Antioxidant activity-guided isolation of flavonoids from Silene gallica aerial parts

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Sara Bechkri[a,b], Abdulmagid Alabdul Magid[b], Assia Khalfallah[a], Laurence Voutquenne-Nazabadioko[b], Ahmed Kabouche[a], Charlotte Sayagh[b], Dominique Harakat[b], Zahia Kabouche[a*]

[a]Université des frères Mentouri-Constantine 1, Département de chimie, Laboratoire d'Obtention de Substances Thérapeutiques (LOST), Campus Chaabet-Ersas, 25000 Constantine, Algeria

[b]Université de Reims Champagne Ardenne, CNRS, ICMR UMR 7312, 51097 Reims, France

*** Corresponding author.**

E-mail address: zahiakabouche@umc.edu.dz

Abstract

A bio-guided fractionation of the 80% aqueous ethanolic extract of the aerial parts of *Silene gallica* L. (Caryophyllaceae), growing in North-Eastern Algeria, was performed to evaluate its antioxidant activity using DPPH, hydroxyl radical scavenging and CUPRAC assays. Successive chromatographic separations of the most antioxidant *n*-BuOH soluble fraction yielded four acylated flavone *C*-glycosides, vitexin 2"-*O*-*β*-D-(4"',6"'-di-acetyl)-glucopyranoside (**1**), orientin-2"-*O*-*β*-D-(4"',6"'-di-acetyl)-glucopyranoside (**2**), orientin-2"-*O*-*β*-D-(6"'-feruloyl)-glucopyranoside (**3**), and orientin-2"-*O*-*β*-D-(6"'-sinapoyl)-glucopyranoside (**4**), as well as six known compounds including four flavonoids (**5**-**8**), a phenylpropanoid glycerolglucoside (regaloside A) (**9**), and a phytoecdysteroide (20-hydroxyecdysone) (**10**). Their structures were established by UV, 1D, 2D NMR, and HR-ESI-MS spectral data, in addition to comparison with literature data. The antioxidant activity of the crude extracts, fractions and compounds **1**-**8** was evaluated. Two acylated orientin glycosides (**3** and **4**) displayed the strongest antioxidant activity.

Keywords

Silene gallica L.; flavonoid; antioxidant; DPPH; HORAC, CUPRAC.

1. Introduction

The *Silene* genus (Caryophyllaceae) is one of the largest genera of the world's flora (Greuter, 1995). *Silene gallica* L., native to central Europe, is an annual growing to 40 cm in height (Asai and Fujimoto 2010). Previous investigations of *S. gallica* led to the isolation of eleven triterpene saponins (silenegallisaponin A-K) (Bechkri et al., 2020) from the aerial parts and ten cyclic fatty acyl glycosides (gallicasides A-J) from the glandular trichome exudate (Asai and Fujimoto 2010). In continuation of our investigation on Caryophyllaceae species (Voutquenne-Nazabadioko et al., 2013; Gevrenova et al., 2019; Bechkri et al., 2020), we studied the phenolic constituent of this species, growing in North-Eastern Algeria. This paper focuses on the bio-guided isolation and structure determination of ten compounds (**1-10**) including four undescribed acylated flavone *C*-glycosides (**1-4**) and six known compounds (**5-10**) from the 80 % aqueous ethanolic extract of the aerial parts of this plant. Their structures were elucidated by spectroscopic methods including 1D- and 2D-NMR experiments (^{1}H, ^{13}C, HSQC, HMBC, COSY and NOESY) in combination with HR-ESI-MS. The antioxidant capacity of extracts from different solvents (80% EtOH, petroleum ether, $CHCl_3$, EtOAc and *n*-BuOH), fractions and isolated compounds was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radicals scavenging (HORAC) and by cupric ion reducing antioxidant capacity assays (CUPRAC).

2. Results and discussion

The 80% aqueous ethanol extract of the aerial parts (SGE) of *S. gallica* was partitioned successively between H_2O and petroleum ether, chloroforme, ethyl acetate and *n*-butanol. The free radical scavenging activities of SGE was tested and the resulting extracts were determined by the DPPH, and HORAC assays and the cupric reducing capacity was assessed by the CUPRAC assay. The most active fraction (Sg-*n*-BuOH) was subjected to vacuum liquid chromatography (VLC) on silica gel, eluting with CH_2Cl_2-MeOH-H_2O (95:5:0 to 0:1:0) to obtain 8 fractions (B1-B8). These fractions were evaluated for their antioxidant activity and the fraction B4 showed the highest

