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C-glycosylflavones from the aerial parts of *Silene colorata*

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Abstract

Phytochemical investigation of *Silene colorata* Poir. aerial parts ethanolic extract led to the isolation of two undescribed C-glycosylflavones along with eight known compounds. The NMR and MS spectroscopic analysis and their comparison with published data led to the identification of nine C-glycosylflavones : apigenin 6-C-[α -L-arabinofuranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl]-7-O- β -D-glucopyranoside, luteolin 6-C-[α -L-arabinofuranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl]-7-O- β -D-glucopyranoside, schaftoside, vitexin, isoorientin, vicenin-3, vicenin-1, saponarin, isovitexin and one ecdysterone; integristerone A. The lack of chemical and biological investigations on this plant encouraged us to carry out the above-mentioned work.

Keywords

Caryophyllaceae, Ecdysterone, Flavonoids, *Silene*.

1. Subject and source

The Caryophyllaceae family comprises 104 genera and more than 2000 species and divided into 3 subfamilies Alsinoideae, Caryophylloideae and Paronychioidea (Bittich, 1993; Harbaugh et al., 2010). In the flora of Northern Africa, Caryophyllaceae is represented by 31 genera, corresponding to 382 taxa (Dobignard and Chatelain, 2011). *Silene* L., a genus of the subfamily Caryophylloideae, includes several

herbaceous plants and, more rarely, small shrubs or subshrubs, primarily distributed in temperate regions of the Northern Hemisphere of Eurasia and America, but also in Africa and contain about 850 species (Hussein et al., 2020; Mamadaliyeva et al., 2014; Zhou et al., 2001). The genus *Silene* is represented in Northern Africa by 144 species or subspecies, among them 56 are endemic (Dobignard and Chatelain, 2011). Algeria is one of the richest *Silene* species hosting aereas with 71 taxa, of which 12 are strictly endemic to the northern part of the country (Véla and Benhouhou, 2007, Quezel and Santa., 1962) and 13 taxa are endemic and shared with other North African countries (Dobignard and Chatelain, 2011). *Silene* species are known for their richness in triterpenoids saponins, flavonoids and phytoecdysteroids (Bechkri et al., 2020; Bechkri et al., 2022; Mamadaliyeva et al., 2014). *Silene colorata* Poir. is a hermaphrodite annual plant, which shows a nocturnal anthesis period. It normally grows in arable lands and its flowering and fructing period ranges from January to June (Terrab et al., 2007). As a part of our ongoing investigation on *Silene* plants (Bechkri et al., 2020; Bechkri et al., 2022; Bechkri et al., 2023), we chose *Silene colorata* Poir. for investigation, due to lack of phytochemistry reports.

In the present study, the aerial parts of *S. colorata* Poir. were collected and identified by Mr. Kamel

Kabouche in June 2017 from El Mansourah, Constantine (North-Eastern Algerian) and the taxonomic identification was further authenticated by Professor Salima Benhouhou from the Herbarium Department of Botanic of the National Superior School of Agronomy of El Harrach (ENSA, Algiers, Algeria), where a voucher specimen (ENSA13417) was deposited at their herbarium.

2. Previous work

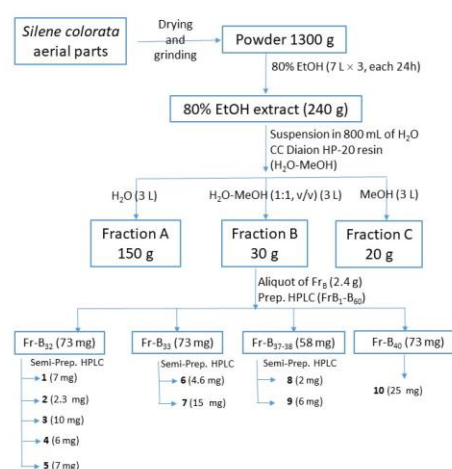
To the best of our knowledge, no data exist on the chemical constituents in *S. colorata*, except the occurrence of a phytoecdysteroid, 20-hydroxyecdysone (Zibareva et al., 1996; Zibareva et al., 2003).

