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FLAVONOIDS AND ANTIOXIDANT ACTIVITY OF *Thapsia garganica* FROM ALGERIA

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In continuation of our investigation of flavonol glycoside distribution in Algerian medicinal plants [1–12], we report here our study on the aerial parts of *Thapsia garganica* L. (Apiaceae) [13]. This is the first time that flavonoids are isolated from this species from which the roots are traditionally used to treat rheumatism [14].

The aerial parts of *Thapsia garganica* L. (Apiaceae) were collected during the flowering period in May (2010) at Constantine, Algeria. A voucher specimen was deposited at the Herbarium of the Laboratory under the code number LOST.Tg.05.05.

Air dried aerial parts of *Thapsia garganica* (2000 g) were macerated three times with 70% MeOH solution. The hydroalcoholic solution was concentrated under reduced pressure to dryness, and the residue was dissolved in hot water (1000 mL) and kept in the cold overnight. After filtration, the aqueous solution was successively extracted with EtOAc once and with *n*-BuOH for three times; then the EtOAc and *n*-BuOH extracts were evaporated to dryness.

The butanolic extract (20 g) was subjected to column chromatography on polyamide SC6 with a gradient of toluene–MeOH of increasing polarity. Preparative TLC on Polyamide DC6 using the system toluene–MeOH–methyl ethyl ketone (4:3:3) and Whatman PC N°3MM, followed by flash column chromatographies on Sephadex LH20, eluted with MeOH, led to ten compounds (**1–10**).

Acid Hydrolysis of 1–10. Each compound (5 mg) was refluxed with 5% H_2SO_4 (5 mL) in water for 1 h. The reaction mixture was diluted with water and fractionated by EtOAc. Each EtOAc-soluble fraction was concentrated and examined by TLC with authentic samples. Each remaining aqueous layer was adjusted to pH 7 with $NaHCO_3$ and filtered. The filtrate was concentrated and examined by TLC with authentic sugars.

Antioxidant Activity. The DPPH radical-scavenging activity of the methanolic extract of the flowers (FMETG) and that of the leaves (LMETG) of *Thapsia garganica* was assayed by a slightly modified method of Blois [15]. After 30 min, the absorbance of the solution was measured at 660 nm and the antioxidant activity calculated using the following equation: DPPH radical-scavenging activity % = [(Absorbance of the control – Absorbance of the sample)/Absorbance of the control] × 100.

Compounds **1–10** were identified by the use of spectroscopic techniques (NMR, UV, mass spectrometry) and acid hydrolysis; they were divided into five flavonol 3-*O*-glucosides (**1–5**), three flavone 7-*O*-glucosides (**6–8**), and two diglycosides (**9–10**).

The pyranose structure of the sugars was confirmed by periodate oxidation [8] of the glycosides. Partial hydrolysis of the glycoside with 1% H_2SO_4 yielded xylose and a glycoside, which on further hydrolysis with 6% HCl yielded D-glucose and diosmetin (compound **9**) or luteolin (compound **10**). This indicated that xylose is present as the terminal sugar. The ^1H and ^{13}C NMR spectra of compounds **9–10** exhibited the same signals for the sugar moieties and the typical signals of diosmetin and luteolin, respectively.

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TABLE 1. DPPH Radical-Scavenging Activity of FMETG and LMETG, %

Sample	1	10	50	100	200
	μg/mL				
FMETG	28.5	41.9	43.8	62.3	73.2
LMETG	28.3	25.0	41.8	52.8	68.7

Ten flavonoid glycosides have been isolated from the leaves of *Thapsia garganica* L., from which five were 3-*O*-glucosides of kaempferol (**1**), quercetin (**2**), rhamnetin (**3**), isorhamnetin (**4**) [16–18], and rhamnazin (**5**) [19]. Three others were 7-*O*-glucosides of apigenin (**6**), luteolin (**7**) [16–18], and diosmetin (**8**) [20, 21], while the last two were diglycosides, diosmetin 7-*O*-*β*-D-xylosyl-(1→6)-*β*-D-glucoside (**9**) and luteolin 7-*O*-*β*-D-xylosyl-(1→6)-*β*-D-glucoside (**10**). Their structures were established on the basis of physical and spectroscopic analysis and by comparison with the literature data. The free radical DPPH scavenging potential of the flowers and leaf extracts was investigated.

Diosmetin 7-*O*-*β*-D-Xylosyl-(1→6)-*β*-D-glucoside (9) [20]. Yellow powder; mp 262–264°C. IR (KBr, ν_{max}, cm^{-1}): 3358 (OH), 1653 (C=O), 1606, 1586, 1495 (C=C), 1258, 1198, 1177 (C-O). UV (MeOH, λ_{max}, nm): 254, 340. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 12.99 (1H, s, 5-OH), 7.64 (1H, dd, J = 2.3, 8.7, H-6′), 7.51 (1H, d, J = 2.3, H-2′), 7.19 (1H, d, J = 8.7, H-5′), 6.87 (1H, s, H-3), 6.86 (1H, d, J = 2.2, H-8), 6.53 (1H, d, J = 2.2, H-6), 5.09 (1H, d, J = 7.2, glucosyl anomeric H), 4.21 (1H, d, J = 7.4, xylosyl anomeric H), 3.92 (3H, s, OCH_3). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 182.43 (C-4), 164.62 (C-7), 163.43 (C-2), 161.62 (C-5), 157.44 (C-9), 151.79 (C-4′), 147.23 (C-3′), 123.34 (C-1′), 119.50 (C-6′), 113.62 (C-2′), 112.67 (C-5′), 105.92 (C-3), 104.60 (C-10), 104.25 (C-1‴), 100.42 (C-1″), 100.11 (C-8), 95.28 (C-6), 76.97 (C-5″), 76.68 (C-3″), 76.10 (C-3‴), 73.82 (C-2‴), 73.53 (C-2″), 69.95 (C-4″), 69.80 (C-4‴), 68.88 (C-6″), 66.10 (C-5‴), 56.26 (OCH_3). (+) FAB-MS *m/z* 595 $[M + H]^+$.

Compound **10** was characterized as luteolin 7-*O*-*β*-D-xylosyl-(1→6)-*β*-D-glucoside [22].

Compounds **1–10** are reported for the first time from the genus. Compounds **9** and **10** have been reported from a few plants.

As shown in Table 1, the FMETG and LMETG markedly quenched the DDPPH radical by 73.2% and 68.7%, respectively, at a concentration of 200 μg/mL. From these results, we can conclude that the leaves and flowers of *Thapsia garganica* possess an equivalent high antioxidant activity.

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