antioxidant potential in DPPH (EC_{50} 29.3 ± 1.2 µg/mL), HORAC (EC_{50} 126.3 ± 5.1 µg/mL) and CUPRAC (EC_{50} 170.0 ± 8.2 µg/mL) assays (Table 1). In order to determine which compounds were responsible for these activities, fraction B4 was purified using preparative and semi-preparative HPLC to yield four acylated flavone *C*-glycosides (**1**-**4**) along with six known compounds (Fig. 1). The known compounds were identified as vitexin 2''-*O*-*β*-D-glucopyranoside (**5**) (Pauli and Junior, 1995), orientin 2''-*O*-*β*-D-glucopyranoside (**6**) (Gluchoff-Fiasson et al., 1989), vitexin 2''-*O*-*β*-D-(6'''-acetyl)-glucopyranoside (**7**) (Faiella et al., 2012), orientin 2''-*O*-*β*-D-xylopyranoside (**8**) (Gluchoff-Fiasson et al., 1989), 20-hydroxyecdysone (**9**) (Girault et al., 1988), and regaloside A (**10**) (Shimomura et al., 1988), by comparison of physical data with literature values and from spectroscopic evidence.

2.1. Structure elucidation of compounds 1-4

Compound **1** was isolated as a yellow amorphous powder. Its UV spectrum exhibited absorption bands characteristic for flavonoids at 334 and 272 nm (Harborne, 1989). Its positive HR-ESI-MS showed an $[M+Na]^+$ ion at *m/z* 701.1702 (calcd for $C_{31}H_{34}O_{17}Na$, 701.1694) consistent with the molecular formula $C_{31}H_{34}O_{17}$. The ^1H NMR spectrum (Table 2) showed an AA'BB' spin system at δ_H 6.96 (2H, d, *J* = 8.4 Hz) and 8.02 (2H, d, *J* = 8.4 Hz), together with two singlets at δ_H 6.27 and 6.58, a sugar chain [two anomeric protons at δ_H 4.46 (1H, d, *J* =7.7 Hz) and 5.07 (1H, d, *J* = 10.0 Hz)]. Analysis of ^{13}C NMR spectrum (Table 2) combined with COSY, NOESY, HSQC-TOCSY and HSQC experiments suggested that **1** was a vitexin derivative and that a *β*-glucopyranosyl unit was assigned starting from the anomeric proton at δ_H 4.46. The H-1, H-2, H-3 and H-4 of glcII exhibited large vicinal coupling (*J* =7.8 Hz) indicating that all the protons are axial (Pauli and Junior, 1995; Faiella et al., 2012; Benmerache et al., 2020). The diaxial coupling constants between the anomeric protons and H-2 in ^1H NMR indicated that all glucosyl units were *β*-linked (Agrawal, 1992). ^1H NMR displayed two acetyl groups at δ_H 1.95 and 1.97 (each 3H, s).The exact locations of the sugars and the acetyl moieties were revealed by the HMBC correlations observed between the

anomeric proton signal at δ_H 5.07 (glcI-H-1) and C-8 (δ_C 103.8), C-9 (δ_C 156.9), and C-7 (δ_C 163.0) and between the anomeric proton signal at δ_H 4.46 (glcII-H-1) and glcI-C-2 (δ_C 77.8). NOE connectivities were found between the resonances at δ_H 4,46 of the anomeric proton (glcII-H-1) and at δ_H 4.35 of the proton (glcI-H-2) confirming the interglycosidic linkage from (glcII-H-1) to (glcI-H-2). The β configuration of the two glucose moieties was confirmed by observation of NOE correlations from H-1" to H-3" and H-5"and from H-1'" to H-3'" and H-5'". The downfield shift for glcII-H-4 (δ_H 4.58) and the upfield shift for glcII-C-5 (δ_C 71.2) indicated the presence of acylation at glcII-C-4 and glcII-C-6, respectively. The location of the acetyl groups was confirmed by the HMBC correlations observed between the glcII-H-4 and the carbonyl carbon at δ_C 170.3 and between the glcII-H-6 (δ_H 3.97) and the carbonyl carbon at δ_C 171.3. Therefore, the structure of **1** was determined to be vitexin 2"-*O*-β-D-(4'",6'"-di-acetyl)-glucopyranoside.

