ABSTRACT

The chemosystematic relationship of four Diplotaxis species; Diplotaxis acris, Diplotaxis erucoides, Diplotaxis harra and Diplotaxis 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 (1D & 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.

1. Introduction

The genus Diplotaxis DC. belongs to the most economically important tribe of family Brassicaceae; Brassicaceae (Warwick et al. 2009), the number of species vary from 27 to 36 and
they are distributed from Europe, Mediterranean region to north-west of India (Boulos 1999; The Plant List 2010; Grillo et al. 2012; Oueslati et al. 2015). Four taxa occurred in Egypt; *Diplotaxis acris* (Forssk.) Boiss. *Diplotaxis erucoides* (L.) DC. *Diplotaxis harra* (Forssk.) Boiss. and *Diplotaxis muralis* (L.) DC. (Boulos 1999). *D. acris* is a winter annual herb, known as salad rocket; it tastes like *Eruca sativa* Mill. and grazed by animals, it grows in desert slopes, stony and sandy desert valleys (Boulos 1999; Warwick et al. 2009). In addition to its economic importance, *D. acris* has antioxidant, hepatoprotective and antinociceptive activities (Atta & Abo 2004; Atta et al. 2006). Little publications dealt with isolation and characterisation of flavonoids from the studied taxa, flavonol and flavone compounds and their glycosides were identified (Hussiney et al. 1998; El-Sayed et al. 1999).

To the best of our knowledge, the chemosystematic value of *D. acris* was not discussed before, moreover as the global interest in finding a new valuable medicinal and food sources was increased, the present study aims to reinvestigate the phytochemical constituents of *D. acris* and to study the infraspecific relationship with three related species; *D. erucoides*, *D. harra* and *D. muralis* from the flavonoids point of view.

### 2. Results and discussion

#### 2.1. Flavonoid investigation

Eleven flavonoid compounds were separated from *D. acris* and identified as; luteolin (4), luteolin 7-O-α-rhamnopyranoside (5), kaempferol (8), 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) (see structural elucidation). Compounds (4, 8, 13, 16, 20, 22 and 32) were isolated for the first time from *D. acris*. The structures of these compounds were elucidated by their chromatographic behaviours, chemical and spectroscopic analysis using UV and ESI/MS, ¹H NMR, ¹³C NMR and HMBC (Mabry et al. 1970; Markham 1982; Agrawal 1989; Markham & Geiger 1994). The isolated compounds were summarised with others reported previously from related species (Table 1) to be useful characters in the current chemotaxonomic study.

#### 2.2. Chemosystematic significance

Taxonomically, the genus *Diplotaxis* belongs to family Brassicaceae (3709 species, 338 genera and 13–19 tribes); tribe Brassiceae (242 species & 48 genera) and considered as one of the core genera of subtribe Brassicinae (Warwick et al. 2009). On the basis of the molecular characters, the species of *Diplotaxis* are divided into three subgenera; *Hesperidium* (DC) Mart.-Lab. (*D. acris* & *D. harra*), *Rhynchocarpum* (Prantl) Mart.-Lab. (*D. erucoides*) and *Diplotaxis* (*D. muralis*) (Gómez-Campo 1999). Due to the stability and availability of flavonoid compounds in genus *Diplotaxis*, they are used as a taxonomic marker. From the flavonoid point of view, 33 compounds were reported (Table 1) and represented as 7 flavones and 26 flavonols. The flavones are based on apigenin, luteolin and diosmetin skeletons, while the flavonols are represented as kaempferol, quercetin, rhamnetin and isorhamnetin and their glycosides. Flavonols were recognised in both *D. acris* and *D. harra* and were absent from the...
other taxa, only apigenin and apigenin-7-O-rhamnoside were reported in *D. harra* (Hussiney et al. 1998; Mohammed et al. 2013) while six compounds were found in *D. acris* and characterized by three core flavone nucleus; apigenin, luteolin and diosmetin as well as their glycosylation at position 7 (Hussiney et al. 1998; El-Sayed et al. 1999). All the studied taxa have flavonol compounds; *D. harra* is the most rich species in flavonol content (19 compounds; Table 1) (Sánchez-Yélamo 1994; Hussiney et al. 1998; Atta et al. 2011; Kassem et al. 2013; Mohammed et al. 2013). The flavonol profile of *D. erucoides* and *D. muralis* is different. The former is characterised by the presence of –OCH₃ group at position 7; rhamnetin 3, 3′-di-O-glucoside (Salah et al. 2015) and the glycosylation take place at position 3 or 7. In *D. muralis*, the –OCH₃ group is found at position 3′ (isorhamnetin compounds) and the glycosylation at position 3 is predominant (Sánchez-Yélamo & Martínez-Laborde 1991). The present study agrees with Gómez-Campo (1999) in the separation of the studied taxa into three