3. Present study

The dried and powdered aerial parts (1300 g) of *S. colorata* were macerated in 80% aqueous ethanol (7 L \times 3, each 24h). After filtration and evaporation procedures, the 80% EtOH extract (240 g) was dissolved in 800 mL of water and then subjected to Diaion HP-20 resin (Sigma Aldrich) column chromatography (650 \times 55 mm), eluted by MeOH-H₂O (0:1, 1:1 and 1:0, 3 L, each) to give fractions Fr_A-Fr_C, respectively (Scheme 1). An aliquot of Fr_B (2.4 g) was subjected to preparative HPLC using a gradient from 10 to 20% CH₃CN in H₂O as mobile phase, in 60 min (Merck prepacked Lichrospher RP_{C-18} column, 250 \times 50 mm, 12 μ m, flow rate 40 mL/min) to give 60 subfractions (Fr_{B1}-Fr_{B60}). Sub-fractions Fr_{B32} (73 mg) was purified by semi-preparative HPLC (Phenomenex RP_{C-18} column, 250 \times 10 mm, Luna 5 μ m, flow rate 5 mL/min), using 15% CH₃CN in H₂O as a mobile phase to afford compounds **1** (t_R 11.0 min, 7 mg), **2** (t_R 9.9 min, 2.3 mg), **3** (t_R 10.0 min, 10 mg), **4** (t_R 10.6 min, 6 mg), and **5** (t_R 11.5 min, 7 mg). Sub-fraction Fr_{B33} (73 mg) was purified by semi-prep. HPLC using a

gradient from 15 to 18% CH₃CN in water as a mobile phase, in 15 min, to yield compounds **6** (t_R 12 min, 4.6 mg), whereas compound **7** (15 mg) was obtained as a precipitate in this fraction. In the same condition as Fr_{B33}, the purification of Fr_{B37-38} (58 mg) by semi-prep. HPLC furnished compounds **8** (t_R 12.7 min, 2 mg) and **9** (t_R 13.7 min, 6 mg). Fr_{B40} contained compound **10** (25 mg) (Scheme 1).

In the present work, we report the isolation and identification of two new C-glycosylflavones (**1-2**), seven known C-glycosylflavones (**3-9**) and one ecdysteroid (**10**) from the 80% EtOH extract of *S. colorata* (Fig. 1) Their structures were proposed after careful analysis of ¹H and ¹³C NMR, COSY, HMBC, HSQC spectra and confirmed with HR-ESI-MS data, as well as comparison with published data in the relevant literature. The structure of the known compounds were identified as vicenin-3 (**3**) (Fiorentino et al., 2008), vicenin-1 (**4**) (Fernando et al., 2019), saponarin (**5**) (Krauze-Baranowska and Cisowski, 1994), isovitexin (**6**) (Peng et al., 2005), vitexin (**7**) (Zhang et al., 2003), schaftoside (**8**) (Fernando et al., 2019), isoorientin (**9**) (Davoust et al., 1980) and integristerone A (**10**) (Simon et al., 2004). All the isolated compounds were reported for first time from this plant. This is the first report on isolation of secondary metabolites from *S. colorata*.



Scheme 1. Extraction and purification protocol for the 80% EtOH extract from the aerial parts of *Silene colorata*.

