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Phytochemical study of ethyl acetate extract and antioxidant activity of *Genistaquadriflora Munby* (Fabaceae)

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ABSTRACT

*This study concerned a chemical screening of the aerial parts of GenistaquadrifloraMunby(endemic species in Algeria and Morocco), in which the presence of several chemical groups was revealed (flavonoids, isoflavonoids, triterpens and others). The chromatographic study on silica gel of the ethyl acetate soluble part of the aqueous-MeOH extract of the aerial partsof this plant, allowed the isolation and the structural elucidation offive known compounds: thevetiaflavone (**1**), genkwanin (**2**), genistein (**3**),biochanin A (**4**) andisoprunetin (**5**). The structures were established on the basis of physical and spectroscopic analysis, and by comparison with the literature data. Antioxidant properties were assayed in terms of antioxidant activity of chloroform and ethyl acetate soluble parts. The scavenging ability on 1, 1-diphenyl-2-picrylhydrazyl (DPPH), is determined with spectrophotometric method.*

Keywords: Isoflavonoid; Flavonoid; Antioxidant activity; *Genistaquadriflora*.

INTRODUCTION

Genista species contain a variety of secondary metabolites of various types, especially isoflavonoids, which have been shown to be biologically active [1-2]. The genus *Genista* (Fabaceae) is represented by 25 species and sub-species in Algeria from which 11 are endemic. These species are distributed in particular, in the eastern and southeastern parts of the country [3]. In continuation of our phytochemical and biological works on Algerian *Genista* [4-8], we report here the chemical screening of *Genistaquadriflora*Munbywhich has not been previously investigated, the structures of isolated flavonoids and isoflavonoids from the ethyl acetate extract and the evaluation of antioxidant activity of the chloroform and the ethyl acetate extracts.

MATERIALS AND METHODS

Plant Material

Aerial parts of *Genistaquadriflora*Munby(Fabaceae) were collected during the flowering phase in May 2009 in eastern Algeria and authenticated by Dr. D. Sarri on the basis of Quezel and Santa [3].

A voucher specimen has been deposited in the Herbarium of the *VARENBIOMOL*research unit, University of Constantine1 under N° 05/2009/GQ.

Phytochemical analysis

The phytochemical screening of both flowers and stems of *G. Quadriflora*was performed using standard procedures based on the colorimetric method [9]. This study showed the presence of several chemical groups. The symbols like ++, + and – denote present, moderately present and not present respectively.

Extraction and isolation

Dried aerial parts of *Genistaquadriflora*(1130 g) were macerated withMeOH–H_2O (80:20, v/v) for 24 hours three times.The crude extract was concentrated at room temp. and diluted with 500 ml H_2O. The remaining aqueous solution was successively extracted with petroleum ether, $CHCl_3$, EtOAc and*n*-BuOH. The ethyl acetate extract (3.5g) was chromatographed on silica gel column using $CHCl_3$-MeOH with increasing polarities, as eluent, to give16 fractions (F_1-F_{16}). Compounds **1**(thevetiaflavone)(106 mg) and **2**(genkwanin) (56mg) were obtained from fraction F_4 (272.2 mg) ($CHCl_3$-MeOH, 99.4:0.6), which was chromatographed on preparative silica gel 60 GF_{254}plates(*n*-hexane/EtOAc, 30:70). Compound **3 (**genistein)(87 mg) was obtained from fraction F_7 (138.2mg) ($CHCl_3$-MeOH, 98.5:1.5), after purification on preparative silica gel 60 GF_{254}plates (*n*-hexane/EtOAc, 30:70). Compound **4 (**biochanin A)(60mg) was obtained as white powder from fraction F_{11}($CHCl_3$/MeOH, 80:20)after filtration. The mother liquor of this fraction was chromatographed on preparative silica gel 60 GF_{254}plates (*n*-hexane/EtOAc, 30:70), to give compound **5** (isoprunetin) (18 mg).

Antioxydant activity

Radical scavenging activity using DPPH method

The model of scavenging DPPH radical is a widely used method to evaluate the free radical scavenging activities of antioxidants. This assay uses stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical as a reagent.Various concentrations of the samples in methanol were added to 3ml of a 0.004% methanol solution of DPPH. After a 30 mn incubation period at room temperature the absorbance was read against a blank at 517 nm using spectrophotometer.

Inhibition of DPPH free radical in percent (I %) was calculated in following way:

$$I\ \% = (A_{blank}\text{-}A_{sample}/A_{blank}) \times 100$$

Where $A_{blank}$is the absorbance of the control reaction (containing all reagents except the test compound), and $A_{sample}$is the absorbance of the test compound.

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Tests were carried out in triplicate [10].

RESULTS AND DISCUSSION

The steams and flowers were screened for the presence or absence of different phytochemical groups (Table 1).

Table 1:Phytochemical screening of *G. Quadriflora*

Chemical Groups	Flowers	stems
Leucoanthocyanins	-	-
Saponins	+	+
Quinones	-	-
Anthocyanins	-	-
Alkaloids	+	+
Coumarins	+	+
Sterols	+	+
Triterpenes	++	++
Terpenes	+	+
Flavonoids	++	++
Tannins	-	-

Isolated and identified compounds

Compound 1.$C_{16}H_{12}O_5$, UV (MeOH, λ_{max}, nm): 266, 345; +NaOH: 263, 407; +$AlCl_3$: 270, 350; + $AlCl_3$/HCl: 270,306, 350; +NaOAc: 275, 378; + NaOAc/H_3BO_3: 269, 351; ^{1}HNMR (250 MHz, CD_3OD, δ, ppm, *J*/Hz): 7.57(2H, d, *J*=8.2, H-2'&H6'), 6.97 (2H, d, *J*=8.2, H-3'&H-5'), 6.67 (1H, s,H-3), 6.50 (1H, d, *J*=2.1, H-8), 6.24 (1H, d, *J*=2.1, H-6), 3.90 (3H, s, OCH_3). Characterized as apigenin5-methyl ether (thevetiaflavone)[11].