| Subgenera/Species | Hesperidium | Rhynchocarpum | Diplotaxis |
|-------------------|-------------|---------------|------------|
| Apigenin (1)      | *D. acris*  | *D. harra*    |            |
| Ap.-7-O-diglucoside (2) | <sup>+</sup><sup>d</sup>   | <sup>+</sup><sup>i</sup> |
| Ap.-7-O-rhamnoside (3) | <sup>+</sup><sup>f</sup> |
| Luteolin (4)      | *D. acris*  | *D. harra*    |            |
| L.-7-O-rhamnoside (5) | <sup>+</sup><sup>d</sup>   | <sup>+</sup><sup>a</sup>|
| L.-7-O-diglucoside (6) | <sup>+</sup><sup>d</sup> |
| Diosmetin 7-O-rhamnoside (7) | <sup>+</sup><sup>f</sup> |
| Kaempferol (8)    | *D. acris*  | *D. harra*    |            |
| K.-3-O-glucoside (9) | <sup>+</sup><sup>d</sup>   | <sup>+</sup><sup>i</sup>   |
| K.-3-O-rhamnoside (10) | <sup>+</sup><sup>i</sup> |
| K.-3-O-diglucoside (11) | <sup>+</sup><sup>i</sup> |
| K.-7-O-glucoside (12) | <sup>+</sup><sup>i</sup>   |
| K.-3-O-glucoside-7-O-rhamnoside (13) | <sup>+</sup><sup>i</sup>   |
| K.-3-O-glucoside-4′-O-xyloside (14) | <sup>+</sup><sup>i</sup>   |
| Quercetin (15)    | *D. acris*  | *D. harra*    |            |
| Q.-3-O-glucoside (16) | <sup>+</sup><sup>d</sup>   | <sup>+</sup><sup>i</sup>   |
| Q.-3-O-rhamnoside (17) | <sup>+</sup><sup>i</sup> |
| Q.-3-O-digalactoside (18) | <sup>+</sup><sup>i</sup> |
| Q.-3-O-diglucoside (19) | <sup>+</sup><sup>i</sup> |
| Q.-7-O-glucoside (20) | <sup>+</sup><sup>i</sup>   |
| Rhamnetin 3,3′-di-O-glucoside (21) | <sup>+</sup><sup>i</sup>   |
| Isorhamnetin (22) | *D. acris*  | *D. harra*    |            |
| I.-3-O-glucoside (23) | <sup>+</sup><sup>d</sup>   | <sup>+</sup><sup>i</sup>   |
| I.-3-O-rhamnoside (24) | <sup>+</sup><sup>i</sup> |
| I.-7-O-glucoside (25) | <sup>+</sup><sup>i</sup> |
| I.-7-O-rhamnoside (26) | <sup>+</sup><sup>i</sup>   |
| I.-3-O-rutinoside (27) | <sup>+</sup><sup>i</sup> |
| I.-3,7-di-O-glucoside (28) | <sup>+</sup><sup>i</sup>   |
| I.-3′,4′-di-O-glucoside (29) | <sup>+</sup><sup>i</sup>   |
| I.-3-O-glucoside-4′-O-rhamnoside (30) | <sup>+</sup><sup>i</sup>   |
| I.-3-O-glucoside-4′-O-xyloside (31) | <sup>+</sup><sup>i</sup>   |
| I.-3-O-glucoside-7-O-rhamnoside (32) | <sup>+</sup><sup>i</sup>   |
| I.-3-O-j2″′-O-acetyl-glucos-yl (1-6)-glucoside (33) | <sup>+</sup><sup>i</sup>   |

<sup>a</sup>Present study.
<sup>b</sup>Kassem et al. (2013).
<sup>c</sup>Atta et al. (2011).
<sup>d</sup>El-Sayed et al. (1999).
<sup>e</sup>Hussiney et al. (1998).
<sup>f</sup>Sánchez-Yélamo and Martínez-Laborde (1991).
<sup>g</sup>Sánchez-Yélamo (1994).
<sup>h</sup>Salah et al. (2015).
<sup>i</sup>Mohammed et al. (2013).
subgenera, where the flavonoid profile of *D. acris* and *D. harra* is similar and the flavonoid pattern of *D. erucoides* is different from *D. muralis*, moreover, more investigation is needed to include the rest species.

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