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Abstract

Six undescribed bis-iridoids named Semipapposiridoids A–F (**1-6**) together with seventeen known compounds were obtained from the roots and aerial parts of *Scabiosa semipapposa* Salzm. ex DC.. Among the known compounds, three lignans were isolated for the first time in *Scabiosa* species, whereas the other known constituents including iridoids, phenol glycosides and flavonoids were previously isolated from *Scabiosa sp*. Their structures were assigned on the basis of extensive 1D- and 2D-NMR experiments (^{1}H, ^{13}C, COSY, TOCSY, NOESY, HSQC, HMBC), mass spectrometry HR-ESI-MS and by comparison of their spectral data with those of literature values. To the best of our knowledge, this should be the first report on the bis-iridoids content of this plant; furthermore, the fact that the plant is rich in mono-and bis-iridoids will support chemotaxonomy of the genus. The total phenolic and flavonoid contents of the 80% MeOH extracts and fractions were evaluated, with their antioxidant activities using three different methods, namely DPPH, ABTS and CUPRAC. Most of the fractions showed a moderate to good antioxidant activity compared to the standards BHA and BHT.

Keywords:

Scabiosa semipapposa Salzm. ex DC., Caprifoliaceae, Bis-iridoid glucosides, Semipapposiridoids A-F, Antioxidant activity.

1. Introduction

The Caprifoliaceae, also known as the honeysuckle family, is a dicotyledonous flowering plant consisting of about 860 species in approximately 42 genera, with a nearly cosmopolitan distribution, with the centers of diversity in eastern North America and eastern Asia, while absent in tropical and southern Africa (Xuand Chang, 2017). Plants of this family are usually shrubs and vines and rarely herbaceous. Many genera of this family like *Cephalaria* (Tabatadze et al., 2007; Pasi et al., 2009; Mustafayeva et al., 2010; Sarikahya and Kirmizigul, 2010; Sarikahya et al., 2021), *Valeriana* (Ming et al., 1997; Tang et al., 2002; Dong et al., 2015; Quan et al., 2020;Liu et al., 2021), *Lonicera* (Lin et al., 2008; Liu et al., 2015;Li et al., 2019;Qiu et al., 2021), and *Scabiosa* (Lehbili et al., 2018a; Lehbili et al., 2018b; Bendamene et al., 2020; Kılınç et al.,2020) have attracted the attention of various authors for their phytochemical and biological properties. The *Scabiosa* genus, belonging to this family, is widespread in the Mediterranean area, including 12 species in Algeria (Quezel and Santa, 1963). Many *Scabiosa* species are used in folk medicine for the treatment of various illnesses such as asthma, influenza, bronchitis, bronchial pneumonia, liver diseases, neurodegenerative diseases, and to treat certain dermatoses, in particular scabies (Girre, 1980; Bammi and Douira, 2002; Rigat et al., 2007; Kose et al., 2015; Moteetee et al., 2016; Pinto et al., 2018). Numerous *Scabiosa* species yielded a great variety of secondary metabolites such as triterpene saponins (Alimbaeva et al., 1977; Baykal et al., 1998; Zheng et al., 2004; Lehbili et al., 2018a; Kılınç et al., 2020; Bendamene et al., 2020), flavonoids, coumarins (Garaev et al., 2008;Al-Qudah et al., 2017; Lehbili et al., 2018b), mono- and bis-iridoid glucosides (Papalexandrou et al., 2003; Polat et al., 2010; Lehbili et al., 2018b) and phenolic compounds (Akar,2022). In our continuous researches on the chemical constituents of *Scabiosa* species growing in Algeria (Lehbili et al., 2018a; Lehbili et al., 2018b; Bendamene et al., 2020), we have investigated *Scabiosa semipapposa* Salzm. Ex DC. roots and aerial parts. After previous isolation of triterpenoid

saponins from the roots of this plant (Bendamene et al., 2020), new phytochemical investigations were performed for its iridoids content. In this paper, we thus describe the isolation and structure elucidation of six undescribed bis-iridoids (**1**–**6**), namely semipapposiridoids A-F (Fig. 1), along with seventeen (**7**-**23**) known compounds. Chemotaxonomy of this species was discussing. In addition, the antioxidant capacity of the 80% methanol extracts and fractions were evaluated using DPPH, ABTS and CUPRAC methods.

2. Results and discussion

The 80% methanol extract of *S. semipapposa* roots was fractionated by vacuum-liquid chromatography (VLC) on RP-C_{18} to give five fractions (R_I to R_V) which were purified by successive chromatographic techniques, including flash chromatography, as well as semi-preparative and preparative high performance liquid chromatography (HPLC) to yield eleven known compounds (**9–19**). In the same manner, the 80% methanol extract of the aerial parts afford six undescribed compounds (**1–6**) (Fig.1) and six known ones (**7, 8, 20–23**). Their structures were established by 1D- and 2D-NMR analysis (^{1}H, ^{13}C, COSY, TOCSY, NOESY, HSQC, HMBC) in combination with mass spectrometry ESI- and HR-ESI-MS, and by comparison of their physical and spectral data with those of literature values. The known compounds were identified as sylvestroside II (**7**) (Jensen et al., 1979), sylvestroside I (**8**) (Jensen et al., 1979; Abdallah,1991), triplotoside A (**9**) (Graikou et al.,2002; Gülcemal et al.,2010), sweroside (**10**) (Jensen et al., 1979), loganin (**11**) (Calis et al., 1984a), secoxyloganin (**12**) (Calis and sticher. 1984b), vanilloloside (**13**) (El-Ghazooly et al., 2003), vanillyl-*β*-D-glucopyranoside (**14**) (Kanho et al., 2005), caffeic acid methylester (**15**)(Rani et Devanan.,2013), 3,4,5-trimethoxyphenyl-1-*O*-*β*-apiofuranosyl-(1"→6')-*β*-glucopyranoside **(=** kelampayosideA) (**16**) (Kanchanapoom et al., 2002; Qingwei et al., 2012), (+)-8-hydroxypinoresinol (**17**) (Tsukamoto et al.,1984a;Tsukamoto et al.,1984b; Cowan et al.,2001),

(+)-8-hydroxypinoresinol-4′-*O*-*β*-D-glucopyranoside) (**18**) (Deyama et al., 1986), (+)-8-hydroxypinoresinol-4-*O*-*β*-D-glucopyranoside) (**19**) (Tsukamoto et al., 1984a; Tsukamoto et al., 1985), apigenin (**20**) (Wawer et al., 2001), luteolin (**21**) (Okamura et al., 1994), lutéoline-7-*O*-*β*-D-glucopyranoside (**22)** (Chiruvella et al., 2007), and kaempferol 3-*O*-(3″,6″-di-*O*-*E*-*p*-coumaroyl)- *β*-D-glucopyranoside (**23**) (Yang et al., 2010).