Compound **1** was obtained as a yellow amorphous powder and its molecular formula was determined as $C_{32}H_{38}O_{19}$, by the HR-ESI-MS $[M + Na]^+$ ion at m/z 749.1915 (calcd for $C_{32}H_{38}O_{19}Na$, 749.1905). Its UV spectrum exhibited absorption bands characteristic for flavone skeleton at 340 and 272 nm (Bechkri et al., 2022). The 1H and ^{13}C NMR spectra exhibited doubling of signals which was found consistently in the literature (Zhou et al., 2017) indicating the existence of two rotamers (Shi et al., 2019). In the 1H NMR spectrum, the proton signals corresponding to aromatic rings of flavone skeleton were observed as two doublets at δ_H 6.96 and 7.91 (each 2H, d, $J = 8.8$ Hz) (Table 1) suggesting the presence of an AA'BB'-type system for the *p*-disubstituted benzene ring, in addition to two singlets at δ_H 6.61 and 6.94, which were assigned to H-8 and H-3 respectively, with the help of HSQC and HMBC analysis suggesting the presence of an apigenin skeleton (Bechkri et al., 2022). Moreover, three anomeric proton signals at δ_H 5.11 (1H, d, $J = 7.8$ Hz), 4.95 (1H, d, $J = 10.0$ Hz) and 5.21 (*brs*) were observed in the 1H NMR spectrum. Characteristic 1H and ^{13}C NMR chemical shift values and coupling constant data indicated that the structure was based on that of saponarin (apigenin 6-*C*- β -D-glucopyranosyl-7-*O*- β -D-glucopyranoside) (Krauze-Baranowska and Cisowski, 1994, Zhou et al., 2017). This was confirmed by the detection of a long-range 1H - ^{13}C connectivity (HMBC) from the anomeric proton of the *C*- β -glucopyranosyl moiety at $\delta_{H-1''}$ 4.95 to the ^{13}C resonance at 109.9 (C-6). The anomeric carbon at $\delta_{C-1''}$ 71.6 in a higher field proved the *C*-glycosidic linkage. The remaining resonances of the primary sugar residue (6-*C*-glc) were assigned from COSY, HSQC and HMBC data using the anomeric proton at $\delta_{H-1''}$ 4.95 as a starting point ((Fig. 2) (Table 1). A second anomeric sugar proton was noted in the 1D 1H NMR spectrum at $\delta_{H-1''}$ 5.11,

with a ^{13}C NMR resonance at $\delta_{C-1''}$ 105.2 by HSQC. Assignment of the corresponding set of sugar resonances identified their origin as a second β -glucopyranosyl moiety *O*-linked to the C-7 of apigenin (Table 1) as deduced by the HMBC correlation observed between H-1'' and C-7 (δ_C 162.9). The H-1, H-2, H-3 and H-4 of the two glucopyranose units exhibited large vicinal coupling constants ($J \geq 7.8$ Hz) in 1H NMR experiment, indicating that all the protons are axial and confirmed that all glucopyranosyl units were β -linked (Agrawal, 1992). The difference in the 1H NMR and ^{13}C NMR data between compound **1** and saponarin suggests that the structure of compound **1** had a third sugar moiety represented by six additional 1H resonances observed at δ_H 5.21 (*brs*, H-1'''), 3.91 (dd, $J = 3.8, 1.4$ Hz, H-2'''), 3.67 (dd, $J = 6.2, 3.8$ Hz, H-3'''), 2.89 (ddd, $J = 6.2, 3.9, 3.2$ Hz, H-4'''), 3.32 (dd, $J = 11.9, 3.9$ Hz, H-5a''') and 3.40 (dd, $J = 11.9, 3.2$ Hz, H-5b''') and five ^{13}C resonances observed at δ_C 108.1 (C-1'''), 81.7 (C-2'''), 76.5 (C-3'''), 83.7 (C-4''') and 60.5 (C-5'''). The deshielded chemical shifts of the anomeric proton (δ_H 5.21) and carbon (δ_C 108.1) of this sugar unit indicated a furanosyl ring (Voutquenne et al., 2005). The third sugar unit was identified as α -L-arabinofuranose from analysis of the 1D- and 2D-NMR spectroscopic data (Agrawal, 1992). The small vicinal coupling constant ($J_{H-1''',H-2'''} 1.4$ Hz) in 1H NMR experiment indicated an α linkage for the arabinosyl anomeric proton. The α -L-arabinofuranose unit was linked to C-2''', as confirmed by the HMBC experiment (H-1''' in correlation with C-2''') and a glycosylation shift of C-2''' (+10 ppm). Based on the above evidence, the structure of compound **1** was established as apigenin 6-*C*-[α -L-arabinofuranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl]-7-*O*- β -D-glucopyranoside.

