

# C-glycosyl flavonoids-rich extract of *Dipcadi erythraeum* Webb & Berthel. bulbs: Phytochemical and anticancer evaluations

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## ABSTRACT

*Dipcadi erythraeum* Webb & Berthel. is a wild edible species belonging to family Asparagaceae and commonly used in folk medicine. The *D. erythraeum* bulbs extract was subjected to chemical investigation using Liquid Chromatography-Electrospray Ionization Mass Spectrometry (LC-ESI-MS) technique to identify its polar active constituents and evaluated against four human carcinoma cell lines; MCF7, HEPG2, A549, and HCT116. The *D. erythraeum* bulbs extract revealed 22 phenolic compounds characterized for the first time from the studied species, 14 of them were identified as C-glycosyl flavonoids. Moreover, the studied extract showed moderate activity against MCF7 and HCT116 at 100 µg/ml with cell viability of 43.6% and 48.4%, respectively. From the chemotaxonomic point of view, the presence of C-glycosyl flavonoids supported that *D. erythraeum* has a closer relationship with the species of Asparagaceae family than Liliaceae.

## INTRODUCTION

The genus *Dipcadi* is recently belonging to the subfamily Scilloideae of the family Asparagaceae. It is reported mainly from Africa, peninsular India, Madagascar, and neighboring Pakistan. Ten species of this genus are reported, of which *D. erythraeum* Webb & Berthel. is distributed in India as well as in different tropical regions of the world such as the Canary Islands, Arabia, Egypt, and Saudi Arabia (Boulos, 2009).

*Dipcadi erythraeum* [Syn. *Dipcadi unicolor* (Stocks) Baker, *Uropetalum unicolor*, Hooker's, *Ornithogalum erythraeum* (Webb & Berthel.) J. C. Manning & Goldblatt, *Uropetalon erythraeum* (Webb & Berthel.) Boiss.] is observed in rocky and gravelly habitats where rainwater collects for some days. The leaves are narrowly linear, while the flowers are greenish in color.

Flowers and fruits appear during the months of August–September (Bhandari, 1990).

*Dipcadi erythraeum* is a medicinal plant with great folk uses. The bulbs and capsules are eaten raw during the famine (Jongbloed *et al.*, 2000; Mandaville, 1990). The leaves are laxative and used as an ointment for wounds (Moussaïd *et al.*, 2013), while the whole plant is used for a cough, biliousness, diabetes, urinary and discharge. Phytochemical screening of *Dipcadi* species revealed the presence of tannins, alkaloids, flavonoids, and saponins (Abdulkareem *et al.*, 2014; Adly *et al.*, 2015; Ali, 2005). El-Shabrawy *et al.* (2016) reported the isolation of two flavonol aglycones (kaempferol and quercetin), one flavonol glycoside (quercetin 3-*O*-rutinoside-7-*O*- $\alpha$ -rhamnopyranoside), and four C-glycosyl flavones (vitexin, isovitexin, orientin, and isoorientin) from the defatted aqueous methanol extract of *D. erythraeum* whole plant.

There are no biological activities reported for *D. erythraeum*, therefore, the objective of the present study is to further our knowledge about its phytochemical constituent using Liquid Chromatography-Electrospray Ionization Mass Spectrometry (LC-ESI-MS) and evaluate its cytotoxic activity against breast (MCF7), hepatocellular (HEPG2), lung (A549), and colon (HCT116) cell lines.

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## EXPERIMENTAL

### Plant material and extraction

*Dipcadi erythraeum* was collected 120 km Cairo-Alexandria desert road, in March 2017 and identified by M. El-Shabrawy. A voucher specimen (s.n. MS6) was deposited in the herbarium of the National Research Center (CAIRC). The bulbs were dried and grinded, then extracted three times with 70% MeOH/H<sub>2</sub>O (Mabry *et al.*, 1970). The solvent was evaporated under reduced pressure at 50°C and then the dried extract was defatted with petroleum ether.

### LC-ESI-MS analysis

*Dipcadi erythraeum* aqueous methanol extract was analyzed by LC-ESI-MS system [High Performance Liquid Chromatography (Waters Alliance 2695) and mass spectrometry (Waters 3100)] according to the methods of Hussein *et al.* (2018). Known peaks were identified by comparing their retention time and mass spectrum with the flavonoid standards (95% purity; UV, Nuclear Magnetic Resonance) which were obtained from our research group (Phytochemical and Plant Systematic Department, NRC) (El-Shabrawy *et al.*, 2016; El-Sherei *et al.*, 2018; Hussein *et al.*, 2017, 2018; Ibrahim *et al.*, 2013; Marzouk *et al.*, 2010). Other peaks were tentatively identified by comparing the mass spectrum with the literature.