Compound **1** was obtained as a yellow amorphous powder, with a molecular formula of $C_{44}H_{56}O_{22}$ based on HR-ESI-MS (*m/z* 959.3170 $[M+Na]^+$, calcd for 959.3161). The infrared (IR) spectrum indicated the presence of the hydroxyl functions (3356 cm^{-1}), and *α*,*β* unsaturated ester carbonyl (1695 and 1629 cm^{-1}). The ^1H and ^{13}C NMR spectra (Table 1) in combination with the HSQC and ^1H-^1H COSY correlations showed two distinct parts, indicated as units A and B (Fig. 1). The signals corresponding to unit A included one olefinic methine [δ_H7.52, s (H-3a); δ_C153.9 (C-3a)], one vinyl [δ_H5.80, ddd (*J*=17.4, 10.4, 8.6 Hz,H-8a); δ_C135.7 (C-8a), δ_H5.28, dd (*J*=10.4, 1.5 Hz, H-10a), 5.33, d(*J*=17.4 Hz, H-10a); δ_C119.6 (C-10a)], one methylene oxy [δ_H 4.11, 4.14, (H-7a); δ_C64.1 (C-7a)], one methylene [δ_H 1.86, td (*J*=13.8, 7.4 Hz, H-6a), 2.12, dd (*J*=13.8, 7.2 Hz, H-6a); δ_C30.1(C-6a)], two methines [δ_H2.90, q (*J*=6.3 Hz, H-5a); δ_C 31.3 (C-5a), δ_H2.70, dt (*J*=8.3, 6.1 Hz, H-9a); δ_C 45.4 (C-9a)], one acetal [δ_H5.58, d (*J*=6.6 Hz, H-1a); δ_C97.7 (C-1a)], one carboxy group [δ_C168.2 (C-11a)]and one carbomethoxy group[δc 173.0 (C-12a)].

This ^1H and ^{13}C NMR spectroscopic data of **1** showed that unit A contained a secoiridoid moiety, almost identical to secologanic acid (Kocsis et al., 1993), except that compound **1** had a carbomethoxy group at C-7a [δ_C64.1 (C-7a)] instead of the aldehyde group of secologanic acid, which was also detected from the HMBC correlations from H-5a and H-6a to C-7a (Fig. 2).

The remaining resonances in the ^1H and ^{13}C NMR spectra of **1** attributed to unit B, particularly those arising from an olefinic methine [δ_H7.47, s (H-3b); δ_C152.5 (C-3b)], one methylene

[δ_H1.78, ddd (J=14.1, 7.8, 5.1 Hz), 2.33, ddd (J=14.1, 7.7, 1.5 Hz)(H-6b); δ_C40.3 (C-6b)], three methines [δ_H3.15, q(J=7.9 Hz,H-5b); δ_C32.6 (C-5b), δ_H2.18, m (H-8b); δ_C41.0 (C-8b); δ_H2.13,ddd (J=17.4, 8.8, 4.8 Hz,H-9b); δ_C47.1 (C-9b)], one oxygenated methine [δ_H 5.24, t (J=4.5 Hz, H-7b); δ_C78.4 (C-7b)], one acetal [δ_H5.33, d (J=4.6 Hz, H-1b); δ_C97.6 (C-1b)], one secondary methyl [δ_H1.10, d (J=6.8 Hz, H-10b); δ_C13.8 (C-10b)], one methoxy [δ_H3.71, s (MeO-12b); δ_C51.7 (MeO-12b)] and one carboxy group [δ_C169.3 (C-11b)], indicated the second unit to be loganic-type iridoid (Kocsis et al., 1993).The major differences between them is the downfield shifts of H-7b (δ_H5.24) and C-7b (δ_C78.4). The attachment site between unit A and B was found to be ester linkage between the C-7 of unit B and the carboxyl group (C-11a) of unit A as deduced by the HMBC correlation observed between the oxygenated methine proton (H-7b) and the carboxy carbon (C-11a).

Furthermore, two anomeric protons resonances corresponding to *O*-linked sugars were observed in the ^{1}H NMR spectrum of **1** as two doublets at δ_H4.74 (J = 7.9 Hz) and 4.76 (J =7.9 Hz). The sugar units were identified after analysis of COSY and HSQC spectra as two glucoses [glc-A ($\delta_{H-1'}$ 4.74 and $\delta_{C-1'}$100.1) and glc-B ($\delta_{H-1''}$4.76 and $\delta_{C-1''}$100.2)] (Table 1). The β-anomeric configurations were defined by the $^3J_{H1,H2}$ coupling constants and the comparison of ^{13}C-NMR chemical shifts with those in the literature (Agrawal, 1992) (Table 1).The glc-A unit was linked to C-1a as deduced from the long-range correlation observed between H-1′ and C-1a in the HMBC spectrum, and the glc-B unit was linked to C-1b as deduced from the long-range correlation observed between H-1″ and C-1b in the HMBC spectrum(Fig. 2). These ^{1}H and ^{13}C NMR spectroscopic data of **1** are very similar to sylvestroside II (Soeren et al., 1979), except for the signals for an additional trans-*p*-coumaroyl unit in **1** with two coupled *trans* double-bond protons [δ_H6.39, H-8‴ and 7.69, H-7‴, each d, J = 15.9 Hz] and four aromatic protons at δ_H7.49 and 6.83 (each 2H, d, J = 8.6 Hz) assignable to H-2‴/6‴ and H-3‴/5‴ respectively, as well as to an ester carbonyl (δ_C168.5) in the ^{13}C NMR spectrum. The chemical

shifts of C-4″ in the glucopyranosyl moiety (glc-B) were downfield shifted from δ_C71.6 to δ_C72.4 relative to those of sylvestroside II (Soeren et al., 1979). Additionally, the location of the *E*-*p*-coumaroyl unit was established by the key cross-peaks between the H-4″ (δ_H4.87) of glucosyl and carbonyl carbon C-9‴ (δ_C168.5) of the coumaroyl in the HMBC spectrum. The relative configuration of **1** was further determined by the NOESY experiment. The NOE correlations observed between δ_H 2.90 (H-5a) with 2.70 (H-9a), 2.13 (H-9b) with 1.10 (H-10b), 3.15 (H-5b) with 2.13 (H-9b) and 5.24 (H-7b) with 2.18 (H-8b) reveal that they were *β*-oriented, as is usually observed in the iridoid skeleton (Jensen et al., 1979; Kocsis et al., 1993; Tomassini et al., 2000; Dinda et al., 2006; Ji et al., 2012). Based on the above findings, compound **1** was identified as 4″-*O*-*E*-*p*-coumaroyl-sylvestroside II, named semipapposiridoid A shown in Fig. 1 and differs from sylvestroside II, reported *in Dipsacus sylvestris* (Soeren et al., 1979), by an additional *E*-*p*-coumaroyl group at C-4″.

Semipapposiridoid B (**2**) was isolated as a yellow powder. The HR-ESI–MS, *m/z* 959.3169 $[M+Na]^+$ was identical to that obtained for compound **1**, suggesting that both compounds were isomers with molecular formula $C_{44}H_{56}O_{22}$. The IR spectrum indicated the presence of the hydroxyl functions (3352cm^{-1}) and *α*,*β* unsaturated ester carbonyl (1688 and 1630 cm^{-1}), which are responsible for the maximum UV absorption of the compound at 231 nm (Tian et al., 2006). Comparison of ^{1}H and ^{13}C NMR values and the analysis of the ^{1}H-^{1}H-COSY, NOESY, HSQC and HMBC revealed that compound **2** is structurally closely related to **1** and contains the same skeleton (sylvestroside II) (Table 1). The only significant difference is the location of the *E*-*p*-coumaroyl group. The *E*-*p*-coumaroyl group was linked to the C-6″of glc-B unit in **2** instead of the C-4″of glc-B in **1**. The HMBC spectrum gave further evidence of this by showing long-range correlations between the C-6″ methylene protons at δ_H4.45 and 4.54 of glucosyl and the carbonyl carbon C-9‴ at δ_C168.9. The relative configuration of **2** was established as identical

to **1** by the NOESY data of **2**. Hence, the structure of **2** was elucidated as 6″-*O*-*E*-*p*-coumaroyl-sylvestroside II (Fig. 1).