Compound **2** was also isolated as a yellow powder with a molecular formula $C_{31}H_{34}O_{18}$ as determined by HR-ESI-MS ($[M+Na]^+$ at *m/z* 717.1651]) (calcd for $C_{31}H_{34}O_{18}Na$, 717.1643). The UV spectra exhibiting absorption bands at 346 and 256 nm were very similar to those of **1**, suggesting a flavone glycoside structure. A comparison of its NMR spectroscopic data with those of **1** (Table 2) indicated that the only difference between **2** and **1** was the aglycone moiety. As in **1**, two singlets at δ_H 6.26 and 6.50, two anomeric protons at δ_H 4.47 (d, J =7.8 Hz) and 5.07 (d, J = 10.0 Hz)], and two acetyl groups at δ_H 1.94 and 1.97 (each 3H, s). The ^{1}H and COSY NMR spectra of **2** exhibited in aglycone region an ABX system at δ_H 7.58 (1H, d, J= 2.0 Hz, H-2'), 7.53 (1H, dd, J= 8.3 and 2.0 Hz, H-6') and 6.93 (1H, d, J= 8.3 Hz, H-5'), due to a 3',4'-disubstituted B-ring. Full assignment of the remaining aglycone resonances in the ^{13}C NMR spectrum of **2** was achieved by analysis of HSQC and HMBC data, which confirmed the presence of an orientin derivative and a β-glucopyranosyl unit (Sientzoff et al., 2015). Each assignment of the aglycone moiety was confirmed by 2D NMR experiments, which also revealed that the saccharide moiety of compound **2** was identical to that of compound **1** (Table 2). Therefore, compound **2** was identified as orientin-2"-*O*-β-D-(4'",6'"-di-acetyl)-glucopyranoside.

Compound **3** was obtained as a yellow powder and exhibited UV absorptions at 249, 272, 295, and 332 nm. The HR-ESI-MS spectrum displayed a molecular ion peak $[M + Na]^+$ at *m/z* 809.1913 (calcd for $C_{37}H_{38}O_{19}Na$, 809.1905), in agreement with a molecular formula of $C_{37}H_{38}O_{19}$. The ^{1}H-NMR and COSY spectra of **3** was very similar to that of **2**, except for the absence of acetyl signals and the presence of three aromatic protons of an ABX system at δ_H 7.04 (d, *J*= 8.0 Hz, H-6‴), 6.86 (d, *J*= 8.0 Hz, H-5‴), 7.21 (s, H-2‴), a signal at δ_H 3.96 (3H, s), and two olefinic protons of an AX system at δ_H 7.53 (d, *J*= 15.9 Hz, H-8‴), and 6.85 (d, *J*= 15.9 Hz, H-7‴) indicative of a feruloyl moiety (Table 2) (Gossan et al., 2015). The large value of coupling constant *J* = 15.9 Hz indicates *trans* geometry of the double bond. The presence of an *E*-feruloyl moiety was evident from the connectivities observed in the HMBC spectrum between the *trans* double-bond and the 1,3,4-substituted aromatic ring as well as the ester carbonyl (δ_C 167.8), and from the protons of the methoxy group and the C-3‴ (δ_C 147.9) of the feruloyl moiety. The HMBC correlation between glcII-H_2-6 (δ_H 3.75, 4.11) and the carbonyl carbon of the feruloyl moiety defined glcII-C-6 as the site of acylation. The complete assignment of the proton and carbon resonances of **3** was achieved by analysis of COSY, HSQC and HMBC spectra (Table 2). Consequently, the structure of **3** was elucidated as orientin-2"-*O*-*β*-D-(6"'-feruloyl)-glucopyranoside.

Compound **4**, isolated as a yellow powder, displayed a molecular ion peak $[M+Na]^+$ at *m/z* 839.2020 in the positive HR-ESI-MS (calcd for $C_{38}H_{40}O_{20}Na$, 839.2011), in agreement with the molecular formula of $C_{38}H_{40}O_{20}Na$ and suggesting an additional methoxy group compared to **3**. UV spectra exhibiting absorption bands at 245, 275, 295 and 332 nm were very similar to those of **3**, suggesting a flavone glycoside structure. Analysis of 2D NMR experiments of **4** and comparison of its ^{1}H and ^{13}C NMR data with those of **3** showed that **4** was orientin-2"-*O*-*β*-D-glucopyranoside acylated with sinapic acid derivative, and also showed that **4** contained the same disaccharide chain linked to C-8 of luteolin (Table 2). This disaccharide was elucidated as above as *β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside. Thus, the additional methoxy group should be located at the sinapoyl derivative. This latter was represented on the ^{1}H NMR spectrum by three signals

comprising two doublets for coupled *trans* double-bond protons as in compound **3**, one singlet at δ_H 6.93 integrating for two protons, and one singlet at δ_H 3.94 integrating for six protons suggesting the presence of *E*-sinapoyl moiety. The ^{1}H and ^{13}C resonances of this *E*-sinapoyl unit were assigned using HMBC spectrum showing connectivities from the *trans* double-bond to the 1,3,4,5-substituted aromatic ring as well as to an ester carbonyl, and from the methoxy groups to the C-3′′′′ and C-5′′′′ of the sinapoyl residue (Table 1) (Bendaikha et al., 2014). The protons of the methoxy groups were also correlated to the protons 2′′′′ and 6′′′′ at δ_H 6.93 in the NOESY spectrum. Full assignment of the spectra was carried out by analyzing the 1D and 2D NMR spectra of compound **4**. In the HMBC spectra of **4**, correlations defining the site of acylation, glycosylation, and interglycosidic linkages were similar to those detected for **3**. Thus, compound **4** was concluded to be orientin-2''-*O*-*β*-D-(6'''-sinapoyl)-glucopyranoside.

