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# Secondary metabolites from *Genista aspalathoides* Lamk ssp. *aspalathoides* M.

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**ABSTRACT**

*Phytochemical investigation of the ethyl acetate and n-butanol soluble parts of the aqueous-MeOH extract of the aerial parts of Genista aspalathoides Lamk ssp. aspalathoides M. collected from El Kala in the eastern Algeria led to the isolation of isoprunetin, genistein 8-C-β-glucopyranoside and orobol 8-C-β-glucopyranoside. The structures were established by spectral analysis, mainly ESIMS, UV and 2D-NMR experiments (COSY, HSQC and HMBC). The free radical scavenging effect of the n-butanol extract has been evaluated using DPPH method.*

**Keywords:** Isoflavone; Isoflavone *C*-glucoside; *Genista aspalathoides;* Fabaceae.

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## INTRODUCTION

The genus *Genista* (Fabaceae) consisting in about 100 species predominately distributed in the Mediterranean area [1] is present in Algeria with 25 species and sub-species [2]. Many species of this genus showed important biological activities [3-7] and a remarkable wealth of bioactive secondary metabolites in particular isoflavonoids [8-11], flavonoids [12, 13] and alkaloids [14]. As a part of our on-going program of research on Algerian plants [15-22] we investigated *Genista aspalathoides* Lamk ssp. *aspalathoides* M. [2] on which no reports on the isolation of any secondary metabolites are available to date. In this paper, we report the isolation of three known secondary metabolites **1-3** (Figure 1) and the free radical scavenging activity of the *n*-butanol soluble part of the aqueous-MeOH extract of the aerial parts of this sub-species.

## MATERIALS AND METHODS

### General Procedures

Melting points were determined on a Büchi B-540 apparatus and are uncorrected. 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-400 MHz, AMX-500

MHz and Avance DPX-250, 250 MHz spectrometers; chemical shifts (δ) are given in ppm using TMS as internal standard and coupling constants (*J*) are given in Hz. Mass spectra: Q-TOF micro (waters) spectrometer.

**Plant material**

*Genista aspalathoides* Lamk ssp. *aspalathoides* (Fabaceae) was collected in June 2010 from the area of El Kala (Cap Rosa), in the eastern Algeria and authenticated by Doctor Djamel Sarri. A voucher specimen (GA/112/06/10) has been deposited at the Herbarium of the VARENBIOMOL research unit, University of Constantine 1.

**Extraction and Isolation**

Air-dried aerial parts (5 kg) of *G. aspalathoides* Lamk ssp. *aspalathoides* were macerated at room temperature with MeOH–$H_2O$ (70:30, v/v) for 24 h, three times. After filtration, the filtrate was concentrated (1500 ml) and dissolved in $H_2O$ (2000 ml) under magnetic agitation. The resulting solution was filtered and successively extracted with $CHCl_3$, EtOAc and *n*-butanol. The organic phases were dried with $Na_2SO_4$, filtered and concentrated *in vacuo* at room temperature to obtain the following extracts: chloroform (37.07 g), EtOAc (66.88 g) and *n*-butanol (278.07 g).
An aliquot of the EtOAc extract (25 g) was chromatographed on a silica gel column eluted with a mixture of $CH_2Cl_2$-$(CH3)_2CO$ with increasing polarity to give 22 fractions ($F_1$–$F_{22}$) according to their TLC behaviour. Fraction $F_8$ (300 mg) ($CH_2Cl_2$-$(CH3)_2CO$ 85:15) gave after concentration a yellowish white precipitate which was washed with $CH_2Cl_2$ to give isoprunetin (90 mg) (**1**) [23]. Fractions $F_{16}$ (215 mg) ($CH_2Cl_2$-$(CH3)_2CO$ 75:25), $F_{17}$ (3000 mg) and $F_{18}$ (2900 mg) ($CH_2Cl_2$-$(CH3)_2CO$ 70:30) which had the same composition were combined and concentrated to obtain a yellowish powder which was washed with pure acetone to yield orobol 8-*C*-*β*-glucopyranoside **(**2.940 g) (**3**) [24, 25].