Semipapposiridoid C (**3**) was isolated as a mixture with semipapposiridoid A (**1**) in the ratio 1:1(*Z*:*E*), based on the integral intensity of the corresponding signals in their ^{1}H NMR. Both components of the mixture had the same molecular formula $C_{44}H_{56}O_{22}$ [HR-ESI–MS, *m/z* 959.3169 [M+ Na] $^{+}$]. The ^{1}H and ^{13}C NMR spectra (Table 1) of **3** were extremely close to those of compound **1**. The only significant difference was the value of the vinyl proton coupling constants [δ_H5.83 and 6.94 (each d, *J* = 12.8 Hz)]. HMBC correlations of the ester carbonyl at δ_C167.3 to the *Z*-vinyl protons [δ_H5.83 and 6.94 (each d, *J* = 12.8 Hz)] and Glc-H-4″ [4.86 (t, *J* =9.5 Hz)] indicated that the structure of semipapposiridoid C (**3**) was 4″-*O*-*Z*-*p*-coumaroyl-sylvestroside II (Fig. 1).

The ^{1}H-NMR and ^{13}C-NMR spectra (Table1) of semipapposiridoid D (**4**) were very similar to those of semipapposiridoid B (**2**) and again showed that they were a mixture of sylvestroside II esterified at the C-6″ position and revealed the presence of *Z*- and *E*-*p*-coumaroyl groups. They had the same molecular formula, $C_{44}H_{56}O_{22}$, according to their HR-ESI–MS. The *Z*-*p*-coumaroyl was located at the C-6″ position in **4**, based on HMBC correlations from the ester carbonyl at δ_C 170.8 to the *Z*-vinyl protons [δ_H5.80 and 6.90 (each d, *J* =12.9 Hz)] and Glc-H-6″ [4.37 (dd, *J* =12.0, 6.5 Hz) and 4.50 (dd, *J* =12.0, 2.1 Hz)]. The relative configuration of **4** was established as identical to **2** by the NOESY data of **4**. Therefore, the chemical structure of semipapposiridoid D was determined to be 6″-*O*-*Z*-*p*-coumaroyl-sylvestroside II (Fig. 1).

Semipapposiridoid E (**5**) was obtained as a yellowish amorphous powder. Its molecular formula $C_{26}H_{40}O_{12}$ was obtained from its HR-ESI-MS (*m/z* 567.2413; $[M + Na]^{+}$, calcd 587.3298). The IR spectrum of **5** showed absorption bands of the hydroxy group at 3377cm^{-1} and the carbonyl groups at 1692 and 1629cm^{-1}, respectively. The UV spectrum showed absorption maxima at λ 235 nm. The ^{1}H- and ^{13}C-NMR spectroscopic signals of **5** were almost identical to those of

abelioside B (Murai et a., 1985) except for the presence of signals at δ_H3.42 (3H, s, 3-OCH_3), δ_C56.0 attributed to the methoxy group, instead of the signal for an ester carbonyl group in abelioside B. In the HMBC spectrum, a correlation between the protons at δ_H3.42 and carbons at δ_C102.9 (C-3) were observed, indicating that this methoxy group was located at C-3. The relative configuration of **5** was established by analysis of the NOESY spectrum showing correlations between H-5 and H-9β axial and between H-1*β* axial and CH_3-10 which indicated that H-5, H-9 and CH_3-10 were all *β*-oriented, and between H-7 and H-8 α-oriented. The NOESY correlations observed between H-3 and H-1 *β* axial indicated a *β*-axial orientation of H-3 and an *α*-axial orientation of methoxyl group at the C-3 position. Based on the above evidence, the structure of semipapposiridoid E (**5**) was established as depicted in Fig. 1.

Semipapposiridoid F (**6**) was isolated as a yellowish amorphous powder. It has the same molecular formula as compound **5** ($C_{26}H_{40}O_{12}$) and differs from the latter only by the orientation of the methoxy group at C-3. The IR spectrum displayed absorption bands at 3376, 1693 and 1629 cm^{-1} attributed to the hydroxyl and carbonyl functions. The ^{13}C NMR spectra (see Table 2) of **6** showed great similarities to those of **5**, except for the signals of C-1 δ_C59.3 (Δ−4.9 ppm), C-3 δ_C99.3 (Δ−3.6 ppm) and C-4 δ_C33.1 (Δ−2.9 ppm). The lack of correlation between H-3 and H-1 in the NOESY spectrum indicates the *β*-axial orientation of the methoxyl group at C-3 (Breitmaier and Voelter, 1987). Therefore, the structure of **6** (semipapposiridoid F) was elucidated as shown in Fig. 1.

The total phenolic and flavonoid contents of the 80% MeOH extracts of the roots (R)and aerial parts (A) of *S. semipapposa* and their fractions R_I to R_V (for the roots), A_I to A_V (for the aerial parts), obtained after VLC fractionation on RP-C_{18}, were quantitatively estimated using a calibration curve established in terms of gallic acid (GAE) and quercetin equivalents (QE), respectively (Table 3). Moderate phenolic and flavonoid contents were found in the 80% MeOH extracts with values of 109.0 µg GAE/mg and 12.8 µg QE/mg for the roots and 169.6 µg

GAE/mg and 19.5 µg QE/mg for the aerial parts. The highest contents of total phenolics and flavonoids were found in the fraction R_I with values of 361.7 µg GAE/mg and 74.6 µg QE/mg, while the fraction R_V containing saponins (Bendamene et al., 2020) was the poorest (32.3 µg GAE/mg and 5.7 µg QE/mg). Previous research carried out on organic extracts of the species *S. tschiliensis* (Wang et al. 2013), *S. arenaria* (Hlila et al. 2015) and *S. stelatta* (Mouffouk et al. 2018), indicated lower levels of phenol and flavonoid contents compared to the results of the present study. Here, we identified in the roots (fractions R_I-R_{IV}), three phenyl glucosides (**13-14, 16**), caffeic acid methyl ester (**15**), three lignans (**17-19**) and four iridoids (**9-12**), and in the aerial parts (A_{III} to A_V), eight bis-iridoids (**1–8**) and four flavonoids (**20-23**), thus justifying these results.

Several *in vitro* test procedures were carried out to estimate the antioxidant activity of crude plant extracts, but there is no simple universal method to assess antioxidant capacity accurately and quantitatively. Therefore, in the present study, three methods (DPPH, ABTS and CUPRAC assays), based on different principles, were applied to determine the antioxidant capacity of samples (Table 3). The antioxidant activity of the 80% MeOH extracts and fractions varied in a dose-dependent manner in all tests. The antioxidant activity of *S. semipapposa* samples ranged from 4.9 to 315.8 µg/mL when measured by the DPPH assay, from 8.9 to 124.0 µg/mL when measured by the $ABTS^{\bullet+}$ assay, and from 10.2 to 378.5 µg/mL when measured by the CUPRAC assay. These results are in agreement with those reported by Mouffouk et al. (2018). The total antioxidant capacity values from $ABTS^{\bullet+}$ were higher than those of the $DPPH^{\bullet}$ and CUPRAC assays. This may result from the presence of antioxidants that interact more selectively with fast reacting $ABTS^{\bullet+}$radicals. Many studies reported that the solubility of the tested extract to different test systems and the stereoselectivity of radicals affect the ability of extracts to react against different radicals (Yu et al. 2002). The moderate antioxidant activity of extract and fractions of the aerial parts is correlated with the presence of amounts of iridoids, which in their

molecule have no (or few) phenolic OH groups, which neutralize free radicals. This is consistent with the study of Pacifico et al. (2009) and Wu et al. (2009) who reported that sweroside (**10**) and iridoids did not exhibit good radical scavenging ability.