Spectra of flavonoids **1**-**4** (see supporting material) exhibited extensive doubling of signals (6/4 ratio). This is due to the existence of obvious rotational isomerism in these spectra, which is a general characteristic of 8-*C*-hexosyl-flavones. Several studies have observed the rotational isomerism of 8-C-glycosyl flavonoids through NMR experiments (Markham et al., 1987, Zhou et al.,2019).

2.2. Biological assays

A study guided by the antioxidant activity of the 80% aqueous ethanolic extract of *S. gallica* was conducted to tentatively determine the active constituents. The free radical scavenging activity of SGE and the resulting fractions was determined by the DPPH and hydroxyl radicals (HORAC) assays and the cupric reducing capacity was evaluated by the CUPRAC assay. Liquid-liquid partitioning in petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH, combined with biological screening, identified the *n*-BuOH soluble fractions (Sg- *n*-BuOH) as the most active parts of the crude methanol extract. The best antioxidant activity was observed for the extracts in this decreasing order: Sg- *n*-BuOH > Sg-EA > SgE > Sg-$CHCl_3$ >Sg-PE, as shown in Table 1. The SG- *n*-BuOH fraction, with the most interesting antioxidant activity, was fractionated by VLC on silica gel and the resulting fractions (B1-B8) were tested for their antioxidant properties (Table 1). Fraction B4

showed the best antioxidant activity in DPPH (EC_{50}: 29.3 ± 1.2 µg/mL), HORAC (EC_{50} 126.3 ± 5.1 µg/mL) and CUPRAC (EC_{50}: 170.0 ± 8.2 µg/mL) assays (Table 1). In order to determine the compounds responsible for this activity, fraction B4 was further purified using preparative and semi-preparative HPLC to obtain compounds **1**-**10**. As summarized in Table 1, compounds **2**, **3**, **4**, **6** and **8** had the highest DPPH radical scavenging activity (EC_{50} from 15.0 to 23.2 µg/mL), similar to that of ascorbic acid used as reference compound (EC_{50} 18.3 ± 1.3 µg/mL). Compounds **3**, **4**, and **6** showed the best hydroxyl radical scavenging activity (EC_{50} from 95.0 to 106.6 µg/mL) compared to quercetin used as reference compound (EC_{50}: 52.2 ± 1.8 µg/mL). Compound **3** showed good cupric ion reducing antioxidant capacity (EC_{50} 18.0 ± 0.7 µg/mL) compared to the reference compound, Trolox (EC_{50} 10.4 ± 0.2 µg/mL), while compound **4** was moderately active (EC_{50} 51.3 ± 1.1 µg/mL) (Table 1). These results showed that compounds **3** and **4** have good antioxidant capacity according to the three methods tested: DPPH, HORAC and CUPRAC assays.

In general, the antioxidant activity of flavonoids is influenced by hindrance effect, the planar skeleton, the B ring OH moiety, and the number of OH groups in the structures (Bendaikha et al., 2014). In most cases, flavonoids with two free *ortho* OH groups at positions 3' and 4' of the B-ring exhibited higher antioxidant activity than compounds with one OH group (Schmitt et al., 2020). Our results confirm this relationship since the most active compounds **2**, **3**, **4**, **6** and **8** were luteolin glycosides.

Compounds **2**, **3** and **4** and **6** shared the orientin 2"-*O*-*β*-D-glucopyranoside, but it can be noted that acylated flavonoids **3** (feruloyl) and **4** (sinapoyl) were more active than **2** (acetylated) or **6** (without acyl group), indicating that acylation with feruloyl (**3**) or sinapoyl (**4**) moieties might contribute to antioxidant activity. Compound **3** was slightly more active than **4**, suggesting that acylation with the feruloyl moiety (**3**) was more favorable for the antioxidant activity than acylation with the sinapoyl (**4**) residue. Unacylated compound **8** was slightly more active than **6**, suggesting that xylose (**8**) was more favorable to antioxidant activity than glucose (**6**).