### Cell culture and sample treatment

The investigated human carcinoma cell lines were breast (MCF7), hepatocellular (HEPG2), lung (A549), and

colon (HCT116). They were purchased from American Tissue Culture Collection. HEPG2, MCF7, and HCT116 cells lines were cultured in RPMI 1640 medium, while A549 cell line was cultured in DMEM media. Media and cell culture preparations and treatments in addition to the *in vitro* cytotoxic activity of the plant extract were measured and followed the same method of Ibrahim *et al.* (2013).

## RESULTS AND DISCUSSION

### Identification of phenolics using LC-ESI-MS analysis

Twenty-two compounds were identified in the bulbs extract of *D. erythraeum* (Fig. 1, Table 1), all of them were characterized for the first time in the studied species.

Peak 2 (*m/z* 195) was characterized as gluconic acid. Its spectrum showed a fragment ion at *m/z* 129 which corresponds to the loss of H<sub>2</sub>O and CO<sub>2</sub> molecules [M-H-CO<sub>2</sub>-H<sub>2</sub>O]<sup>-</sup> (Felipe *et al.*, 2014). Peak (3) at *m/z* 335 showed a fragment ion at *m/z* 173 [M-162-H]<sup>-</sup>, after the loss of hexose unit, indicative for shikimic acid which showed other two fragments after the loss of H<sub>2</sub>O molecules; *m/z* 155 [M-162-H-H<sub>2</sub>O]<sup>-</sup> and 137 [M-162-H-2H<sub>2</sub>O]<sup>-</sup>. Accordingly, compound 3 was tentatively identified as shikimic acid hexoside (Spínola *et al.*, 2015). Peak (4) showed [M-H]<sup>-</sup> ion at *m/z* 487 and produced fragments at *m/z* 325 [M-H-hexose]<sup>-</sup> (coumaric acid hexoside), 163 [M-H-2hexose]<sup>-</sup> (coumaric acid), and *m/z* 119 [M-H-2hexose-COOH]<sup>-</sup> (decarboxylated coumaric acid). Thus, compound 4 was tentatively identified as coumaric acid dihexoside (Simirgiotis *et al.*, 2015). Peak (5) showed a molecular ion at *m/z* 503 and revealed fragments at *m/z* 341 [M-H-

