SUPPLEMENTARY MATERIAL

Chemosystematic significance of flavonoids isolated from *Diplotaxis acris* (Brassicaceae) and related taxa

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Abstract

The chemosystematic relationship of four *Diplotaxis* species; *Diplotaxis acris*, *D. eruroides*, *D. harra* and *D. muralis* were surveyed from the flavonoids point of view. These species were found to produce 33 flavonoids (7 flavones and 26 flavonols), including 11 compounds were isolated in the present study from *D. acris*. Among them, seven flavonoids were identified for the first time; luteolin (**4**), kaempferol (**8**), kaempferol 3-*O*- β -glucopyranoside-7-*O*- α -rhamnopyranoside (**13**), quercetin 3-*O*- β -glucopyranoside (**16**), quercetin 7-*O*- β -glucopyranoside (**20**), isorhamnetin (**22**) and isorhamnetin 3-*O*- β -glucopyranoside-7-*O*- α -rhamnopyranoside (**32**). Their structures were recognized on the basis of chemical and spectroscopic techniques (1 & 2D NMR, UV, EI & ESI/MS). The isolated flavonoids may provide useful taxonomic characters at the infraspecific levels of classification where the flavonoid profile of *D. acris* and *D. harra* is similar and different from the other species.

Keywords: *Diplotaxis*; Brassicaceae; Brassiceae; flavonoid patterns; chemotaxonomy.

Experimental

General

1D and 2D NMR experiments (^1H , ^{13}C and HMBC) were recorded on a Jeol EX-500 spectrometer: 500 MHz (^1H NMR), 125 MHz (^{13}C NMR). UV spectrophotometer (Shimadzu UV-240), EIMS: Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS spectrometer, ESIMS: Waters 3100 spectrometer. CC (Column Chromatography) Polyamide 6S (Riedel-De-Haen AG, Seelze Haen AG, Seelze Hanver, Germany) using MeOH/H₂O as eluent. CC Silica gel 60 (Merck, 0.063-0.2mm) using CHCl₃/MeOH (2:3). PC (Paper Chromatography) (descending) Whatman No. 1 and 3 MM papers, using solvent systems; H₂O, 15% HOAc (H₂O-HOAc 85:15), 30% HOAc (H₂O-HOAc 70:30), BAW (n-BuOH-HOAc-H₂O 4:1:5, upper layer). CC Sephadex LH-20 (Pharmazia) using MeOH as eluent. Authentic samples were obtained from the department of phytochemistry and plant systematics, NRC. Complete acid hydrolysis (2 N HCL, 2 h, 100°C) were carried out and followed by paper co-chromatography with authentic samples to identify the aglycones and sugar moieties.

Plant material

Fresh samples of *D. acris* were collected on the Ras-Ghareb, Hurgada desert road, Egypt, in March 2013. The sample was identified by Dr Mona M. Marzouk. A voucher specimen (No. MZ920) has been deposited in the herbarium of the National Research Centre (CAIRC).

Extraction and isolation

Air-dried, ground, aerial parts of *D. acris* (750g) were defatted with petroleum ether (40-60°C) then extracted three times with 70% methanol/water at room temperature. The methanol extract was evaporated under reduced pressure at 60°C then the crude extract (109g) subjected to a polyamide 6S column (85x5 cm) starting with water as eluent then decreasing the polarity by increasing the concentration of methanol by 10%. A total of 54 fractions were collected, each of about 250 ml. Similar fractions were combined according to their PC properties using H₂O, 15% HOAc, CAW and BAW as eluents to give five main fractions (A-E). Fraction A (30% MeOH/H₂O) was applied to a Silica gel column (35x2.5 cm) using CHCl₃-MeOH (2:3).

Fractionation gave rise to a two major fractions. They were applied to PC using BAW then purified on a Sephadex LH-20 column (30x1.5 cm) using methanol yielded compounds **13** and **32**. Fraction B (50% MeOH/H₂O) was rechromatographed on polyamide column (35 x2.5 cm) using saturated BuOH yielded four major fractions. They were purified on a Sephadex LH-20 column using MeOH yielded compounds **5**, **9**, **16** and **23**. Fraction C (70% MeOH/H₂O) yielded compounds **4** and **20** by separation on PC using 15% HOAc followed by BAW. Fractions D and E (90-100% MeOH/H₂O) were chromatographed on PC using 15% HOAc followed by 30% HOAc yielded compounds **8**, **15** and **22**.

Structural elucidation

32 flavonoids were detected in the aerial parts of the studied taxa (Figure S1); eleven compounds were isolated in the present study from *D. acris* and identified as; luteolin (**4**), luteolin 7-*O*- α -rhamnopyranoside (**5**), kaempferol (**7**), kaempferol 3-*O*- β -glucopyranoside (**9**), kaempferol 3-*O*- β -glucopyranoside-7-*O*- α -rhamnopyranoside (**13**), quercetin (**15**), quercetin 3-*O*- β -glucopyranoside (**16**), quercetin 7-*O*- β -glucopyranoside (**20**), isorhamnetin (**22**), isorhamnetin 3-*O*- β -glucopyranoside (**23**) and isorhamnetin 3-*O*- β -glucopyranoside-7-*O*- α -rhamnopyranoside (**32**). The UV spectral data revealed that the two former compounds belonging to flavone nuclei, while the other are flavonols. All compounds have free OH groups at 5 and 4' positions, but compounds **5**, **13**, **20** and **32** showed a substitution at position 7. In addition to *ortho* dihydroxy groups are assigning to compounds **4**, **5**, **15**, **16** and **20** (Mabry et al., 1970; Markham, 1982). ¹H-NMR spectrum revealed the presence of luteolin nucleolus in compounds **4** and **5**, which represented as aglycone and 7-*O*- α -rhamnopyranoside (H-1" at δ 5.42, d, *J*=2.0 Hz), respectively (Markham and Geiger, 1994). Compounds **8**, **9** and **13** are characterized by a kaempferol nucleolus, represented as aglycone, 3-*O*- β -glucopyranoside (H-1" at δ 5.33, d, *J*=7.5 Hz), and 3-*O*- β -glucopyranoside-7-*O*- α -rhamnopyranoside (H-1" at δ 5.48 , d, *J*=7.5 Hz; H-1'" at δ 5.56, d, *J*=2.0 Hz), respectively. The ¹H NMR data of compounds **15**, **16** and **20** revealed the presence of quercetin moiety as aglycone, 3-*O*- β -glucopyranoside (H-1" at δ 5.43, d, *J*=7.5 Hz) and 7-*O*- β -glucopyranoside (H-1" at δ 5.06, d, *J*=7.5 Hz), respectively. Also isorhamnetin is the skeleton of compounds **22**, **23** and **32** which elucidated as aglycone, 3-*O*- β -glucopyranoside (H-1" at δ 5.45, d, *J*=7.5 Hz), and 3-*O*- β -glucopyranoside-7-*O*- α -rhamnopyranoside (H-1" at δ 5.5, d, *J*=7.0 Hz & H-1'" at δ 5.55, d, *J*=2.0 Hz), respectively (Markham and Geiger, 1994). The ¹³C NMR and

HMBC data of compounds **13** and **32** confirmed the 3-*O*- β -glucopyranoside-7-*O*- α -rhamnopyranoside of kaempferol and isorhamnetin, respectively (Agrawal, 1989).

appreciated.

References

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Figure S1. Chemical structure of flavonoids isolated from *Diplotaxis acris* and its related taxa