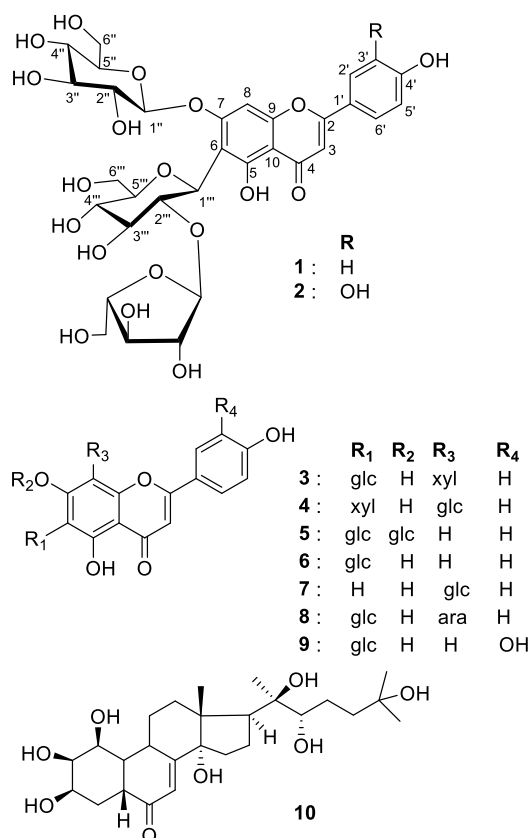


Fig. 1. Chemical structure of compounds **1-10**, isolated from *S. colorata*.

Compound **2** was isolated as a yellow amorphous powder with a molecular formula $C_{32}H_{38}O_{20}$ as determined by HR-ESI-MS ($[M + Na]^+$ at m/z 765.1864 (calcd for $C_{32}H_{38}O_{20}Na$, 765.1854). The UV spectra exhibiting absorption bands at 350, 270 and 256 nm were very similar to those of **1**, suggesting a flavone glycoside structure. The 1H and COSY NMR spectra of **2** exhibited in aglycone region, in addition to two singlet at δ_H 6.61 (H-3) and 6.94 (H-8), an ABX system at δ_H 7.44 (d, $J = 2.1$ Hz, H-2'), 7.46 (dd, $J = 8.3, 2.1$ Hz, H-6') and 6.96 (d, $J = 8.2$ Hz, H-5'), due to a 3',4'-disubstituted B-ring. Full assignment of the remaining aglycone resonances in the 1H and ^{13}C NMR spectrum of **2** was achieved by analysis of HSQC and HMBC data, which confirmed the presence of a luteolin derivative (Bechkri et al., 2022). Extensive analysis of the 1D- and 2D-NMR spectra of **2** and comparison of the 1H and ^{13}C NMR values of the

sugar part of **2** with those of **1** indicated that **2** had, as in **1**, a β -D-glucopyranose *O*-linked to C-7 and an α -L-arabinofuranosyl-(1 \rightarrow 2)- β -D-glucopyranose *C*-linked to C-6 (Fig. 2) (Table 1). Therefore, compound **2** was identified as luteolin 6-*C*-[α -L-arabinofuranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl]-7-*O*- β -D-glucopyranoside.

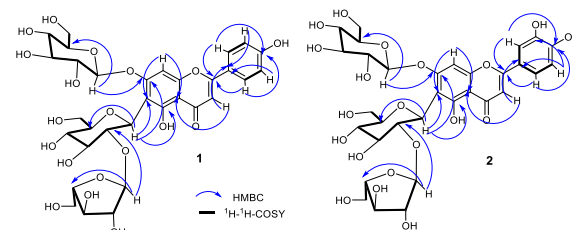


Fig. 2. Key 1H - 1H -COSY and HMBC correlations (H \rightarrow C) of **1** and **2**.

The hydrolysis of compounds **1** and **2** afforded D-glucose and L-arabinose, which verified the conclusion. The details of the acid hydrolysis procedure can be found in the Supplementary Material. The 1H and ^{13}C NMR spectrums obtained at room temperature exhibited doubling of signal indicating the existence of rotational isomers for these two compounds, as separate sets of signals, as previously observed for similar *C*-glycosylflavones (Bechkri et al., 2022; Cheng et al., 2000; Hosoya et al., 2005; Zhou et al., 2017).

