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Preliminary Phytochemical Analysis and Chemical Constituents from *Genista aspalathoides* Lamk. ssp. *erinaceoides* (Lois.) M. (Fabaceae).

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ABSTRACT

The preliminary phytochemical screening of the aerial parts of *Genista aspalathoides* (Lamk) ssp. *erinaceoides* (Lois.) M. showed the presence of alkaloids, sterols, triterpenoids, terpenoids, saponins and flavonoids. The presence of these secondary metabolites signified the potential of this plant as a source of therapeutic agent. Therefore, it was of interest to investigate the chemical constituents of this species. The chromatographic study on silica gel of chloroform extract allowed us to isolate and identify 2 secondary metabolites: derrone 4'-methyl ether **1** and 5, 4'-dihydroxy-7, 3'-dimethoxyflavanone (eriodictyol 7, 3'-dimethyl ether) **2.** The structures were established by the combination of their spectroscopic data, notably the analysis of UV, ^{1}H, ^{13}C and 2D-NMR. All these results were described for the first time from this species. Compounds **1** and **2** are new for the genus *Genista*.

Keywords: Phytochemical screening; Isoflavonoid; Flavanone; *Genista aspalathoides*.

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INTRODUCTION

The genus *Genista* (Fabaceae) consisting in about 100 species predominately distributed in the Mediterranean area [1], this genus is present in Algeria with 25 species and sub-species [2]. Many species of this genus showed important biological activities [3] and a remarkable wealth of bioactive secondary metabolites in particular isoflavonoids and flavonoids [4-6]. In continuation of our phytochemical study on Algerian genus *Genista* [7-11], we report here the chemical screening of the aerial parts of *G. aspalathoides* (Lamk) ssp. e*rinaceoides* (Lois.) M. and the structures of isolated compounds from the chloroform extract. Knowing that this subspecies has not been previously investigated.

MATERIALS AND METHODS

Plant material

Aerial parts of *G. aspalathoides* (Lamk) ssp. *erinaceoides* (Lois.) M. were collected during the flowering phase in May 2009 near M'Sila in the eastern Algeria and authenticated by Dr. D. Sarri on the basis of Quezel and Santa [2]. A voucher specimen has been deposited in the Herbarium of the VARENBIOMOL unit research, University of Constantine1 under n° 05/2009/GA.

Phytochemical analysis

The phytochemical screening of aerial parts of *G. aspalathoides* (Lamk) ssp. *erinaceoides* (Lois.) M. was performed using standard procedures based on the colorimetric method [12]. This study showed the presence of several chemical groups. The symbols like + and – denote present, and not present respectively in Table 1.

Extraction and isolation

Air-dried aerial parts 2700 g of *G. aspalathoides* (Lamk) ssp. *erinaceoides* (Lois.) M. were macerated at room temperature with (EtOH/H_2O; 80:20; v/v) for 72 h, three times. After filtration, the filtrate was concentrated and dissolved in H_2O (800 ml) under magnetic stirring. The resulting solution was filtered and successively extracted with $CHCl_3$, EtOAc and *n*-butanol. The organic layers were dried with Na_2SO_4, giving after removal of solvents under reduced pressure the following extracts: chloroform (4.0 g), EtOAc (14.0 g) and *n*-butanol (80.0 g). The chloroform extract was fractionated by column chromatography (silica gel; Petroleum ether/ethyl acetate step gradients) to yield 6 fractions (K1-K6) obtained by combining the eluates on the basis of TLC analysis. Compound **1** (71 mg) was obtained from fraction K1 (118 mg) and purified by crystallization in chloroform and a little amount of MeOH. Compound **2** (21 mg) was obtained from fraction K6 (120 mg) after purification on preparative plates of silica gel 60 GF_{254} eluted with $CHCl_3$/MeOH (90:10).

RESULTS AND DISCUSSION

Table 1: Phytochemical screening of *G. aspalathoides* (Lamk) ssp. *erinaceoides* (Lois.) M.

Chemical Groups	Aerial parts
Alkaloids	+
Coumarins	-
Sterols	+
Triterpenes	+
Terpenes	+
Flavonoids	+
Leucoanthancyanins	-
Tannins	-
Saponins	+
Quinones	-
Anthocyanins	-

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The results of the screened aerial parts from *G. aspalathoides* (Lamk) ssp. *erinaceoides* (Lois.) M. are shown in the Table 1.

