviflora* Desf. led to the isolation of 13 secondary metabolites : 5-hydroxy-6,7,3',4'-tetramethoxyflavone (**1**), eupatilin (**2**), eupatorin (**3**), cirsilineol (**4**), jaceosidin (**5**), 5Hα,6Hβ,7Hα-15-hydroxy-8α-(1',2'-dihydroxyethyl-acryloxy)-elema-1(2),3(4),11(13)-trien-6,12-olide (**6**), genkwanin (**7**), thevetiaflavone (**8**), cnicin (**9**), ethyl-*O*-α–*L*-arabinofuranoside (**10**), cornicinine (**11**), syringin (**12**) and nicotiflorin (**13**).The structures were established by the combination of their spectroscopic data, notably the analysis of UV, ^{1}H, ^{13}C NMR, DEPT, HSQC, NOESY, HMBC spectra as well as by HRESI-MS. All these compounds were described for the first time from this species. Compound (**11**) is new for all the reign plant, while compound **10** is new for the genus *Centaurea.*

Keywords: Flavonoids; Sesquiterpene lactones; Syringin; Cornicinine; *Centaurea parviflora*; Asteraceae.

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INTRODUCTION

Many species of the genus *Centaurea* have been used in folk medicine to cure various ailments [1]. Several studies on the species of this genus showed various biological activities [2-4]. As a part of our continuing phytochemical studies on the species of this genus [5-9], we report our results on *Centaurea parviflora* Desf., an endemic species for Algeria and Tunisia [10] on which no previous phytochemical study has been carried out.

In the present work on the chemical constituents of the chloroform and *n*-butanol soluble parts of the aqueous-EtOH extract of the flowering aerial parts of *Centaurea parviflora* Desf, we have isolated 13 compounds from which: 8 flavonoids, 2 sesquiterpene lactones, a phenylpropanoid glucoside, an alcane glycoside and a glucosylated triketide δ-lactone. We report here the chemical study of these compounds.

MATERIALS AND METHODS

General experimental procedure:

TLC: pre-coated aluminium foil silica gel 60 F_{254} (Merck). Column chromatography (CC): silica gel 60 (Merck 200–400 mesh). UV Spectra (MeOH): Shimadzu (190–3200 nm, UV-3101PC) spectrophotometer. NMR spectra: Bruker AMX-500 MHz, ARX 400 and AC 300 spectrometers; chemical shifts (δ) are given in ppm using TMS as internal standard and coupling constants (*J*) are given in Hz. HRESI-MS and ESIMS: Micromass ZAB-spectrometer.

Plant material:

Flowering aerials parts of *Centaurea parviflora* Desf. were collected near Oum El Bouaghi in the eastern Algeria and authenticated by Prof. O. Rached-Mosbah (Ecology Department, University of Constantine 1, Algeria). A voucher specimen (CPC17/06/06) has been deposited in the Herbarium of the of the VARENBIOMOL research unit, University of Constantine 1.

Extraction and isolation:

Air-dried leaves and flowers (2397 g) of *Centaurea parviflora* Desf. were macerated at room temperature with EtOH–H_2O (70:30 v/v) for 48 h, three times. After filtration, the filtrate was concentrated (800 ml) and dissolved in H_2O (960 ml). The resulting solution was extracted successively with $CHCl_3$, EtOAc and *n*-butanol. The organic phases were dried with Na_2SO_4, filtered and concentrated in vacuum at room temperature to obtain the following extracts: chloroform (25 g), EtOAc (6.46 g) and *n*-butanol (28.2 g).

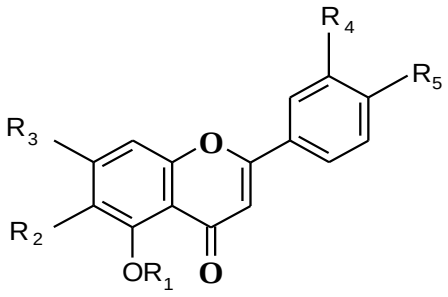
An aliquot of the chloroform extract (12 g) was fractionated by CC (silica gel; $CHCl_3$/Me_2CO step gradients and then with increasing percentages of MeOH) to yield 13 fractions (1-13) obtained by combining the eluates on the basis of TLC analysis. Fraction 2 (210 mg) ($CHCl_3$ 100%) gave, by crystallization in ether and a little amount of $CHCl_3$, 5-hydroxy-6,7,3',4'-tetramethoxyflavone **(1)** as needles (90 mg) [11]. Fraction 3 (220 mg) ($CHCl_3$ 100%) was submitted to preparative plates of silica gel eluted with (petroleum