Compound 2.$C_{16}H_{12}O_5$, UV (MeOH, λ_{max}, nm): 276, 343; +NaOH: 324, 405; +$AlCl_3$: 277,396; + $AlCl_3$/HCl: 279,396; +NaOAc: 276, 319; + NaOAc/H_3BO_3: 276, 345; ^{1}HNMR (250 MHz, CD_3OD, δ, ppm, *J*/Hz): 7.63(2H, d, *J*=8.1, H-2'&H6'), 7.03 (2H, d, *J*=8.1, H-3'& H-5'), 6.72 (1H, s,H-3), 6.56 (1H, d, *J*=2.1, H-8), 6.27 (1H, d, *J*=2.1, H-6), 4.01 (3H, s, OCH_3). Characterized as apigenin7-methyl ether (genkwanin)[12].

Compound 3.$C_{15}H_{10}O_5$, UV (MeOH, λ_{max}, nm):261, 323(sh); +NaOH: 278, 321 (sh); +$AlCl_3$: 270, 306(sh); + $AlCl_3$/HCl: 270,306(sh); +NaOAc: 269, 328 (sh); + NaOAc/H_3BO_3: 266, 323 (sh); ^{1}HNMR (250 MHz, CD_3OD, δ, ppm, *J*/Hz): 7.90 (1H, s, H-2), 7.35 (2H, d, *J*=8.7, H-2'& H-6'), 6.85 (2H, d, *J*=8.7, H-3'& H-5'), 6.10 (1H, d, *J*=2.1, H-8), 6.07 (1H, d, *J*=2.1, H-6).Identified as 4', 5, 7-trihydroxyisoflavone (genistein)[13].

Compound 4.$C_{16}H_{12}O_5$, UV (MeOH, λ_{max}, nm): 257, 318(sh); +NaOH: 258, 318 (sh); +$AlCl_3$: 255, 318(sh); + $AlCl_3$/HCl: 255,319(sh); +NaOAc: 258, 318 (sh); + NaOAc/H_3BO_3: 257, 318 (sh); ^{1}HNMR (250 MHz, CD_3OD, δ, ppm, *J*/Hz): 7.95 (1H, s, H-2), 7.34 (2H, d, *J*=8.7, H-2'&H-6'), 6.85 (2H, d, *J*=8.7, H-3'& H-5'), 6.40 (2H, brs, H-8, H-6), 3.90 (3H, s, OCH_3). Identified as genistein 4'-methyl ether (biochanin A)[14].

Compound 5.$C_{16}H_{12}O_5$, UV (MeOH, λ_{max}, nm): 257, 318(sh); +NaOH: 266, 318 (sh); +$AlCl_3$: 254, 318(sh); + $AlCl_3$/HCl: 263,319(sh); +NaOAc: 263, 318 (sh); + NaOAc/H_3BO_3: 257, 318 (sh); ^{1}HNMR (250 MHz, CD_3OD, δ, ppm, *J*/Hz): 7.95 (1H, s, H-2), 7.34 (2H, d, *J*=8.7, H-2'& H-6'), 6.85 (2H, d, *J*=8.7, H-3'& H-5'), 6.40 (2H, brs, H-8, H-6), 3.90 (3H, s, OCH_3). Identified asgenistein5- methyl ether (isoprunetin)[15].

The structures of these compounds were elucidated by the use of combined spectral methods (UV-Vis, ^{1}H NMR) as well as by comparing their spectroscopic data with those reported in the literature[16, 17].

Antioxydant activity

The DPPH radical accepts an electron or hydrogen radical to become a stable diamagnetic molecule with a different color. Thus, the degree of its discoloration from purple to yellow is attributed to the hydrogen donating ability of the added compound.The DPPH scavenging activities of antioxidants are attributed to their hydrogen donating abilities which is indicative of its radical scavenging potential [18].

In the present study, the DPPH radical scavenging ability of different extracts of *G. quadriflora* was evaluated in a dose-dependent manner. Different extracts of *G. quadriflora*proved to be an effective scavenger of DPPH radicals compared to ascorbic acid used as reference. The scavenging effect of the samples on the DPPH radical decreased in the order of ascorbic acid >chloroformextract> ethyl acetate extract, and was concentration-dependent. From table 2, the IC_{50} values of chloroform and ethyl acetate extracts of this plant showed moderate antioxidant activity in comparison with ascorbic acid used as standard.

Table 2. Antioxidant activity of chloroform and ethyl acetate extracts

IC_{50} DPPH (µg/ml)	
Chloroformextract	58.97
Ethylacetateextract	61.64
Ascorbic acid	5.18

CONCLUSION

The present study depicts the presence of different phytoconstituents such as alkaloids, flavonoids, saponins, coumarins, sterols and terpenoids in both the flowers and steams of *G. quadriflora*. Five flavonoids namely, thevetiaflavone (**1**); genkwanin (**2**); genistein (**3**); biochanin A (**4**); isoprunetin (**5**)have been isolated from the ethyl acetate extract. The Chloroform and ethyl acetate extracts of this plant were examined for their *in vitro* antioxidant properties using DPPH test. The results showed that both extracts had moderate antioxidant activity *in vitro*. All These results were reported for the first time from *GenistaQuadriflora*Munby.

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