Among the fractions tested, the fraction R_I showed significant antioxidant activity in all tests. Moreover, the results also indicated that this fraction showed higher activity (FRS_{50} 4.9 µg/mL) (Table 3) than those of the antioxidant standards BHA and BHT (FRS_{50} 8.7, 15.9 µg/mL, respectively) in the DPPH assays. The good antioxidant activity of the fraction R_I could be associated with its high content of phenolic compounds (361.7±1.2 µg GAE/mg). Indeed, phenolic compounds have been the most studied for their antioxidant activity and have been reported to protect against many diseases through their ability to neutralize free radicals. (Jayaprakasha et al., 2001; Gali and Bdjou, 2019). In the fraction R_I, the iridoids sweroside (**10**) and secologanine (**12**) were purified as well as the phenolic glucosides (**13, 14, 16**). Vanilloloside (**13**) was found to be a moderately active scavenger of DPPH FRS_{50} 43.3 µg/mL) and moderate CUPRAC activity (0.397 mM TRg^{-1}) (Sarikahya et al., 2011), while vanillyl β-D-glucopyranoside (**14**), showed DDPH scavenger activity (FRS_{50} 30.88 µg/mL) (Shi et al., 2012).

3. Conclusions

In summary, the systematic phytochemical study on the roots and the aerial parts of *S. semipapposa* afforded six undescribed components, semipapposiridoids A-F (**1**–**6**) (Fig. 1), and three known bis-iridoid glucosides (**7**–**9)**, three iridoids (**10**–**12)**, three phenolic glucosides (**13**–**14, 16)**, one caffeic acid methyl ester (**15**), three lignans (**17**–**19)** and four flavonoids (**20**–**23**) (Fig. S49 see supplementary data). These results expand our knowledge of chemical diversity and provide evidence to further analysis of their potential chemotaxonomic significance.

Compounds **7**–**12, 20**– **23,** have been previously reported from different species of the genus *Scabiosa* (Horn et al.,2001; Papalexandrou et al.,2003; Christopoulou et al.,2008; Wang

etal.,2015; Lehbili et al.,2018a) and might reveal the close chemotaxonomic relationships between *S. semipapposa* and other *Scabiosa* species. It is also interesting to note that lignans (**17**–**19)** are described for the first time in the genus *Scabiosa*. They could serve as potential chemotaxonomic markers to differentiate *Scabiosa* from other genera in the Caprifoliaceae family.

Among the bis-iridoids, compounds (**1**–**4, 7–9)** isolated from *S. semipapposa* were found to possess secoiridoid/iridoid-subtype skeletons consisting of secologanic acid condensed to the 7-OH of loganin or loganin-like iridoids. These results also showed the close relationship between *Scabiosa* species (Papalexandrou et al.,2003; Lehbili et al.,2018a).

In addition, the antioxidant activities of the 80% MeOH extracts and the fractions (R_I to R_V and A_I to A_{IV}) were evaluated by DPPH, ABTS and CUPRAC assays. Our results demonstrate that the fraction R_I has particularly high antioxidant and free radical scavenging activities, probably due to the presence of vanillyl alcohol glucosides.

4. Experimental

4.1. General experimental procedures

UV spectra were measured on a Shimadzu UV/Vis U-2450 spectrophotometer. Optical rotations of pure compounds were measured in CH_3OH using a MCP 5100 AntonPaar Polarimeter. IR spectra were recorded on a Nicolet Avatar 320 FT-IR spectrometer with KBr disks. The 1D and 2D NMR spectra (^1H and ^{13}C NMR, ^1H-^1H COSY, NOESY, HSQC and HMBC) were performed using a Bruker Avance III 600 spectrometer (^1H at 600 MHz and ^{13}C at 150 MHz) equipped with a 5 mm TCI cryoprobe. 2D-NMR experiments were performed using standard Bruker microprograms (TopSpin 3.5 software). ESI-MS and HR-ESI-MS experiments were performed using a Micromass Q-TOF micro instrument (Manchester, UK). Flash chromatography (FC) was carried out on a Grace Reveleris system equipped with dual UV and ELSD detection using Grace® cartridges (Silica gel or RP-C_{18}). The medium-pressure

liquid chromatography (MPLC) was employed using a Buchi pump system AP250/500 (Buchi, France), with a RP-C_{18} silica gel MERCK column (15 × 230 and 26 × 460 mm). Semi-preparative HPLC was realized on a Dionex apparatus equipped with an ASI-100 automated sample injector, a STH 585 column oven, a P580 pump, a diode array detector UVD 340S and the Chromeleon® software version 6.8. A prepacked RP-C_{18} column (Phenomenex 250 x 10 mm, Luna 5 µ) was used for semi-preparative HPLC. The eluting mobile phase consisted of H_2O with TFA (0.0025%) and CH_3CN with a flow rate of 5 mL/min and the chromatogram was monitored at 205 and 215 nm. Analytical HPLC experiments were performed using a Thermofisher Ultimate 3000 (Thermo Fischer Scientific, Villebon sur Yvette, France), equipped with a 4 ways pump LPG 3400 SD, an automatic injector WPS 3000 SL, a UV/visible diode array detector 3000 and the Chromeleon® software version 6.8. A prepacked C_{18} column Uptisphere Strategy C_{18} (Interchim, 4.6×250 mm, 5µ) was used for analytical HPLC and the mobile phase consisted of H_2O with TFA (0.0025% v/v) and CH_3CN. A gradient elution method was applied from 5% to 80% of CH_3CN in 30 min with a flow rate of 1 mL/min and the chromatograms were monitored at 205, 254, 300 and 360 nm.TLC was performed on pre-coated silicagel 60 F_{254} Merck and compounds were visualized by spraying the dried plates with 50% H_2SO_4, followed by heating.

The antioxidant activity assays were carried out at the Center of Biotechnology Research (Contantine, Algeria) on a 96-well microplate reader, Perkin Elmer Multimode Plate Reader EnSpire (Perkin Elmer, France). Butylated hydroxylanisole (BHA), butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$), 2,2′-azinobis (3-ethyl-benzothiazoline-6-suphonic acid) diammonium salt ($ABTS^{\bullet+}$) and Ciocalteureagent (FCR) were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals and solvents were of analytical grade.

4.2. Plant material

The whole plant *Scabiosa semipapposa* Salzm. ex DC. was collected in May 2017 from Alguemas, Constantine, North-Eastern Algerian (latitude 36.3479 and longitude 6.650773).The plant material was identified by Mr. Kamel Kabouche. A voucher specimen (LOST Ss.05/17) has been deposited at the herbarium of LOST Laboratory, University Frères Mentouri-Constantine, Algeria.