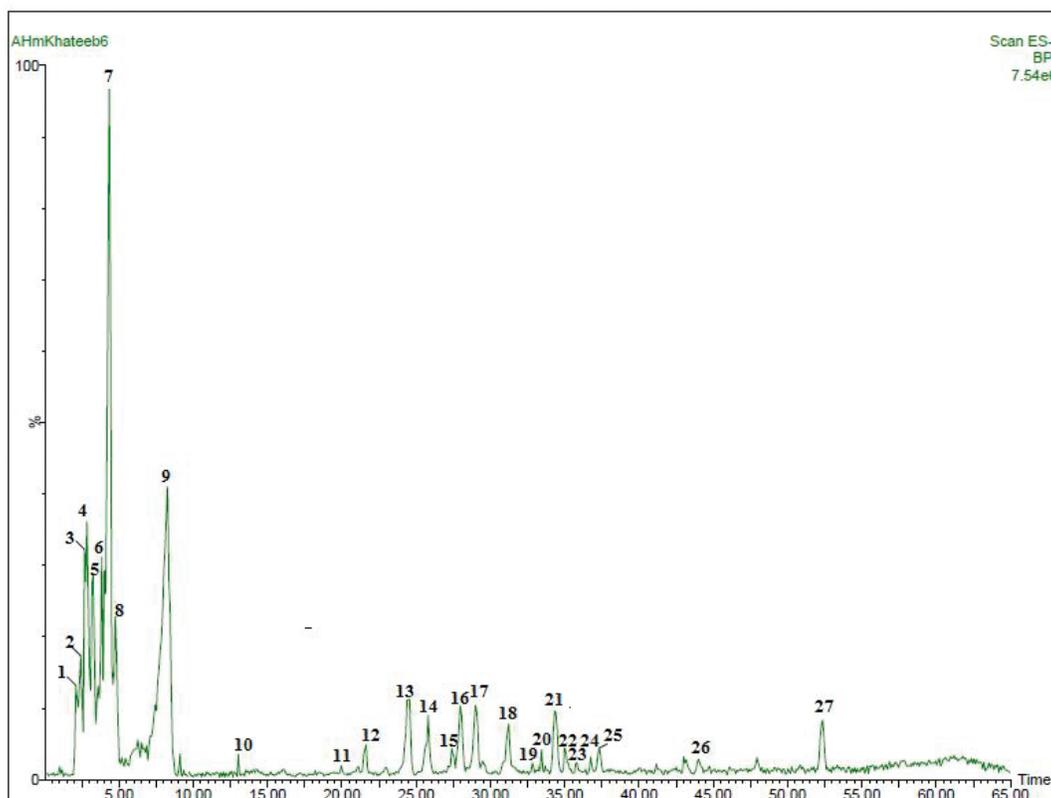


Figure 1. LC-ESI-MS chromatogram of *D. erythraeum* bulbs.

**Table 1.** Tentative identification of chemical compounds in *D. erythraeum* bulbs.

No.	Rt (min)	M	[M-H] <sup>-</sup>	m/z fragments	Tentative identification
1	2.09	306	305	289, 273, 175, 159	Unknown
2	2.42	196	195	177, 159, 129	Gluconic acid
3	2.67	336	335	173, 155, 137, 93	Shikimic acid hexoside
4	2.84	488	487	325, 163, 119	Coumaricaciddihexoside
5	3.25	504	503	341, 179, 135	Caffeicaciddihexoside
6	3.84	317	316	171, 128	Unknown
7	4.34	196	195	80, 96	Unknown
8	4.76	192	191	173, 128, 85	Quinic acid
9	8.3	422	421	337, 173, 113	Malonylcoumaroylquinic acid
10	13.03	356	355		Ferulic acid hexoside
11	19.82	610	609	447, 357, 327	Isoorientin-7- <i>O</i> -β-glucopyranoside (lutonarin)*
12	21.5	562	561	470	Apigenin 6,8-di- <i>C</i> -rhamnoside*
13	24.55	594	593	503, 473	Apigenin 6,8-di- <i>C</i> -glucoside*
14	25.8	572	571	481, 459, 257	Unknown
15	27.47	490	489	447, 429, 327	Isoorientin X''- <i>O</i> -acetyl
16	27.9	606	605	503, 473, 443	Apigenin 6- <i>C</i> -pentoside-8- <i>C</i> -hexoside X''- <i>O</i> -acetyl
17	28.9	639	638	506, 302	Delphinidin- <i>O</i> -hexoside X''- <i>O</i> -acetyl- <i>O</i> -pentoside
18	31.2	580	579	489, 459	Luteolin 6- <i>C</i> -β-glucopyranoside-8- <i>C</i> -α-arabinopyranoside (carlinside)*
19	33.3	578	577	457	Apigenin 6- <i>C</i> -β-glucopyranoside-8- <i>C</i> -α-rhamnopyranoside (violanthin)*
20	33.48	678	677	533, 473, 443, 383, 353	Apigenin 6,8-di- <i>C</i> -pentoside- <i>O</i> -rhamnoside
21	34.4	564	563	503, 473, 443	Apigenin 6- <i>C</i> -hexoside-8- <i>C</i> -pentoside
22	35.15	594	593	533, 503, 473	Luteolin- <i>O</i> -methyl ether-6- <i>C</i> -pentoside-8- <i>C</i> -hexoside
23	35.8	548	547	478	Apigenin 6- <i>C</i> -pentoside-8- <i>C</i> -rhamnoside
24	36.74	694	693	547, 478	Apigenin 6- <i>C</i> -pentoside-8- <i>C</i> -rhamnose- <i>O</i> -rhamnoside
25	37.4	578	577	487, 473, 457	Apigenin 6- <i>C</i> -α-rhamnopyranoside-8- <i>C</i> -β-glucopyranoside(isoviolanthin)*
26	41.4	754	753	591, 501, 487, 471	Apigenin- <i>O</i> -methyl ether-6- <i>C</i> -hexoside-8- <i>C</i> -rhamnoside- <i>O</i> -hexoside
27	52.36	1052	1051	869	Unknown

\*Compounds identified by comparing their retention times and mass spectrum with the authentic.

\*Compounds identified based on the mass spectral data cited in the literature.

hexose]<sup>-</sup>, 179 [M-H-2 hexose]<sup>-</sup>, and 135 [M-H-2 hexose-COOH]<sup>-</sup>, indicating the presence of caffeic acid di-hexoside (Simirgiotis *et al.*, 2015). Compound (8) at *m/z* 191 was existing as quinic acid, confirmed by the presence of the fragment ion at *m/z* 127 [M-H-CO-2H<sub>2</sub>O]<sup>-</sup> (Taamalli *et al.*, 2015).