3. Conclusions

In this study, antioxidant activity detection was applied to fractions of an 80% EtOH extract of *S. gallica* aerial parts containing components of different polarities. Purification of the active fractions by preparative and semi-preparative HPLC afforded four new compounds (**1**-**4**) and six known compounds. All flavonoids (**1**-**8**) showed antioxidant potential. Two flavonoids were identified as the main antioxidant compounds (**3** and **4**) which showed a good effect in DPPH, HORAC and CUPRAC assays.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured in MeOH using a Perkin-Elmer 341 Polarimeter. UV-Vis spectra were acquired in MeOH with Shimadzu UV-Vis U-2450 spectrophotometer. NMR spectra were recorded in CD_3OD on a Bruker AvanceIII-600 spectrometer (Karlsruhe, Germany) (^1H at 600 MHz and ^{13}C at 150 MHz). HR-ESI-MS spectra were recorded on a Micromass Q-TOF micro instrument (Manchester, UK). Flash chromatography was carried out on a Grace Reveleris system equipped with dual UV and ELSD detection using Grace® cartridges (Silica gel or RP-C_{18}). Preparative HPLC was performed on Armen Instrument apparatus equipped with an AP 250 pump and a Knauer (Merck) detector UV K-2501. The mobile phase was composed of H_2O acidified with TFA (0.025%) and CH_3CN with a flow rate of 5 mL/min for the semi-prep HPLC and 75 mL/min for the preparative HPLC and the chromatograms were monitored at 255 nm. Semi-preparative HPLC purification was carried out on a Dionex apparatus equipped with an ASI-100 automated sample injector, a STH 585 column oven, a P580 pump, and a UVD 340S diode array detector, all driven by the Chromeleon® software version 6.8. A prepacked C_{18} column (Interchim, 250 × 10 mm, 5 μ) was used for semi-preparative HPLC. Thin-layer chromatography (TLC) was carried out on silica gel 60 F_{254} pre-coated aluminum plates (0.2 mm, Merck), using $CHCl_3$/MeOH/H_2O (14/6/1, v/v/v) as mobile phase. After developing with solvent systems, spots were visualized under

UV light (254 and 366 nm) and sprayed with 50% H_2SO_4 followed by heating. A FLUOstar Omega spectrophotometer (BMG LABTECH) was used for absorbance measurement in antioxidant assays.

4.2. Plant material

The aerial parts of *Silene gallica* L. were collected from Djebel El-Ouahch, Constantine (North-Eastern Algerian (GPS: x=6.671694, y=36.394611, z=888 m) in May 2016. Plant specimen was identified by Mr. Kamel Kabouche. Voucher specimen (LOST Sg.05/16) are on files in the herbarium of LOST Laboratory, University frères Mentouri-Constantine 1, Algeria.

4.3. Extraction and isolation

The dried and powdered aerial part of *S. gallica* (1 kg) was extracted three times with 80% aqueous ethanol (each 5 L, 24 h), and concentrated under vacuum in order to evaporate the ethanol and obtain an aqueous solution. The aqueous solution (700 mL) was extracted successively with petroleum ether, $CHCl_3$, EtOAc and *n*-ButOH (3 × 300 mL, each), then dried under reduced pressure to yield 4 g of Sg-PE, 0.8 g of Sg-$CHCl_3$, 2.3 g of Sg-EtOAc and 33.2 g of Sg-*n*-BuOH extracts, respectively. A part of Sg-*n*-BuOH extract (10.8 g) was subjected to vacuum liquid chromatography (VLC) over silica gel (9 cm × 5.5 cm) eluted with the system CH_2Cl_2-MeOH (95:5, 9:1:0, 8:2, 7:3), CH_2Cl_2-MeOH-H_2O (14:6:1, 6:4:0.7, 1:1:0) and finally by 100% MeOH to obtain 8 fractions B1-B8, respectively. A part of fraction B4 (1.75 g) was subjected to preparative HPLC eluted with CH_3CN-H_2O (15-20% CH_3CN, in 45 min) to yield compounds **10** (t_R=22 min, 125 mg), **2** (t_R=27 min, 34.2 mg) and **3** (t_R=40 min, 16 mg). Fractions 9-13 (111 mg) were purified by flash chromatography over silica gel, eluted with MeOH-CH_2Cl_2 (20-80%, in 40 min) to afford compound **6** (30 mg). Fractions 14-15 (67 mg) were purified by semi-prep. HPLC eluted with CH_3CN-H_2O (16:84) to yield compound **9** (t_R=9.8 min, 4 mg). Fractions 18-19 (70 mg) were purified by semi-prep. HPLC eluted with CH_3CN-H_2O (17-20% CH_3CN, in 20 min) to yield compounds **8** (t_R=8.0 min, 12 mg) and **5** (t_R=10.0 min, 15 mg). Fraction 23 (59 mg) was purified in the same manner as fractions 18-19 to yield compounds **7** (t_R=15.3 min, 10 mg). Fraction 34 (13.9 mg) was purified by semi-prep. HPLC eluted with CH_3CN-H_2O (2:8) to afford compound **1**

(t_R=18.0 min, 3.7 mg). Fraction 37 (32 mg) was purified by semi-prep. HPLC eluted with CH_3CN-H_2O (25:75) to give compound **4** (t_R=6.0 min, 12 mg).