4.3. Extraction and isolation

The aerial parts and the roots of *S. semipapposa* were studied apart from each other. The dried and powdered roots of *S. semipapposa* (1 kg) were macerated in 80% MeOH (3 ×10 L, 24 h) at room temperature, followed by rotary evaporation at 45 °C under reduced pressure to give the crude extract (100 g). A part of this (55 g) was subjected to RP-C_{18}vacuum liquid chromatography (VLC) eluted successively with MeOH-H_2O (3:7, 4:6, 6:4, 8:2 and 10:0), to provide five major fractions (R_I to R_V, respectively). Fraction R_I (1.3 g) was subjected to High Performance Flash Chromatography (HPFC), over silica gel, eluted by a gradient system of CH_2Cl_2-MeOH-H_2O (10:0:0 to 7:3:0.5), in 39 min to afford 69 sub-fractions (R_{I-1} to R_{I-69}). Sub-fractions R_{I-29} to R_{I-34}were combined (27.5 mg) and purified by semi-preparative HPLC using a gradient (10-30% CH_3CN, in 20 min) to furnish compounds **14** (1.4 mg, t_R 5.9 min), **10** (3.5 mg, t_R 11.1 min). Then the combined sub-fractions $R_{I-36-37}$ (23 mg) were purified by semi-preparative HPLC (10-30% CH_3CN, in 20 min) to afford compounds **13** (1 mg, t_R 4.5 min), **16** (1.9 mg, t_R 13.7 min) and **12** (3.4 mg, t_R 18.4 min). Fraction R_{II} (1.3 g) was fractionated by silica gel CC, using a gradient of EtOAc–MeOH (1:0–6:4) to give 230 sub-fractions. Sub-fractions $R_{II-50-55}$ contained the pure compound **11** (31.3 mg). Sub-fractions $R_{II-59-63}$ (38.8 mg) was purified by semi prep HPLC using an isocratic elution of 15% CH_3CN during 25 min to give compounds **10** (14.3 mg, t_R 9.9 min) and **18** (6.4 mg, t_R 19.4 min) whereas the purification of the $R_{II-70-80}$ (58.2 mg) in the same condition gave **19** (2.9 mg, t_R 22.1 min). Fraction R_{III} (1.2 g)

was applied to HPFC on normal phase silica gel, eluted with the system CH_2Cl_2-MeOH-H_2O [10:0:0 to 60:40:7], in 48 min to afford 60 sub-fractions. The purification of the sub-fractions $R_{III\text{-}10\text{-}14}$ (24.8 mg) by semi prep HPLC in isocratic elution with 25% CH_3CN during 20 min, gave compounds **17** (4.7 mg, t_R 11.1 min), and **15** (6.9 mg, t_R 8.1 min). Fraction R_{IV} (198 mg) was purified by silica gel CC, eluted with the gradient of the mixture of CH_2Cl_2-MeOH-H_2O [95:5:0-70:30:5], to afford compound **9** (11.3 mg) as a white precipitate in pure form. Fraction R_V was purified previously (Bendamene et al., 2020).

The same protocol was used for the extraction of the aerial parts of *S. semipapposa* (700 mg). A part of the resulting 80% MeOH extract (50 g) was submitted to VLC over RP-C_{18} eluted successively with MeOH-H_2O (3:7, 4:6, 6:4, 8:2 and 10:0), to give 5 main fractions (A_I to A_V, respectively) and a pure compound **22** (3mg) obtained as a precipitate in fraction A_{III}. This latter (4 g) was fractionated by flash chromatography over silica gel, using a binary gradient of CH_2Cl_2–MeOH [10:0 to 6:4] in 55 min to afford 146 sub-fractions. Sub-fractions $A_{III\text{-}51\text{-}52}$ (51 mg), $A_{III\text{-}57\text{-}60}$ (50 mg) and $A_{III\text{-}72}$ (33.4 mg), were purified each one by MPLC on RP-C_{18} eluted by gradient system 10%–50% CH_3CN, in 45 min to yield compounds **5** (4.4 mg) and **6** (2.6 mg), from $A_{III\text{-}51\text{-}52}$, **10** (8.5 mg), and a mixture of **3** and **1** (1.7 mg) from $A_{III\text{-}57\text{-}60}$, and **8** (15.2 mg) from $A_{III\text{-}72}$. Fraction A_{IV} (1.9g) was purified by flash chromatography over silica gel, eluted by a gradient system of CH_2Cl_2-MeOH [10:0 to 6:4] in 45 min to afford 100 sub-fractions. Compounds **21**(1mg), **1** (23.2 mg) and **7** (24.4 mg) were obtained as pure compounds in fractions $A_{IV\text{-}25}$, $A_{IV\text{-}45}$ and $A_{IV\text{-}66\text{-}67}$, respectively, while the sub-fraction $A_{IV\text{-}22}$ (31.5 mg) was purified by MPLC (20-55% CH_3CN, in 18 min) to yield 3.4 mg of compound **20**. Sub-fractions $A_{IV\text{-}32}$ to $A_{IV\text{-}35}$ (30 mg) were pooled and purified by MPLC (30-60% CH_3CN, in 20 min) to afford **23** (6 mg). The remaining sub-fraction $A_{IV\text{-}53\text{-}54}$ (28.5 mg) was chromatographed by MPLC (20-50% CH_3CN, in 20 min) to furnish the pure compound **2** (8.5 mg), and the mixture of compounds **3** and **1** (1.9 mg), and **4** and **2** (1 mg).

*4.3.1. Semipapposiridoid A (**1**)*

Yellowish solid; $[\alpha]_D^{25}$-97.7(*c*1, MeOH); IR (KBr) ν_{max} (cm^{-1}):3356, 2924, 1695, 1629, 1603, 1514, 1440, 1371, 1260; UVλ_{max} (MeOH) (log ε): 231 (0.177), 313 (0.193) nm; ^{1}H (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) data: see Table1. HR-ESI-MS *m/z*: 959.3170$[M+Na]^+$ (calcd for $C_{44}H_{56}O_{22}$, 959.3161).

*4.3.2. Semipapposiridoid B (**2**)*

Yellowish solid; $[\alpha]_D^{25}$-40(*c* 0.16, MeOH); IR (KBr) ν_{max} (cm^{-1}):3352, 2925, 1688, 1629, 1605, 1515, 1440, 1380, 1282; UVλ_{max} (MeOH) (log ε): 228 (0.845), 308 (0.250) nm; ^{1}H (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) data: see Table1. HR-ESI-MS *m/z:* 959.3169 $[M+Na]^+$ (calcd for $C_{44}H_{56}O_{22}$, 959.3161).

*4.3.3. Semipapposiridoid C (**3**)*

Yellowish solid; $[\alpha]_D^{25}$-66(*c* 0.17, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3357, 2924, 1695, 1630, 1067; UVλ_{max} (MeOH) (log ε): 231 (1.266), 312 (0.630) nm; ^{1}H (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) data: see Table 1. HR-ESI-MS *m/z:* 959.3156 $[M+Na]^+$ (calcd for $C_{44}H_{56}O_{22}$,959.3161).

*4.3.4. Semipapposiridoid D(**4**)*

Yellowish solid; $[\alpha]_D^{25}$13.3(*c*0.18, MeOH); IR(KBr) ν_{max}(cm^{-1}):3358, 2920, 1695, 1630,1068; UVλ_{max} (MeOH) (log ε): 231 (0.724), 312 (0.304) nm; ^{1}H (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) data: see Table1. HR-ESI-MS *m/z*: 959.3169 $[M+Na]^+$ (calcd for $C_{44}H_{56}O_{22}$, 959.3161).

*4.3.5. Semipapposiridoid E(**5**)*

Yellowish solid: $[\alpha]_D^{25}$-58 (*c*0.44, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3377, 2926, 1692, 1629, 1449, 1386, 1277; UV λ_{max} (MeOH) (log ε): 235 (0.580) nm; ^{1}H (600 MHz, CD_3OD) and ^{13}C NMR

(150 MHz, CD_3OD) data: see Table 2. HR-ESI-MS *m/z*: 567.2413 $[M+Na]^+$ (calcd for $C_{26}H_{40}O_{12}$,567.2417).

*4.3.6. Semipapposiridoid F (**6**)*

Yellowish solid; $[\alpha]_D^{25}$-14 (*c*0.26, MeOH);IR (KBr) ν_{max} (cm^{-1}):3376, 2926, 1693, 1629, 1451, 1387, 1262; UV λ_{max} (MeOH) (log ε): 235 (1.761) nm;^{1}H (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) data: see Table 2. HR-ESI-MS m/z: 567.2408 $[M+Na]^+$ (calcd for $C_{26}H_{40}O_{12}$, 567.2417).