4. Chemotaxonomic significance

In genus *Silene*, the major constituents are ecdysteroids, flavonoid glycosides (especially *C*-glycosides), alkaloids, saponins and glycolipids (Mamadaliyeva et al., 2014). In this study, nine *C*-glycosylflavonols (**1-9**) of which **1** and **2** were previously undescribed and one ecdysteroid (**10**) were isolated from *S. colorata*. Notably, this is the first isolation study to report all these compounds from this species. From a chemotaxonomic viewpoint, *Silene* species (Caryophyllaceae) are capable of accumulating apigenin and luteolin derivatives including their *C*- and/or *O*-glycosides and the 6-*C*-, 8-*C*-, or 7-*O*-monosaccharides were

often composed of β -D-glucopyranose, β -D-galactopyranose, α -L-rhamnopyranose, β -D-xylopyranose and α -L-arabinopyranose (Mamadaliyeva et al., 2014; Van Genderen and Van Hemert, 1986).

Table 1. ^1H NMR and ^{13}C NMR spectroscopic data of compounds **1-2** in CD_3OD .^a

1		2	
δ_{H} (J)	δ_{C}	δ_{H} (J)	δ_{C}
2	165.3	2	165.3
3 6.61 s	102.9	3 6.72 s	102.7
4	182.9	4	182.7
5	159.7	5	161.0
6	109.9	6	110.9
7	162.9	7	161.6
8 6.94 s	93.7	8 7.01 s	94.5
9	157.4	9	156.6
10	105.3	10	106.0
1'	121.6	1'	122.5
2' 7.91 d (8.8)	128.3	2' 7.44 d (2.1)	113.2
3' 6.96 d (8.8)	115.6	3'	145.8
4' -	161.6	4'	149.8
5' 6.96 d (8.8)	115.6	5' 6.96 d (8.3)	115.3
6' 7.91 d (8.8)	128.3	6' 7.46 dd (8.3, 2.1)	119.3
7-O-glc			
1'' 5.11 d (7.8)	101.2	1'' 5.11 d (7.8)	101.2
2'' 3.59 t (8.5)	73.6	2'' 3.62 dd (9.3, 7.8)	73.6
3'' 3.54 t (8.5)	76.1	3'' 3.54 t (9.3)	76.1
4'' 3.44 t (8.5)	69.6	4'' 3.48 t (9.3)	69.6
5'' 3.62 ddd (8.5, 5.7, 2.2)	77.0	5'' 3.62 ddd (9.3, 5.7, 2.2)	77.0
6'' 3.80 dd (12.2, 5.7)	61.1	6'' 3.80 dd (12.2, 5.7)	61.1
6'' 3.99 dd (12.2, 2.2)		6'' 3.99 dd (12.2, 2.2)	
6-C-glc			
1''' 4.95 d (10.0)	71.6	1''' 4.98 d (9.9)	71.6
2''' 4.40 dd (10.0, 9.6)	75.6	2''' 4.40 dd (9.9, 8.6)	75.6
3''' 3.65 t (9.6)	79.3	3''' 3.65 t (8.6)	79.3
4''' 4.61 t (9.6)	69.6	4''' 4.62 t (8.6)	69.6
5''' 3.40 ddd (9.6, 4.1, 2.0)	80.6	5''' 3.38 ddd (8.6, 4.1, 2.0)	80.6
6''' 3.84 dd (12.1, 4.1)	60.5	6''' 3.84 dd (12.1, 4.1)	60.5
6''' 3.88 dd (12.1, 2.0)		6''' 3.88 dd (12.1, 2.0)	
2'''-ara(f)			
1'''' 5.21 brs	108.1	1'''' 5.21 brs	108.1
2'''' 3.91 dd (3.8, 1.4)	81.7	2'''' 3.90 dd (3.8, 1.4)	81.7
3'''' 3.67 dd (6.2, 3.8)	76.5	3'''' 3.67 dd (6.2, 3.8)	76.5
4'''' 2.89 ddd (6.2, 3.9, 3.2)	83.7	4'''' 2.88 dd (6.2, 3.8, 4.1, 3.4)	83.7
5'''' 3.32 dd (11.9, 3.9)	60.5	5'''' 3.30 dd (12.0, 4.1)	60.5
5'''' 3.40 dd (11.9, 3.2)		5'''' 3.40 dd (12.0, 3.4)	

^a δ in ppm, J in parentheses in Hz.