Peak (9) at *m/z* 421 revealed the fragmentation pattern of coumaroyl quinic acid (*m/z* 337, 191, 173, 163, and 119) (Marzouk *et al.*, 2018) with an extra 84 amu, suggestive for a malonyl group. Therefore, compound 9 was considered as malonylcoumaroyl quinic acid. Peak (10) showed a molecular ion at *m/z* 355 and revealed fragments at *m/z* 193 [M-H-hexose]<sup>-</sup> and 149 [M-H-hexose-COOH]<sup>-</sup>, indicating the presence of ferulic acid hexoside (Simirgiotis *et al.*, 2015). Peak (11) with a molecular anion at *m/z* 609 showed a fragment ion at *m/z* 447 after the loss of a dehydrated hexose unit [M-162-H]<sup>-</sup>, attached as *O*-substitution of the C-glycosyl flavone structure. The fragment ions at *m/z* 327 [447-120-H]<sup>-</sup> and at *m/z* 357 [447-90-H]<sup>-</sup> confirmed the characteristic isoorientin ion. Therefore, peak

11 assigned as isoorientin-7-*O*-hexoside. In comparison with standards, compound 11 was identified as isoorientin-7-*O*-β-glucopyranoside (lutonarin) (El-Sherei *et al.*, 2018).

Compound (12) showed a molecular anion at *m/z* 562 and exhibited losses of (-90) which appeared at *m/z* 472. It was readily assigned as apigenin-6, 8-di-*C*-rhamnoside, in comparison with a standard. Likewise, apigenin-6, 8-di-*C*-glucoside was identified for peak (13) at *m/z* 593 and confirmed by its losses of (-90 amu) and (-120 amu) appearing at *m/z* 503 and 473.

Peak (15) showed typical fragments of isoorientin (*m/z* 327 [M-120-H]<sup>-</sup> and *m/z* 357 [M-90-H]<sup>-</sup>) with an extra 42 amu, suggestive for an acetyl group connected to a glucose moiety and identified as isoorientin X''-*O*-acetyl. Moreover, peak (16) has a molecular ion at *m/z* 605 and showed fragmentation pattern of apigenin 6-*C*-pentoside-8-*C*-hexoside with an extra 42 amu indicative for an acetyl group connected to either hexose or pentose moieties as revealed from its -120 amu and -90 amu losses appearing at *m/z* 443 and 473, respectively, in addition to

–60 amu loss for a C-pentoside appearing at  $m/z$  503 [M-60-H]<sup>-</sup> corresponding to a C-linked pentose. Pentose moiety is suggested to attach at the C-6 position as evident from  $m/z$  503 [M-60-H]<sup>-</sup> appearing at higher intensity relative to  $m/z$  443 [M-120-H]<sup>-</sup> and 473 [M-90-H]<sup>-</sup> (Geiger and Markham, 1986). Therefore, peak 16 was identified as apigenin-6-C-pentoside-8-C-hexoside X"-O-acetyl. Di-C-glycosyl apigenin acetate derivatives were reported previously from some monocotyledon members (Williams, 1975).

Peak (17) with  $m/z$  638 showed a fragment ion at  $m/z$  506 [M-132-H]<sup>-</sup>, after the loss of pentose unit and indicative for delphinidin-O-hexoside with an extra 42 amu indicative for an acetyl group connected to the hexose moiety. Another fragment was observed at  $m/z$  302 for delphinidin aglycone, thus compounds 17 was tentatively identified as delphinidin-O-hexoside X"-O-acetyl-O-pentoside. Delphinidin di-acetyl di-glycoside was reported before from the bulbs of other member of family Hyacinthaceae (Dias *et al.*, 2003).

On the bases of retention time and fragmentation pattern of standards, compound 18 with  $m/z$  579 is identified as luteolin 6-C-β-glucopyranoside-8-C-α-arabinopyranoside (carlinside) (El-Sherei *et al.*, 2018), confirmed by three fragment ions at  $m/z$  519 [M-60-H]<sup>-</sup>,  $m/z$  489 [M-90-H]<sup>-</sup>, and  $m/z$  459 [M-120-H]<sup>-</sup>.