4.4. Spectral data

*4.4.1. Vitexin 2''-O-β-D-(4''',6'''-di-acetyl)-glucopyranoside (**1**)*

Amorphous yellow powder; $[\alpha]_D^{20}$-20.7 (*c* 0.28, MeOH) ; UV (MeOH) λmax (logε) 272 (1.91), 334 (2.05) nm; ^1H and ^{13}C NMR data; see Table 2; HR-ESI-MS *m/z* 701.1702 $[M+Na]^+$ (calcd for $C_{31}H_{34}O_{17}Na$, 701.1694).

*4.4.2. Orientin 2''-O-β-D-(4''',6'''-di-acetyl)-glucopyranoside (**2**)*

Amorphous yellow powder; $[\alpha]_D^{20}$-15.6 (*c* 0.85, MeOH) ; UV (MeOH) λmax (logε) 256 (1.02), 346 (1.12) nm; ^1H and ^{13}C NMR data; see Table 2; HR-ESI-MS *m/z* 717.1651 $[M+Na]^+$ (calcd for $C_{31}H_{34}O_{18}Na$, 717.1643).

*4.4.3. Orientin 2''-O-β-D-(6'''-feruloyl)-glucopyranoside (**3**)*

Amorphous yellow powder; $[\alpha]_D^{20}$-61.1 (*c* 0.83, MeOH) ; UV (MeOH) λmax (logε) 249 (1.51), 272 (1.41), 295 (1.45), 332 (2.28) nm; ^1H and ^{13}C NMR data; see Table 2; HR-ESI-MS *m/z* 809.1913 $[M+Na]^+$ (calcd for $C_{37}H_{38}O_{19}Na$, 809.1905).

*4.4.4. Orientin 2''-O-β-D-(6'''-sinapoyl)-glucopyranoside (**4**)*

Amorphous yellow powder; $[\alpha]_D^{20}$-59.5 (*c* 0.86, MeOH) ; UV (MeOH) λmax (logε) 245 (1.9), 272 (1.35), 295 (1.30), 332 (2.13) nm; ^1H and ^{13}C NMR data; see Table 2; HR-ESI-MS *m/z* 839.2020 $[M+Na]^+$ (calcd for $C_{38}H_{40}O_{20}Na$, 839.2011).

4.5. Acid hydrolysis

An aliquot of the fraction Sg-*n*-BuOH-4 (100 mg) was treated with 2 N TFA (trifluoroacetic acid, aqueous solution, 15 mL) at 90 °C for 6 h. After extraction with CH_2Cl_2 (10 mL × 3), the water-soluble layer was evaporated to dryness (50.1 mg). The sugars mixture was then purified by preparative silica gel TLC eluted with CH_3COOEt:CH_3COOH:CH_3OH:H_2O (65:25:15:15) to afford 6 mg of glucose (R_f 0.48) and 3 mg of xylose (R_f 0.52). The optical rotation of each purified

monosaccharide was measured [glucose: $[\alpha]^{20}_{D}$ +49.3 (*c* 0.5, H_2O); D-xylose:, $[\alpha]^{20}_{D}$ +18.5 (*c* 0.25, H_2O)] and the absolute configurations were determined as D by comparison with $[\alpha]^{20}_{D}$ values of authentic samples of D-xylose and D-glucose (Sigma–Aldrich).

4.6. DPPH radical scavenging activity

Extracts, fractions and compounds **1**-**10** were evaluated for their DPPH radical scavenging activity according to a recently published procedure (Schmitt et al., 2020). Samples were prepared at concentrations of 400, 200, 100, 50, 25, 12.5 and 6.25 µg/mL. Ascorbic acid was used as positive controls. All the tests were conducted in triplicate and EC_{50} were determined by interpolation of concentration versus inhibition curves obtained by a MSExcel calculation sheet.