A part of the *n*-butanol extract (25 g) was fractionated by CC (silica gel; $CH_2Cl_2$-MeOH step gradients) to yield 18 fractions ($F'_1$–$F'_{18}$) obtained by combining the eluates on the basis of TLC analysis. Fraction $F'_{12}$ ($CH_2Cl_2$-MeOH 60:40), showed during its concentration, the formation of a precipitate which was filtered and washed with MeOH to give genistein 8-*C*-β-glucopyranoside (**2)** (402.1 mg) as white powder [25, 26]. Fractions $F'_{14}$ ($CH_2Cl_2$-MeOH 40:60), $F'_{15}$ ($CH_2Cl_2$-MeOH 30:70), $F'_{16}$ ($CH_2Cl_2$-MeOH 20:80) and $F'_{17}$ ($CH_2Cl_2$-MeOH 10:90) which had similar composition were combined. The resulting solution was concentrated to give a yellowish precipitate which was washed with acetone to obtain orobol 8-*C*-*β*-glucopyranoside **(**10.402 g) (**3**) [24, 25].

**Determination of DPPH radical scavenging activity**

The ability to scavenge the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH°) was determined based on the method of Magalhaes *et al.*(2006) [27] with minor modifications. A solution of 0.2 mM DPPH in methanol was prepared and 1 mL of this solution was mixed with 1 mL of extract in methanol (5 to 150 µg/mL).The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. A control sample containing the same volume of solvent in place of extract was used to measure the maximum DPPH absorbance. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid and trolox were used as references. Results were expressed as percentage of inhibition of the DPPH radical according to the following equation:

$$\% \text{ Inhibition of DPPH} = \frac{\text{(Absorbance of control - Absorbance of sample) x 100}}{\text{Absorbance of control}}$$

## RESULTS AND DISCUSSION

**Isolated and identified compounds**

**Compound 1:** UV (MeOH) λmax (nm): 265, 323 ; $^1$H NMR (400 MHz, MeOH-$d_4$) δ (ppm) = 7.79 (1H, s, H-2), 7.23 (2H, d, *J* = 8.8 Hz, H.2'& H-6'), 6.71 (2H, d, *J* = 8.8 Hz , H-3' & H-5'), 6.32 (2H, s, H-6 &H-8), 3.78 (3H, s, 5-$OCH_3$); $^{13}$C NMR (100 MHz, MeOH-$d_4$): *δ* (ppm) = 177.89 (C-4), 164.77 (C-7), 163.06 (C-5), 161.49 (C-9), 152.46 (C-2), 158.53 (C-4'), 131.58 (C-2' & C-6'), 127.03 (C-3), 124.57 (C-1'), 116.08 (C-3' & C-5'), 109.41 (C-10), 97.56 (C-6), 56.48 (5-$OCH_3$). This compound was identified as isoprunetin [23].

**Compound 2***:* UV (MeOH) λmax (nm): 265, 340sh, (MeOH+NaOH) 277, 339sh, the spectrum remains unchanged after 5min and more ; (MeOH+$AlCl_3$) 267, 381 ; (MeOH+$AlCl_3$+HCl) 272, 375 ; (MeOH+NaOAc) 279, 332sh ; (MeOH+NaOAc+$H_3BO_3$) 279, 328sh ; $^1$H NMR (250 MHz, acetone-$d_6$) δ (ppm) = 13.21 (1H, s, OH-5), 8.24 (1H, s, H-2), 7.46 (2H, d, *J* = 8.7 Hz, H-2' & H-6'), 6.91 (2H, d, *J* = 8.7 Hz , H-3' & H-5'), 6.27 (1H, s, H-6), 5.05 (1H, d, *J* = 9.6 Hz, H-1''), 3.90–3.50 (6H, m, H-2''), H-6''a, H-6''b, H-3'', H-5'' and H-4''); $^{13}$C NMR (62.5 MHz, acetone-$d_6$): *δ* (ppm) = 181.04 (C-4), 162.99 (C-7), 162.28 (C-5), 157.54 (C-4'), 155.69 (C-9), 153.32 (C-2), 132.21 (C-2' & C-6'), 122.78 (C-3), 121.96 (C-1'), 115.03 (C-3' & C-5'), 105.25 (C-10), 103.09 (C-8), 99.76 (C-6), 81.58

(C-5''), 80.10 (C-3''), 75.45 (C-1''), 72.89 (C-2''), 71.83 (C-4''), 62.93 (C-6''). This compound was characterized as genistein 8-*C*-β-glucopyranoside [25, 26].