ether/cyclohexane/acetone 3:3:4) to give eupatilin (**2**) (55 mg) [12] and eupatorin (**3**) (40 mg) [11]. Fraction 4 (180 mg) ($CHCl_3$/Me_2CO 99:1) was chromatographed on preparative silica gel TLC eluted with (petroleum ether/cyclohexane/acetone 3:3:4) to give cirsilineol (**4**) (62 mg) [13] and jaceosidin (**5**) (28 mg) [14]. Fraction 7 (145 mg) ($CHCl_3$ / Me_2CO 87:13) was submitted to preparative plates of silica gel eluted with (*n*-hexane/ EtOAc 1:2) to give 5Hα,6Hβ,7Hα-15-hydroxy-8α-(1',2'-dihydroxyethyl-acryloxy)-elema-1(2),3(4),11(13)-trien 6,12-olide (**6**) (33 mg) [15, 16] and a mixture which was rechromatographed on preparative TLC (ether /acetone 2:1) to afford genkwanin (**7**) (15 mg) [17, 18] and thevetiaflavone (**8**) (11.5 mg) [19]. Fraction 11 (430 mg) ($CHCl_3$/Me_2CO 65:35) gave, after purification by crystallization in *n*-hexane and a little amount of acetone, cnicin (**9**) (151 mg) [15].

A part of the *n*-BuOH extract (23 g) was chromatographed on a silica gel (230-400 Mesh) column eluted with ($CHCl_3$/MeOH/ H_2O with increasing polarity) to give 31 fractions (1-31)**.** Fraction 12 ($CHCl_3$/MeOH 92:8) gave after purification on a Sephadex column eluted with MeOH, ethyl *O*-α-*L*-arabinofuranoside (**10**) (30.9 mg) [20]. Fractions 14 and 15 ($CHCl_3$/MeOH 88:12) were purified on preparative plates of silica gel eluted with (Ether/EtOAc/MeOH 2:7:1), to give cornicinine (**11**) (57.8 mg) [21] and syringin (**12**) (52.2 mg) [22, 23] respectively. Fraction 20 ($CHCl_3$/MeOH 75:25) was submitted to preparative TLC (Ether/EtOAc/MeOH 2:7:1) to afford nicotiflorin (**13**) (22.6 mg) [24].

RESULTS AND DISCUSSION

The structures of the compounds were established by chemical and spectral analysis, mainly HRESI-MS, HREI-MS, UV, ^1H, ^{13}C and 2D-NMR (COSY, NOESY, HSQC and HMBC) as well as by comparing their spectroscopic data with those reported in the literature (Figure 1).



Compounds	R_1	R_2	R_3	R_4	R_5
1	H	OMe	OMe	OMe	OMe
2	H	OMe	OH	OMe	OMe
3	H	OMe	OMe	OH	OMe
4	H	OMe	OMe	OMe	OH
5	H	OMe	OH	OMe	OH
7	H	H	OMe	H	OH
8	Me	H	OH	H	OH