4.7. Hydroxyl radical scavenging activity (HORAC)

Extracts, fractions and compounds **1**-**9** were evaluated for their hydroxyl radical scavenging activity at various concentrations, ranging from 1330 to 41.56 µg/mL, according to Schmitt et al. (2020). Ascorbic acid was used as positive controls. All the tests were conducted in triplicate and EC_{50} were determined by interpolation of concentration versus inhibition curves obtained by a MSExcel calculation sheet.

4.8. Power cupric ion reducing (CUPRAC) assay

Extracts, fractions and compounds **1**-**9** were tested for their cupric ion reducing ability. The cupric ion reducing activity (CUPRAC) was determined according to Schmitt et al. (2020). Samples were prepared at concentrations of 572, 286, 143, 71.5, 35.75, 17.87 and 8.94 µg/mL. Trolox (Sigma-aldrich) was used as positive controls. All the tests were conducted in triplicate and EC_{50} were determined by interpolation of concentration versus inhibition curves obtained by a MSExcel calculation sheet.

4.9. Statistical analysis

All data on bioassays were the average of triplicate analyses. The data were recorded as mean values ± standard deviation. The IC_{50} and values were calculated by linear regression analysis (MSExcel calculation sheet).

Conflict of interest: The authors declare no conflict of 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.

Supplementary data: HR-ESI-MS, UV, and NMR data for compounds **1-4**

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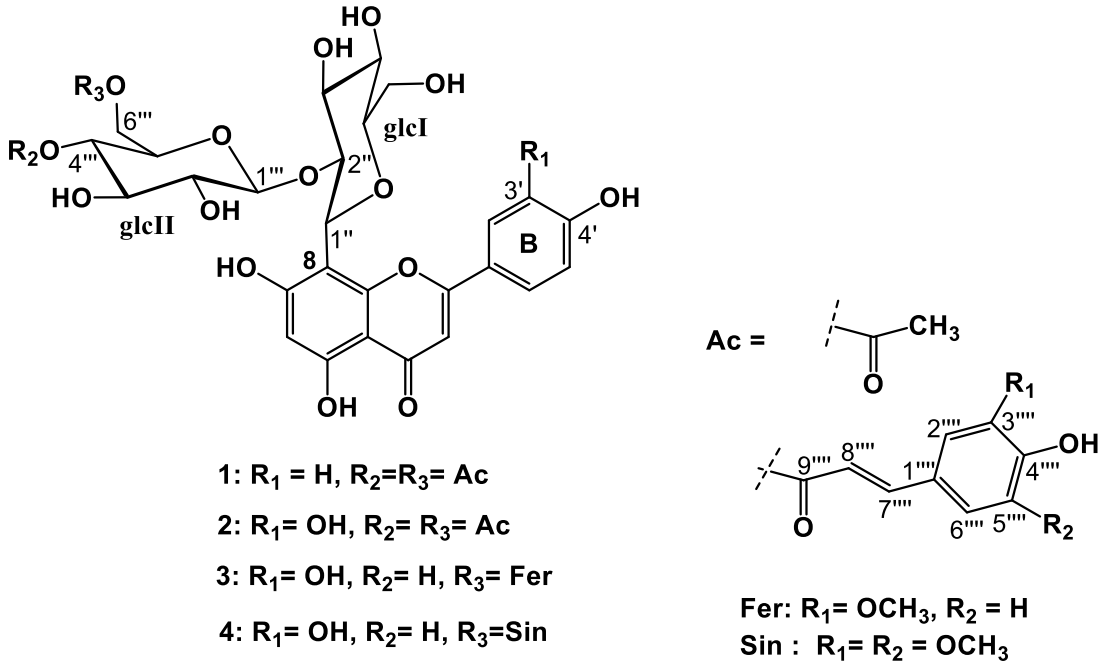

Fig. 1. Chemical structure of new compounds **1**-**4**, isolated from *S. gallica.*

Table 1. Antioxidant activity of extracts, fractions and compounds isolated from *S. gallica*.[a]