Isomer peaks (19) and (25) showed the same molecular ion peak at  $m/z$  577 and displayed the same fragment ions at  $m/z$  487 [M-90-H]<sup>-</sup> and  $m/z$  457 [M-120-H]<sup>-</sup> indicative for a C-hexose unit. Isomers difference was founded on the intensity of  $m/z$  457 [M-120-H]<sup>-</sup> fragment for compound 19, it appeared as a base peak suggesting the attachment of the deoxyhexosyl moiety at the C-6 (Farag *et al.*, 2016). In comparison with standards, compounds 19 and 25 were identified as apigenin 6-C-β-glucopyranoside-8-C-α-rhamnopyranoside (violanthin) and apigenin 6-C-α-rhamnopyranoside-8-C-β-glucopyranoside (isoviolanthin), respectively (El-Sherei *et al.*, 2018).

Peak (20) at  $m/z$  677 showed a fragment ion at  $m/z$  533, after the loss of a dehydrated rhamnose unit [M-146-H]<sup>-</sup>, attached as O-substitution of the flavone di C-glycoside structure ( $m/z$  533). Further fragmentation pattern of  $m/z$  533 was revealed as  $m/z$  473 [M-60-H]<sup>-</sup> and 443 [M-90-H]<sup>-</sup>, indicating the loss for C-pentoside. Therefore, compound 20 was identified as apigenin 6, 8-di-C-pentoside-O-rhamnoside.

Peak (21) at  $m/z$  563 showed fragmentation pattern of flavone-C-hexoside as revealed from its (-120 amu) and (-90 amu) losses appearing at  $m/z$  443 and 473, respectively, in addition to (-60 amu) loss for a C-pentoside appearing at  $m/z$  503 [M-60-H]<sup>-</sup> corresponding to a C-linked pentose. Hexose moiety is suggested to attach at the C-6 position as evident from  $m/z$  443 [M-120-H]<sup>-</sup> appearing as base peak relative to pentose (Geiger and Markham, 1986) and was identified as apigenin-6-C-hexoside-8-C-pentoside.

Peak (23) at  $m/z$  547 revealed a fragment ion at  $m/z$  487 after the loss of -60 amu corresponding to a C-linked pentose; therefore, compound 23 was identified as apigenin-6-C-pentoside-8-C-rhamnoside.

Peak (22) and (24) at 679 and  $m/z$  693 revealed the same fragment ion at  $m/z$  547, after the loss of pentose unit [M-132-H]<sup>-</sup> or rhamnose unit [M-146-H]<sup>-</sup>, respectively. Both pentose and rhamnose are suggested to be attached as O-substitution of the flavone di C-glycoside structure ( $m/z$  547). Further fragmentation pattern of  $m/z$  547 indicates the same structure of compound

23. Therefore, compounds 22 and 24 were similar to peak (23) with an extra mass difference of 132 amu (pentose moiety) and 146 amu (rhamnose moiety) in molecular ions, respectively. Therefore, compounds (22) and (24) were identified as apigenin 6-C-pentoside-8-C-rhamnose-O-pentoside and apigenin 6-C-pentoside-8-C-rhamnose-O-rhamnoside, respectively.

Peak (26)  $m/z$  753 showed a fragment ion at  $m/z$  591, after the loss of a dehydrated hexose unit [M-162-H]<sup>-</sup>, attached as O-substitution of the flavone di C-glycoside structure ( $m/z$  591). Further fragmentation pattern of  $m/z$  591 was similar to that of the peak (25) with an extra mass difference of 14 amu in molecular ions and its fragment masses assigned as apigenin 6-C-rhamnoside-8-C-hexoside-methyl ether (Farag *et al.*, 2016). C-glycosyl apigenin methyl ether derivatives were reported previously from some monocotyledon member; *Iris* species (Iridaceae) (Kawase and Yagishita, 1968).

### In vitro cytotoxic activity

The results indicated that the bulbs aqueous methanol extract of *D. erythraeum* showed moderate activity against MCF7 and HCT116 at 100 µg/ml with cell viability of 43.6% and 48.4%, respectively. However, it exhibited no evident cytotoxicity against A549 and HEPG2 cell lines.

### CONCLUSION

Total 22 compounds including 14 C-glycosyl flavonoids, 6 phenolic acid derivatives (coumaric and caffeic acids derivatives), one organic acid, and one anthocyanin (delphinine derivative) were identified or tentatively characterized, all of them were detected for the first time from *D. erythraeum*. The presence of C-glycosyl flavonoids supported the suggestion achieved by El-Shabrawy *et al.* (2016), which indicate that *D. erythraeum* has a similar biosynthetic pathway and, therefore, a closer relationship with the species of Asparagaceae family than Liliaceae. Moreover, the studied extract showed moderate activity against MCF7 and HCT116 at 100 µg/ml with cell viability of 43.6% and 48.4%, respectively.

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### CONFLICT OF INTERESTS

Author declares that there are no conflicts of interest.

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