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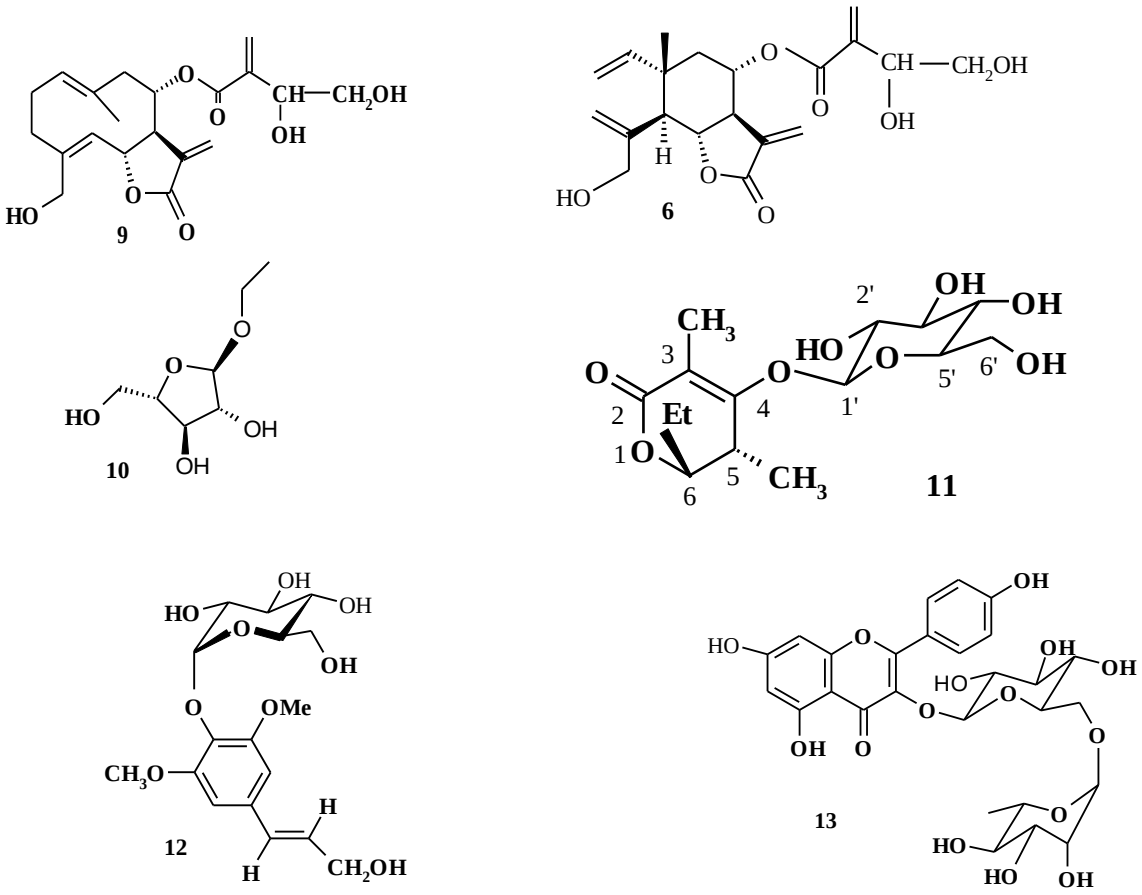

Figure 1: Structures of compounds (1-13)

Sesquiterpene lactones and flavonoids constitute the characteristic secondary metabolites of the genus *Centaurea* [25]. In the present study, eight flavonoids (seven methoxylated flavones and one flavonol glycoside), two sesquiterpene lactones together with a phenylpropanoid glucoside, syringin which is known for the species of this genus, were isolated. The characteristic of the chemical composition of this species is the presence of ethyl-*O*-α-*L*-arabinofuranoside which was previously isolated from mycelial cultures of *Dichomitus squalens* [20] and the glucosylated triketide δ-lactone, cornicinine **11** which is new for all the reign plant. From natural source, it was isolated from the insect *Nephrotoma cornicina* (Linnaeus 1758) [21]. It is important to note that this study is the first report on the chemical composition of *Centaurea parviflora* Desf.

Cornicine (**11**): ESI-MS: *m/z* 687.2 $[2M+Na]^+$; 371.1 $[M+Na]^+$; 333.1 $[M+H]^+$; 171.1 $[M-162]^+$. These results agreed with the molecular formula $C_{15}H_{24}O_8$. ^{1}H-NMR (300 MHz, MeOH-d_4, δ in ppm, *J* in Hz): δ 2.81 (m, H-5), 3.40 (m, H-2', H-4'), 3.45 (m, H-3', H-5'), 3.87 (dd, $^2J_{6'a\text{-}6'b}$=12.3 ; $^3J_{6'a\text{-}5'}$=2.1 H-6'a), 3.63 (dd, $^2J_{6'b\text{-}6'a}$=12.3 ; $^3J_{6'b\text{-}5'}$=6.3, H-6'b), 4.32 (ddd, $^3J_{6\text{-}CH2a}$=8.7 ; $^3J_{6\text{-}CH2b}$=5.7 ; $^3J_{6\text{-}5}$=2.7, H-6), 4.96 (d, $^3J_{1'\text{-}2'}$=7.2, H-1'), 1.78 (s, 3-CH_3), 1.12 (d, $^3J_{CH\text{-}CH3}$=7.0, 5-CH_3), 1.04 (t, $^3J_{CH2\text{-}CH3}$=7.2, 6-CH_3), 1.64 (m, H of CH_2) , 1.78 (m, H of CH_2). ^{13}C-NMR (75 MHz, MeOH-d_4, δ in ppm, *J* in Hz), δ 9.1 (3-CH_3), 8.4 (6-CH_3), 10.0 (5-CH_3), 24.2 (ethyl CH_2), 31.8 (C-5), 61.6 (CH_2 of sugar), 70.1 (C-4'), 73.7 (C-3'), 77.4 (C-5'), 76.8 (C-2'), 80.2 (C-6), 98.6 (C-1'), 104.7 (C-3), 170.2 (C-4), 171.2 (C-2).