**Compound 3**: Yellowish powder; MP. 200°C; UV (MeOH) λmax (nm): 264, 296sh, 328sh ; (MeOH+NaOH) 276, 334sh, the spectrum decomposes after 10 minutes and more ; (MeOH+$AlCl_3$) 270, 300sh, 385 ; (MeOH+$AlCl_3$+HCl) 271, 396 ; (MeOH+NaOAc) 275, 332; $^1$H NMR (400 MHz, MeOH-$d_4$) δ (ppm) = 13.02 (1H, s, OH-5), 8.04 (1H, s, H-2), 7.02 (1H, br s, H-2'), 6.83 (1H, dd, *J* = 8.1, 1.6 Hz , H-6'), 6.81 (1H, d, *J* = 8.1 Hz, H-5'), 6.28 (1H, s, H-6), 4.96 (1H, d, *J* = 9.6 Hz, H-1''), 4.15 (1H, t, *J* = 9.6 Hz, H-2''), 3.92 (1H, br d, *J* = 12.1 Hz, H-6''a), 3.77 (1H, dd, *J* = 12.1; 5.8 Hz, H-6''b), 3.55–3.51 (1H, m, H-3''), 3.53–3.48 (2H, m, H-4'' and H-5''); $^{13}$C NMR (100 MHz, MeOH-$d_4$): *δ* (ppm) = 182.48 (C-4), 164.67 (C-7), 163.36 (C-5), 157.89 (C-9, deduced from HMBC spectrum), 154.79 (C-2), 146.74 (C-4'), 146.14 (C-3'), 124.40 (C-3), 123.72 (C-1'), 121.77 (C-6'), 117.42 (C-2'), 116.44 (C-5'), 106.48 (C-10), 104.38 (C-8), 100.05 (C-6, deduced from HSQC spectrum), 82.58 (C-5''), 80.10 (C-3''), 75.45 (C-1''), 72.89 (C-2''), 71.83 (C-4''), 62.93 (C-6''); $^1$H NMR (500 MHz, DMSO-$d_6$) δ (ppm) = 13.21 (1H, s, OH-5), 8.30 (1H, s, H-2), 6.98 (1H, d, *J* = 1.6 Hz, H-2'), 6.80 (1H, dd, *J* = 8.1, 1.6 Hz , H-6'), 6.78 (1H, d, *J* = 8.1 Hz, H-5'), 6.29 (1H, s, H-6), 4.65 (1H, d, *J* = 9.6 Hz, H-1''), 3.96 (1H, t, *J* = 9.6 Hz, H-2''), 3.70 (H-6''a, obscured by the signal of the water of the solvent, deduced from HSQC spectrum), 3.42 (1H, m, H-6''b), 3.24–3.16 (3H, m, H-3'', H-5'', H-4''); $^{13}$C NMR (125 MHz, DMSO-$d_6$): *δ* (ppm) = 180.88 (C-4), 163.50 (C-7), 161.42 (C-5), 156.80 (C-9), 154.12 (C-2), 145.82 (C-4'), 145.19 (C-3'), 122.48 (C-3), 121.93 (C-1'), 120.36 (C-6'), 116.81 (C-2'), 115.81 (C-5'), 105.00 (C-10), 104.43 (C-8), 99.04 (C-6), 81.87 (C-5''), 79.01 (C-3''), 73.52 (C-1''), 70.97 (C-2''), 70.93 (C-4''), 61.75 (C-6'') ; ESIMS (positive mode) *m/z*: 449.1 $[M+H]^+$ ; ESIMS (negative mode) *m/z*: 447.1 $[M-H]^-$ ; Q-TOF-MS/MS of $[M+H]^+$: 449.1 $[M+H]^+$, 431.1 $[M+H-H_2O]^+$, 413.1$[M+H-2H_2O]^+$, 395.1 $[M+H-3H_2O]^+$, 383.1 $[M+H-2H_2O-CH_2O$(from sugar)$]^+$, 367.1 $[M+H-3H_2O-CO]^+$, 353.1 $[M+H-3H_2O-CH_2CO$(from sugar)$]^+$,329.1 [M+H-120(from sugar)]$^+$, 311.1$[M+H-H_2O$-120(from sugar)$]^+$. On the basis of all these results, this compound was characterized as orobol 8-*C*-*β*-glucopyranoside [24, 25].