Isolated and identified compounds

Compound 1. $C_{21}H_{18}O_5$, UV (MeOH, λmax, nm): 283; +NaOH: 292; +$AlCl_3$: 283; + $AlCl_3$/HCl: 283; +NaOAc: 283; +NaOAc/H_3BO_3: 283; ^{1}H-NMR (400 MHz, acetone-d_6, δ (ppm), *J* (Hz): 8.78 (1H, s, H-2), 8.06 (2H, d, *J*= 8.0, H-2'& H-6') , 7.51 (2H, d, *J*= 8.0, H-3'& H-5'), 7.28 (1H, d, *J*= 10.0, H-3"), 6.97 (1H, s, H-6), 6.37 (1H, d, *J*= 10.0, H-4"), 3.53 (3H, s, OCH_3), 1.48 (6H, s, $2CH_3$). ^{13}C-NMR (100 MHz, acetone-d_6): δ(ppm) = 181.86 (C-4), 158.51 (C-7), 158.17 (C-5), 160.29 (C-4'), 157.79 (C-9), 154.67 (C-2), 131.20 (C-2' & C-6'), 122.90 (C-3), 124.10 (C-1'), 115.03 (C-3' & C-5'), 106.76 (C-10), 104.00 (C-8), 95.42 (C-6), 27.49 (C-5''), 129.42 (C-3''), 78.86 (C-2''), 115.10 (C-4''). This compound was identified as **derrone 4'-*O*-methyl ether** [13].

Compound 2. $C_{17}H_{16}O_6$, ^{1}H-NMR (400 MHz, DMSO-d_6, δ, (ppm), *J* (Hz): 7.06 (1H, d, *J*=1.5, H-2'), 6.88 (1H, dd, *J*=6.5; 1.5, H-6'), 6.77 (1H, d, *J*= 6.5, H-5'), 6.06 (1H, d, *J*= 1.7, H-8), 5.97 (1H, d, *J*= 1.7, H-6), 5.32 (1H, dd, *J*= 10.2; 2.2, H-2β), 3.3 (1H, dd, *J*=13.1; 10.2, H-3β), 3.0 (1H, dd, *J*=13.1; 2.2, H-3β partialy obscured by the signal of the water of the solvent, deduced from COSY spectrum), 3.78 (3H, s, OCH_3), 3.74 (3H, s, OCH_3). ^{13}C-NMR (100 MHz, DMSO -d_6): δ (ppm)= 78.29 (C-2), 44.83 (C-3), 93.20 (C-8), 119.39 (C-6'), 129.85 (C-1'), 146.74 (C-3'), 111.02 (C-2'), 115.14 (C-5'), 147.48 (C-4'), 95.58 (C-6), 104.42 (C-10), 162.16 (C-5), 164.19 (C-7), 187.77 (C-4), 55.66 (OCH_3-3'), 55.57 (OCH_3-7).

This compound was identified as **5, 4'-dihydroxy-7, 3'-dimethoxyflavanone (eriodictyol 7, 3'-dimethyl ether)** [14].

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

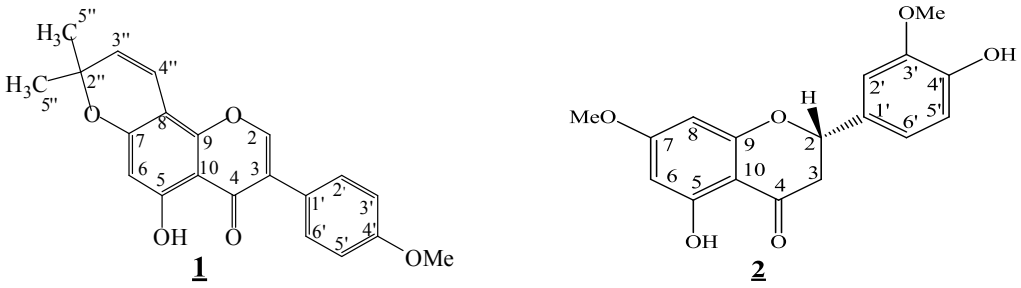

Figure1: Structures of compounds 1 and 2

CONCLUSION

The present study depicts the presence of different phytoconstituents such as alkaloids, sterols, triterpenoids, terpenoids, saponins and flavonoids in the aerial parts of *G. aspalathoides* (Lamk) ssp. *erinaceoides* (Lois.) M. Two flavonoids namely derrone 4'-*O*-methyl ether **1** and 5,4'-dihydroxy-7,3'-dimethoxyflavanone (eriodictyol 7,3'-dimethyl ether) **2**, have been isolated from the chloroform extract of this species for the first time and to the best of our knowledge, they are also described for the first time from the genus *Genista.*

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