4.4. Determination of total phenolic (TPC) and flavonoid contents (TFC)

Total phenolic content (TPC) of 80% MeOH extracts of aerial parts, roots, and fractions (R_{I-V} and A_{I-IV}) of *S. semipapposa* (Table 3) was determined spectrophotometrically using Folin-Ciocalteu method as previously reported (Singleton and Rossi, 1965) with slight modifications (Muller et al., 2010). The absorbance was measured at 765 nm and the results were expressed as micrograms of gallic acid equivalent per milligram of extract (µg GAE/mg).

The total flavonoid content (TPC) was analyzed using an adapted aluminum chloride colorimetric reported by Topçu et al. (2007). The absorbance was measured at 415 nm and the results were expressed as mg of quercetin equivalents per milligram of extract (µg QE/mg).

4.5. Antioxidant activities

Evaluation of the antioxidant activities of 80% MeOH extracts of aerial parts, roots, and fractions (R_I-R_V and A_I-A_{IV}) was carried out by three different methods including scavenging of the free radicals of 2,2-diphenyl-1-pycrylhydrasyl (DPPH) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) and cupric reducing antioxidant capacity (CUPRAC) assays.

4.5.1. DPPH free radical scavenging activity

The free radical scavenging activity of the 80% MeOH extracts and the fractions of *S. semipapposa* was determined by the DPPH assay described by Blois (1958) with few

modifications as previously described (Boutaghane et al., 2018). Briefly, 40 µL of either standard or sample solutions was mixed with 160 µL of DPPH solution in microplate. The mixture was then incubated at room temperature in the dark for 30 min at 37°C, and the absorbance was measured at λ 517 nm and expressed as percentage of DPPH radical scavenging calculated as follows: DPPH scavenging% = $[(Ab_{control} - Ab_{sample})/Ab_{control}] \times 100$.

The sample concentration providing 50% free radical scavenging activity (FRS_{50}) was calculated from the graph of DPPH scavenging effect percentage against sample concentration. BHA and BHT were used as positive controls. All the tests were conducted in triplicate. The experimental data were expressed as mean ± standard deviation.

4.5.2. ABTS radical scavenging activity

The ABTS scavenging activity of the 80% MeOH extracts and the obtained fractions was performed spectrophotometrically according to a literature method (Re et al. 1999) with a little modification. The $ABTS^{\bullet+}$ was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 h. Before usage, the $ABTS^{\bullet+}$ solution was diluted to get an absorbance of 0.70 ± 0.02 at 734 nm with methanol. Then 40 µL of either standard or sample solutions was added to 160 µL of $ABTS^{\bullet+}$ solution in microplate). After 10 min, the percentage inhibition at λ 734 nm was calculated for each concentration relative to a blank absorbance (methanol). The scavenging capability of $ABTS^{\bullet+}$ was calculated using the following equation: $ABTS^{\bullet+}scavenging\% = [(Ab_{control} - Ab_{sample})/Ab_{control}] \times 100$.

The sample concentration providing 50% cation radical scavenging activity (CRS_{50}) was calculated from the graph of $ABTS^{\bullet+}$ scavenging effect percentage against sample concentration. BHA and BHT were used as positive controls. All the tests were conducted in triplicate. The experimental data were expressed as mean ± standard deviation.

4.5.3. Power cupric ion reducing (CUPRAC)

80% MeOH extracts and fractions were tested for their power cupric ion reducing (CUPRAC) capacity assay using the method of Apak et al. (2004) with some modifications. Sixty microliters of ammonium acetate buffer solution (1 M, pH = 7.0) were added to 40 μL of a solution of copper (II) chloride (10 mM) and 50 μL of the neocuprine solution (7.5 mM). Different concentrations of extracts, fractions and standards were added to the initial mixture to make a final volume of 200 μL. After 1 h, the absorbance at 450 nm was recorded against a reagent blank by using a 96-well microplate reader. The power cupric ion reducing was calculated as follows: $[1 – A_0/ (A_1 – A_2)] \times 100$, where A_0 is absorbance of the control (without sample), A_1 is absorbance in the presence of the sample and A_2 is absorbance of the blank. All the tests were conducted in triplicate and the reduction capacity of the extracts was compared with BHA and BHT standards.

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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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version.

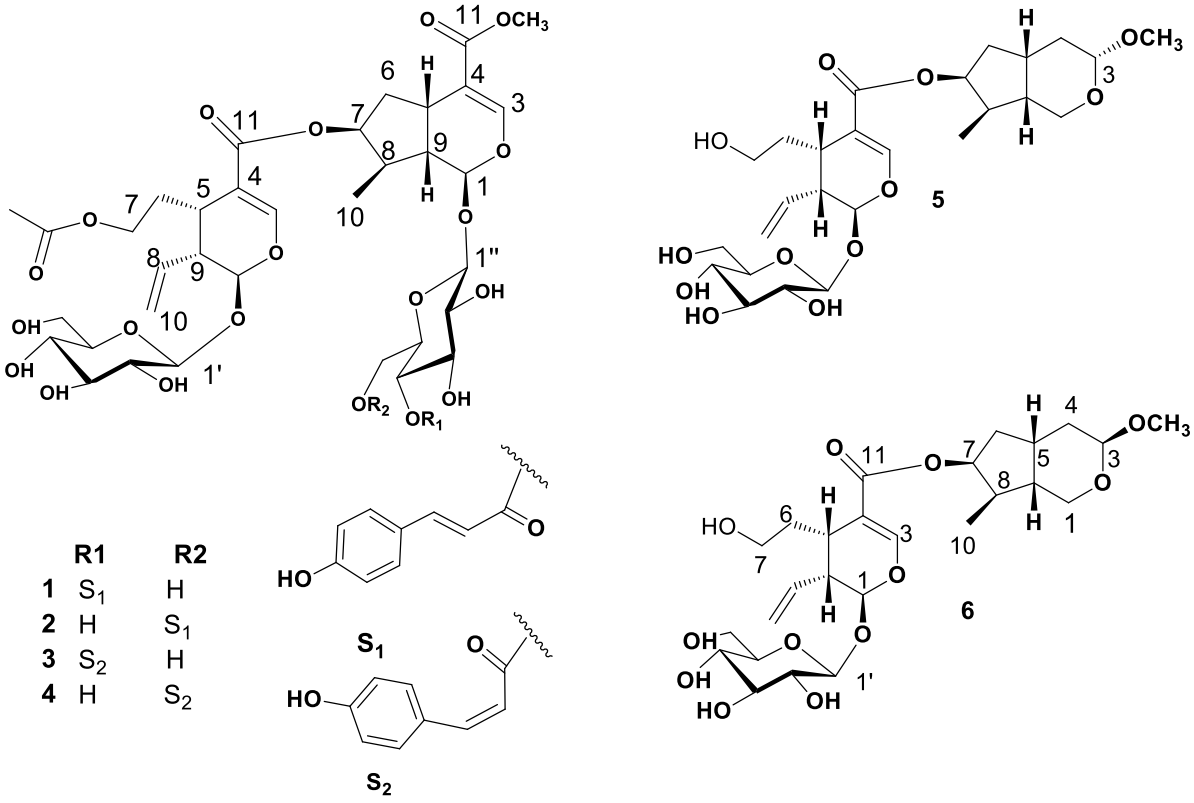

Fig. 1. Structures of undescribed bis-iridoids **1**–**6** isolated from *S. semipapposa*