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REFERENCES

[1] Vázquez FM, Suarez MA, Pérez A. J Ethnopharmacol 1997; 55: 81-85.
[2] Chucla MT, Lamela M, Gato A, Cadavid I. Planta Med 1988; 54: 107-109.
[3] Kaij-A-Kamb M, Amoros M, Girre L. Pharm Acta Helv 1992; 67: 178-188.
[4] Garbacki N, Gloaguen V, Damas J, Bodart P, Tits M, Angenot L. J Ethnopharmacol 1999; 68: 235-241.
[5] Bentamène A, Benayache S, Creche J, Petit G, Bermejo-Barrera J, León F, Benayache F. Biochem Syst Ecol 2005; 33:1061-1065.
[6] Seghiri R, Boumaza O, Mekkiou R, Benayache S, Mosset P, Quintana J, Estévez F, León F, Bermejo J, Benayache F. Phytochem Lett 2009; 2:114-118.
[7] Hammoud L, Seghiri R, Benayache S, Mosset P, Lobstein A, Chaabi M, León F, Brouard I, Bermejo J, Benayache F. Nat prod Res 2012; 26: 203-208.
[8] Kolli EH, León F, Benayache F, Estévez S, Quintana J, Estévez F, Brouard I, Bermejo J, Benayache S. J Braz Chem Soc, 2012; 23: 977-983.
[9] Bicha S, Chalard P, Hammoud L, León F, Brouard I, Garcia VP, Lobstein A, Bentamene A, Benayache S, Bermejo J, Benayache F. Rec Nat Prod, 2013; 7:114-118.
[10] Quezel P, Santa S. Nouvelle flore de l'Algérie et des régions désertiques méridionales. Paris: Editions du CNRS. 1963;1023.
[11] Tomás-Lorente F, Ferreres F, Tomás-Barberán FA, Rivera D, Obón C. Phytochemistry 1989; 28: 2141-2143.
[12] Miski M, Ulubelen A, Johansson C, Mabry TJ. J Nat Prod 1983; 46: 874-875.
[13] Wollenweber E, Schober I, Dennis CW, George Y. Phytochemistry 1985; 24: 2129-2131.
[14] Christensen LP, Lam J. Phytochemistry 1991; 30: 2663-2665.
[15] Bruno M, Herz W. Phytochemitry 1988; 27: 1873-1874.
[16] Bruno M, Vasallo N, Fazio TC, Gedris E, Herz W. Biochem Syst Ecol 1998; 26: 281-283.
[17] Ahmed ZF, Rimpler H, Rizk AM, Hammouda, FM, Ismail SI. Phytochemistry 1970; 9: 1595-1601.
[18] Xie PS, Wu CM. Chin Trad Herb Drugs 1979; 8: 6-6.
[19] Me HK, Zhong Y, Yin JT. Chin Trad Herb Drugs 2007; 38: 669-670.
[20] Xie HH, Xu XY, Dan Y, Wei XY. Chin J Nat Med 2009; 7: 390-393.
[21] Mathieu B, De Hoffmann E, Van Hove C. Glycosylated triketide Delta lactones. International application published under the patent cooperation treaty (PCT), Number WO 2005//054267 A1.
[22] Greca MD, Ferrara M, Fiorentino A, Monaco P, Previtera L. Phytochemistry 1998; 49, 1299-1304.
[23] Konuklugil B, Bahadir O. Turk J Chem 2004; 28: 741-744.
[24] Karamenderes C, Bedir E, Pawar R, Baykan S, Khan IA. Phytochemistry 2007; 68: 609-615.
[25] Gousiadou C, Skaltsa H. Biochem Syst Ecol 2003; 31: 389-396.