Based on literature search, we found that vicenin-3 (**3**) was isolated from *S. boissieri*, *S. chlorantha*, *S. commutata*, *S. cyri*, *S. foliosa*, *S. graminifolia*, *S. jenissensis*, *S. italica*, *S. macrostyla*, *S. nutans*, *S. repens* and *S. wolgensis* (Darmograi, 1977; Mamadaliyeva et al., 2014), vicenin-1 (**4**) was isolated from *S. boissieri*, *S. chlorantha*, *S. commutata*, *S. cyri*, *S. foliosa*, *S. graminifolia*, *S. jenissensis*, *S. italica*, *S. macrostyla*, *S. nutans* and *S. wolgensis* (Darmograi, 1977; Mamadaliyeva et al., 2014), saponarin (**5**) was isolated from *S. italica*, *S. pratensis*, *S. seoulensis* and *S. repense* (Seo et al., 2020; Van Genderen and Van Hemert, 1986) and isovitexin (**6**) was isolated from *S. alba*, *S. armeria*, *S. boissieri*, *S. brachuica*, *S. bupleuroides*, *S. chlorantha*, *S. chlorifolia*, *S. commutata*, *S. compacta*, *S. cretacea*, *S. cubanensis*, *S. cyri*, *S. diclinis*, *S. dioica*, *S. foliosa*, *S. graminifolia*, *S. jenissensis*, *S. italica*, *S. macrostyla*, *S. multifida*, *S. nutans*, *S. polaris*, *S. repens*, *S. supina*, *S. turgida* and *S. wolgensis* (Darmograi, 1977; Heinsbroek et al., 1980; Richardson, 1978; Iwashina and Ootani, 1987), vitexin (**7**) was isolated from *S. alba*, *S. armeria*, *S. brachuica*, *S. bupleuroides*, *S. chlorantha*, *S. chlorifolia*, *S. commutata*, *S. compacta*, *S. cretacea*, *S. cubanensis*, *S. cyri*, *S. diclinis*, *S. foliosa*, *S. graminifolia*, *S. jenissensis*, *S. italica*, *S. macrostyla*, *S. multifida*, *S. nutans*, *S. polaris*, *S. repens*, *S. saxatilis*, *S. supina*, *S. turgida* and *S. wolgensis* (Darmograi, 1977; Heinsbroek et al., 1980; Richardson, 1978; Zemtsova et al., 1975), schaftoside (**8**) was isolated from *Silene schafta*, *S. italic* and *S. repens* (Chopin et al., 1974; Olennikov et al., 2019; Olennikov, 2020; Plouvier, 1967), isoorientin (**9**) was isolated from *S. armeria*, *S. alba*, *S. boissieri*, *S. bupleuroides*, *S. chlorantha*, *S. chlorifolia*, *S. commutata*, *S. compacta*, *S. cretacea*, *S. cubanensis*, *S. cyri*, *S. foliosa*, *S. graminifolia*, *S. jenissensis*, *S. littorea*, *S. italica*, *S. macrostyla*, *S.*

nutans, *S. polaris*, *S. pratensis*, *S. repens*, *S. saxatilis*, *S. viscariopsis* and *S. vulgaris* (Darmograi et al., 1977; Glyzin and Dzhumyrko, 1975; Richardson, 1978; Iwashina and Ootani, 1987; Olennikov, 2020; Niemann, 1981), whereas integristerone A (**10**) was previously isolated from *S. brahuica*, *S. claviformis*, *S. fridvaldszkyana*, *S. gigantea*, *S. italica* ssp. *nemoralis*, *S. nutans*, *S. oites*, *S. repens*, *S. scabrifolia*, *S. supina*, *S. tatarica*, *S. tomentella*, *S. viridiflora* and *S. wallichiana* (Mamadalieiva et al., 214). In summary, the present study certainly enriches the chemical diversity, expands the knowledge about the chemical compositions of *S. colorata* and provides evidence for plant chemotaxonomic studies of the genus *Silene*.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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