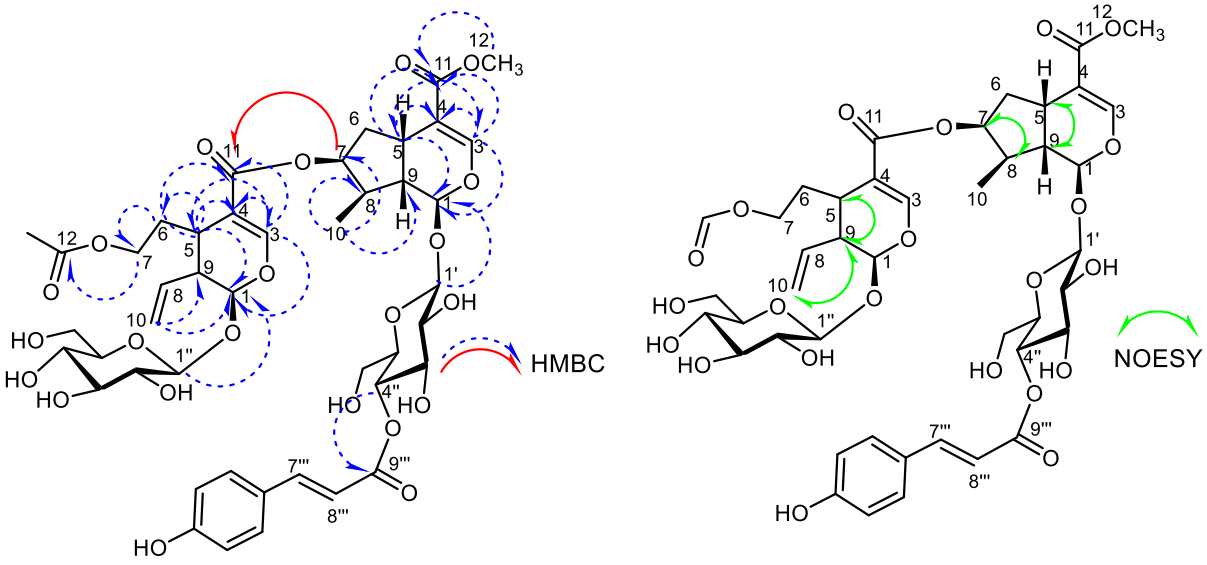

Fig. 2. Selected HMBC and NOESY correlations of compound **1**

Table 1 ^{13}C NMR and ^{1}H NMR spectroscopic data of compounds **1-4** in CD_3OD.[a]

Position	1		2		3		4	
	$\delta_C\delta_H$		$\delta_C\delta_H$		δ_C	δ_H	δ_C	δ_H
Unit A								
1a	97.7	5.58, d (6.6)	97.7	5.56, d (6.5)	97.7	5.59, d (6.5)	97.7	5.56, d (6.6)
3a	153.9	7.52, s	153.7	7.49, d (1.3)	153.7	7.52, (s l)	153.7	7.49, d (2.8)
4a	111.6	-	111.6	-	111.6	-	111.6	-
5a	31.3	2.90, q (6.3)	31.2	2.87, q (6.4)	31.3	2.90, q (6.3)	31.2	2.87, q (6.3)
6a	30.1	1.86, td (13.8, 7.4)	30.1	1.81,dq (13.8, 7.2)	30.1	1.85, dq (13.7, 7.1)	30.2	1.82, dq (13.8, 6.7)
		2.12, dd (13.8,7.2)		2.02, m		2.04,m		2.01, m
7a	64.1	4.11, m	64.1	4.08, m	64.1	4.11, m	64.1	4.08, m
		4.14, m		4.12, m		4.14, m		4.12, m
8a	135.7	5.80, ddd (17.4, 10.4, 8.6)	135.7	5.78, ddd (14.4-10.4-8.9)	135.7	5.80, q (8.5)	135.7	5.78, dd (9.8, 8.8)
9a	45.4	2.70, dt (8.3, 6.1)	45.3	2.68, dd (8.2-6.1)	45.3	2.69, dt (8.2, 5.8)	45.3	2.67, dd (17.2, 10.4, 6.3)
10a	119.6	5.28, dd (10.4-1.5)	119.6	5.26, d (10.4)	119.6	5.28, d (10.7)	119.6	5.25, d (10.4)
		5.33, d (17.4)		5.31, d (14.4)		5.33, dd (14.7, 1.4)		5.32, d (17.2)
11a**C=O**	168.2	-	168.1	-	168.3	-	168.2	-
12a**C=O**	173.0	-	173.0	-	173.0	-	173.0	-
13a**CH_3**	20.9	2.04, s	21.0	2.02, s	20.9	2.04, s	21.0	2.18, s
1a-*O*-glc					1A-*O*-glc		1A-*O*-glc	
1′	100.1	4.74, d (7.9)	100.6	4.68, d (8.0)	100.1	4.74, d (7.9)	100.1	4.71, d (8.0)
2′	74.7	3.23, dd (9.0, 7.9)	74.6	3.22, dd (9.0, 8.0)	74.8	3.22, t (8.7)	74.7	3.22, dd (8.8, 8.0)
3′	78.0	3.40, t (9.0)	78.0	3.39, t (9.0)	78.0	3.39, t (9.1)	78.0	3.39, t (8.8)
4′	71.6	3.30, t (9.2)	71.5	3.29, t (9.5)	71.6	3.29, t (9.2)	71.6	3.29, t (9.6)
5′	78.4	3.33, m	78.4	3.34, m	78.5	3.33, m	78.4	3.35, m
6′	62.8	3.69, dd (11.9, 6.0)	62.8	3.68, dd (11.9, 6.2)	62.8	3.68, dd (11.9, 4.6)	62.1	3.68, dd (12.0, 6.1)
		3.93, dd (11.9, 2.0)		3.92, dd (11.9, 1.9)		3.93, dd (11.9, 1.8)		3.92, d (12.0, 1.3)
Unit B								
1b	97.6	5.33, d (4.6)	97.3	5.08, d (5.7)	97.6	5.34, d (4.8)	98.0	5.09, d (5.5)
3b	152.5	7.47,d (1.3)	152.8	7.44, d (1.1)	152.5	7.45, d (1.2)	152.6	7.44, d (1.3)
4b	113.3	-	112.9	-	113.3	-	112.9	-
5b	32.6	3.15 q (7.9)	33.1	3.09, q (8.4)	32.6	3.15, q (7.9)	32.7	3.10, q (8.0)
6b	40.3	1.78, ddd (14.1, 7.8, 5.1)	40.6	1.61, ddd (14.4, 8.9 ,4.8)	40.3	1.78, ddd (14.5, 7.7, 5.0)	40.5	1.61, ddd, (11.3, 9.0, 5.0)
		2.33, ddd (14.1, 7.7, 1.5)		2.26, dd (14.4, 7.7)		2.33, td (14.5, 7.7)		2.27, m
7b	78.4	5.24, t (4.5)	78.2	5.22, t (4.7)	78.4	5.24, t (4.5)	78.4	5.23, t (4.6)
8b	41.0	2.18, m	41.2	2.12, m	41.1	2.18, m	41.1	2.09, m
9b	47.1	2.13, ddd (17.4, 8.8, 4.8)	46.9	2.02, m	47.1	2.12, ddd (13.8, 8.9, 5.0)	47.1	2.02, m
10b	13.8	1.10, d (6.8)	14.1	1.02, d (7.1)	13.8	1.10, d (6.7)	13.9	1.02, d (7.1)
11b-**C=O**	169.3	-	169.2	-	169.3	-	169.3	-
12b-**OCH_3**	51.7	3.71, s			51.8	3.72, s		
1b-*O*-glc					1B-*O*-glc			
1″	100.2	4.76, d (7.9)	100.1	4.69, d (7.9)	100.2	4.77, d (7.9)	100.6	4.66, d (7.8)
2″	74.8	3.34, dd (9.3, 7.9)	74.6	3.25, dd (9.0, 7.9)	74.8	3.33, m	74.7	3.23, dd (9.5, 7.8)
3″	75.7	3.68, t (9.2)	77.8	3.42, t (9.0)	75.7	3.68, t (9.1)	77.8	3.40, t (8.9)
4″	72.4	**4.87**, t (9.5)	71.7	3.39, t 9.0)	72.0	**4.86**, t (9.5)	71.7	3.30, t (9.0)
5″	76.5	3.60, m	75.7	3.59, m	76.5	3.59, m	75.6	3.55, m
6″	62.5	3.60, m	64.0	**4.45**, dd (11.9, 6.4)	62.5	3.57, m	64.1	**4.37**, dd (12.0, 6.5)
		3.66, m		**4.54**, dd (11.9, 2.1)		3.66, m		**4.50**, dd (12.0, 2.1)
4″- *O-E-P*-coumaroyl			**6″-*O-E-P*-coumaroyl**		**4″-*O-Z-P*- coumaroyl**		**6″-*O-Z-P*-coumaroyl**	
1‴	127.1	-	127.0	-	127.5	-	127.5	-
2‴	131.3	7.49, d (8.6)	131.3	7.48, d (8.5)	134.0	7.72, d (8.6)	133.9	7.68, d (8.7)
3‴	116.9	6.83, d (8.6)	116.9	6.82, d (8.5)	115.8	6.77, d (8.6)	115.9	6.78, d (8.7)
4‴	161.4	-	161.4	-	160.3	-	160.5	-
5‴	116.9	6.83, d (8.6)	116.9	6.82, d (8.5)	115.8	6.77, d (8.6)	115.9	6.78, d (8.7)
6‴	131.3	7.49, d (8.6)	131.3	7.48, d (8.5)	134.0	7.72, d (8.6)	133.9	7.68, d (8.7)
7‴	147.3	7.69, d (15.9)	146.9	7.67, d (15.9)	146.2	6.94, d (12.8)	145.5	6.90, d (12.9)
8‴	114.7	6.39, d (15.9)	114.9	6.37, d (15.9)	115.9	5.83, d (12.8)	116.2	5.80, d (12.9)
9‴	168.5	-	168.9	-	167.3	-	170.8	-