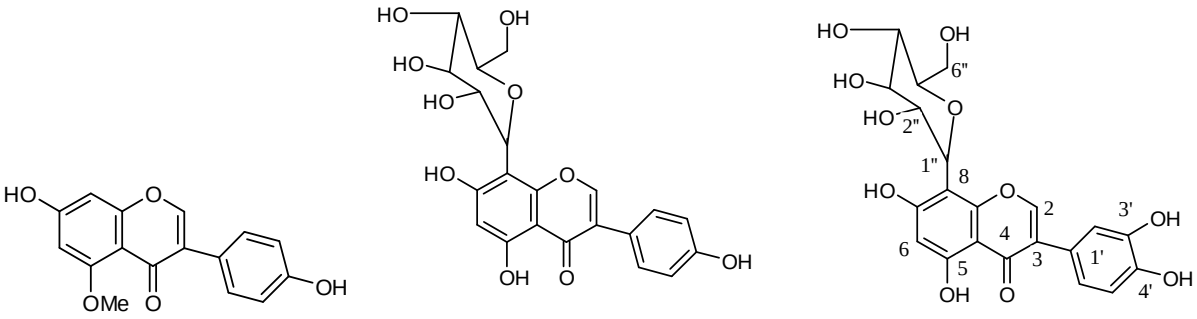


**Figure 1: Structures of the compounds 1 - 3**

**DPPH radical-scavenging activity**

Free radicals are fairly and highly reactive substances able of causing oxidation and sometimes irreversible damage cell playing an important role in pathogenesis of various diseases [28]. One of the mechanisms to investigate antioxidant capacity is to study the scavenging effect on proton radicals using 1,1-diphenyl-2-picrylhydrazyle radical (DPPH°) assay [27]. The scavenging activity was based on the reaction that the purple colour of DPPH solution fades quickly when it reacts with proton radical scavengers [29]. In this investigation, the antioxidant activity of *n*-butanolic extract of *Genista aspalathoides* Lamk ssp. *aspalathoides* (BEG) is expressed as the percentage of scavenging capacity, which measures the reduction in the initial DPPH° absorption caused by the sample.

Our results revealed that the scavenging effect of BEG was increased with increasing concentration, the extent discoloration of reaction exhibited a marked effect (86 %) in a dose response (50 µg/mL) compared to standards, trolox and ascorbic acid (96-97 %) at 20 µg/mL (Figure 2). Figure 3 showed that the extract and standards concentration which was required for 50 % inhibition ($IC_{50}$) was in order of ascorbic acid (2,19±0,26 µg/mL) > trolox (6,87±0,15 µg/mL) > BEG (14,49±0,94 µg/mL). The antioxidant activity of BEG would believed to be influenced by the positions of phenol-hydrogen in the molecules. It was reported that phenolic compounds exhibited redox properties which phytochemicaly play a crucial role in determining the antioxidant properties [30, 31].