[a] in ppm, *J* in parentheses in Hz.

Table 2 ^{13}C NMR and ^{1}H NMR spectroscopic data of compounds **5-6** in CD_3OD.[a]

Position	5		6	
	$\delta_C\delta_H$		$\delta_C\delta_H$	
Unit A				
1a	97.7	5.57, d (6.5)	97.7	5.56, d (6.5)
3a	153.4	7.47 s	153.4	7.48, s
4a	112.0	-	112.0	-
5a	31.0	2.84, q (6.6)	31.0	2.84, q (6.4)
6a	33.1	1.73, ddd (13.7-7.7-6.2)	33.1	1.72, m
		1.88 m		1.86, m
7a	61.2	3.55, m	61.2	3.55, m
		3.60, m		3.60, m
8a	135.9	5.80, ddd (17.4-10.4-8.7)	135.9	5.80, ddd (17.3, 10.7 ,8.7)
9a	45.4	2.65, dt (8.2, 6.1)	45.4	2.65, dt (8.1-6.0)
10a	119.3	5.26, dd (10.4-1.2)	119.3	5.25, d (10.7-1.3)
		5.31, dd (17.4-1.2)		5.30, d (17.3)
11-C=O	168.7	-	168.7	-
1A-*O*-glc				
1′	100.1	4.71, d (7.9)	100.1	4.71, d (7.9)
2′	74.6	3.21, dd (9.1-7.9)	74.6	3.20, dd (9.0-7.9)
3′	78.0	3.38, t (8.8)	78.0	3.38, t (8.8)
4′	71.5	3.28, dd (9.7-8.7)	71.6	3.28, dd (9.7-8.7)
5′	78.4	3.32 m	78.4	3.31 m
6’	62.8	3.68, dd (11.9-6.1)	62.8	3.68, dd (11.9-6.0)
		3.92, dd (11.9-2.0)		3.92, dd (11.9-2.0)
Unit B				
1b	64.2	3.79, dd (12.1, 4.8)	59.3	3.52, dd (11.9-3.7)
		3.83, dd (12.1, 5.0)		3.99, dd (11.9-1.4)
3b	102.9	4.42, dd (8.9, 3.3)	99.3	4.67, t (3.5)
4b	35.4	1.28, ddd (13.3-9.7-8.9)	33.1	1.55, ddd (14.1 ,10.0, 3.9)
		1.84, m		1.68, ddd (14.2-6.2-3.2)
5b	34.4	2.34, m	31.1	2.51, m
6b	39.8	1.84, m	39.8	1.74, ddd (13.6-7.6-6.0)
		1.97, ddd (14.6-8.1-2.5)		1.86, m
7b	79.1	5.29, m	78.7	5.32, m
8b	39.3	2.13, dq (11.5, 6.3)	39.0	2.28, dq (11.1, 6.6)
9b	44.8	1.83, m	44.6	1.98, m
10b	12.8	0.88, d (6.8)	12.9	1.00, d (6.9)
11b-OCH_3	56.0	3.42, s	55.0	3.37, s

[a] in ppm, *J* in parentheses in Hz.

Table 3. Phenolic, flavonoid contents and antioxidant activity by the DPPH$^{\bullet}$, ABTS$^{\bullet+}$, and CUPRAC assays of extracts and fractions from *Scabiosa semipapposa*. FRS_{50}, CRS_{50} and $A_{0.50}$ values represent the means ± S.D of three parallel measurements

Extracts	TPC (µg GAE/mg)	TFC (µg QE/mg)	DPPH FRS_{50} (µg/mL)	ABTS CRS_{50} (µg/mL)	CUPRAC $A_{0.50}$ (µg/mL)
Roots 80% MeOH extract (R)	109.0±0.5	12.8±1.7	80.0±0.6	49.5 ±1.2	122.2±1.0
Fraction R_I	361.7±1.2	74.6±1.4	4.9 ±0.1	8.9±0.3	10.2±0.1
Fraction R_{II}	266.8±0.6	37.4±0.1	34.3±0.6	12.7±0.4	29.1±0.4
Fraction R_{III}	150.8±1.4	10.5 ±0.5	64.6 ±0.1	23.3 ±0.9	54.8±0.8
Fraction R_{IV}	158.9±1.6	43.0±0.6	62.1±0.8	36.2±0.6	44.1±1.9
Fraction R_V	32.3 ±0.3	5.7±0.4	315.8±0.7	124.0±0.9	378.5 ±1.4
Aerial parts 80% MeOH extract (A)	169.6±0.2	19.5±0.0	80.0±0.5	31.1 ±0.7	92.2 ± 1.7
Fraction A_I	190.8±0.9	20.9±1.8	45.1 ± 0.8	27.3±0.7	32.9 ± 1.1
Fraction A_{II}	243.9±0.8	44.2 ±1.6	46.0 ± 1.8	18.8 ±0.6	35.5 ± 0.5
Fraction A_{III}	146.7 ±0.8	57.2 ±1.4	42.1 ± 0.1	23.5 ±1.3	37.1 ±0.8
Fraction A_{IV}	183.9 ± 0.9	62.2 ±2.7	46.9 ± 0.6	41.7 ± 0.9	40.9 ± 0.2
BHT[a]			15.9 ± 0.1	2.7±0.2	5.4±0.3
BHA[a]			8.7 ±0.1	1.8 ± 0.1	3.1 ±0.1

[a]Used as a positive control

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