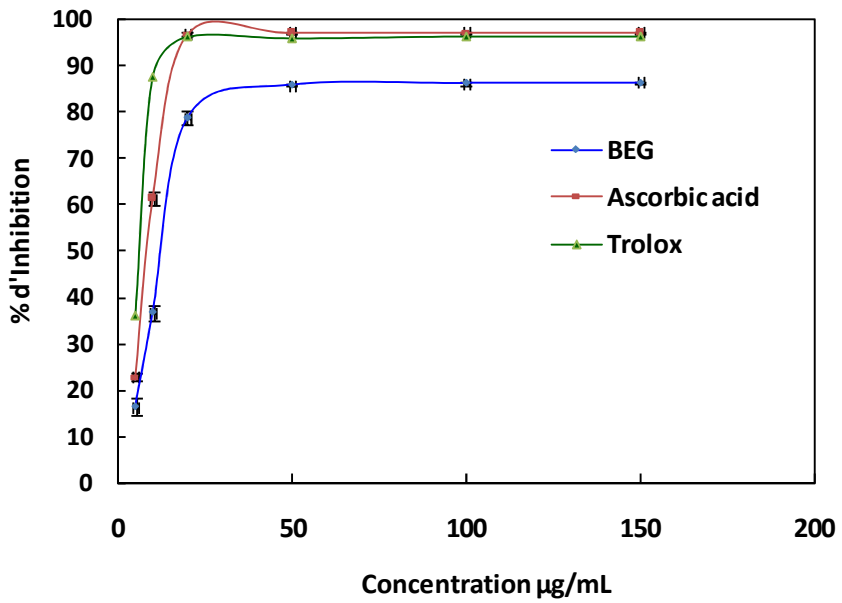


**Figure 2: DPPH radical-scavenging activities of BEG and standards. Each value represents a mean ± SD (n=3), P<0.05**

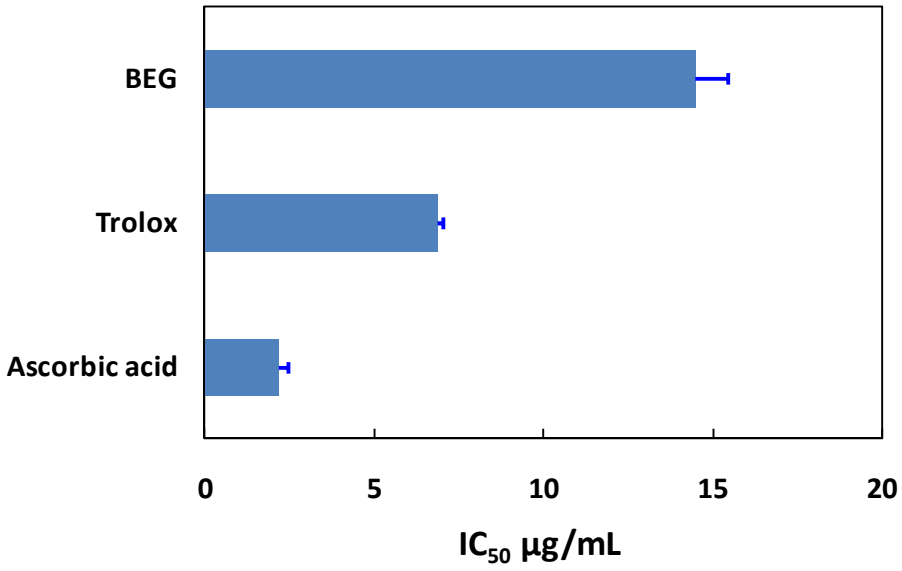


**Figure 3: $IC_{50}$ values of antioxidant activities of BEG and standards. Each value represents a mean ± SD (n=3), P<0.05.**

## CONCLUSION

This study allowed the isolation and the structural elucidation of three isoflavones from *Genista aspalathoides* Lamk ssp. *aspalathoides* M. (Fabaceae) which was not studied before.

Based on these chemical studies, the antioxidant effectiveness of the *n*-butanolic extract of this sub-species could be attributed to these phenolic compounds, in particular to the isoflavone-8-*C*-glucosides which were the major components. It is important to note the remarkable abundance of orobol 8-*C*-*β*-glucopyranoside in this plant.

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