		DPPH	HORAC	CUPRAC
		EC_{50} (µg/mL)	EC_{50} (µg/mL)	EC_{50} (µg/mL)
	SGE	204.4 ± 9.7	366.6 ± 5.1	650.0 ± 10.2
extracts	Sg-PE	250.0 ± 6.2	533.3 ± 8.3	nd
	Sg-$CHCl_3$	195.0 ± 2.8	489.0 ± 11.5	nd
	Sg-EA	106.8 ± 3.8	423.3 ± 11.5	nd
	Sg-BuOH	57.7 ± 2.1	126.6 ± 7.3	330.0 ± 7.3
	B1	167.3 ± 1.7	110.0 ± 5.8	nd
fractions	B2	104.3 ± 5.7	823.3 ± 10.1	nd
	B3	62.2 ± 5.2	366.6 ± 8.21	nd
	B4	29.3 ± 1.2	126.3 ± 5.1	170.0 ± 8.2
	B5	58.3 ± 1.4	320.6 ± 2.8	nd
	B6	109.4 ± 3.6	443.3 ± 15.2	nd
	B7	191.5 ± 7.4	nd	nd
	B8	300.8 ± 10.4	nd	nd
compounds	1	nd	301.6 ± 9.5	nd
	2	16.2 ± 0.9	185.1 ± 5.3	170.0 ±5.6
	3	15.0 ± 0.8	95.0 ± 3.8	18.0 ± 0.7
	4	21.3 ± 1.7	106.6 ± 4.2	51.3 ± 1.1
	5	78.5 ± 5.3	112.5 ± 5.6	nd
	6	23.2 ± 1.1	96.0 ± 2.7	178.6 ± 2.3
	7	77.5 ± 4.5	210.0 ± 7.5	nd
	8	15.2 ± 0.3	135.0 ± 8.2	168.3 ± 3.4
	9	nd	nd	109.0 ± 3.6
	10	nd	nd	nd
controls	Ascorbic acid[b]	18.3 ± 1.3	-	-
	Quercetin[b]	-	52.2 ± 1.8	-
	Trolox[b]	-	-	10.4 ± 0.2

[a] Values expressed are means ± S.D. of three parallel measurements, [b] used as positive control, nd: EC_{50} is not achieved.

Table 2. ^{1}H NMR and ^{13}C NMR spectroscopic data of compounds **1**–**4** in CD_3OD.[a,b]

Position	1		2		3		4	
	δ_H m (*J* in Hz)	δ_C	δ_H m (*J* in Hz)	δ_C	δ_H m (*J* in Hz)	δ_C	δ_H m (*J* in Hz)	δ_C
2	-	164.9	-	164.9	-	164.9	-	164.9
3	6.58 s	102.2	6.50 s	102.2	6.46 s	102.5	6.46 s	102.5
4	-	182.8	-	182.7	-	182.6	-	182.6
5	-	161.1	-	161.0	-	161.0	-	161.0
6	6.27 s	97.9	6.26 s	97.9	6.25 s	97.8	6.23 s	97.8
7	-	163.0	-	163.0	-	162.8	-	162.8
8	-	103.8	-	103.7	-	103.8	-	103.8
9		156.9		156.9		156.9		156.9
10		104.3		104.3		104.3		104.3
1′		122.2		122.7		122.9		122.7
2′	8.02 d (8.4)	128.7	7.58 d (2.0)	113.6	7.57 d (1.7)	113.6	7.56 d (1.3)	113.6
3′	6.96 d (8.4)	115.7	-	145.8	-	145.7	-	145.6
4′	-	161.4	-	149.6	-	149.6	-	149.4
5′	6.96 d (8.4)	115.7	6.93 d (8.2)	115.4	6.85 d (8.2)	115.4	6.87 d (8.5)	115.4
6′	8.02 d (8.4)	128.7	7.54 dd (8.2,2.0)	119.5	7.45 dd (8.2, 1.7)	119.5	7.44 dd (8.5, 1.3)	119.5
glcI								
1″	5.07 d (10.0)	72.3	5.06 d (10.0)	72.4	5.06 d (10.0)	72.4	5.06 d (10.0)	72.4
2″	4.35 t (9.4)	77.8	4.37 dd (9.6, 9.2)	77.7	4.43 t (9.3)	77.3	4.43 t (9.4)	77.2
3″	3.77 t (9.3)	79.1	3.77 t (9.3)	79.2	3.79 t (9.2)	79.2	3.78 t (8.9)	79.3
4″	3.69 t (9.3)	70.7	3.73 t (9.3)	70.7	3.73 t (9.2)	70.7	3.73 t (8.9)	70.7
5″	3.48 m	81.6	3.49 m	81.6	3.48 m	81.6	3.49 m	81.6
6″	3.81 dd (12.2, 5.5)	61.5	3.89 dd (12.3, 5.8)	61.7	3.86 dd (12.5, 5.4)	61.8	3.87 dd (11.4, 5.2)	61.8
	3.97 dd (12.2, 1.8)		4.00 dm (12.3)		3.98		3.99 dd (11.4, 1.8)	
glcII								
1‴	4.46 d (7.7)	103.2	4.47 d (7.8)	103.2	4.48 d (7.7)	103.1	4.49 d (7.7)	103.1
2‴	3.03 dd (9.4, 8.2)	74.1	3.01 dd (9.3, 8.0)	74.1	3.00 dd (8.8, 8.2)	74.3	3.00 dd (8.8, 7.7)	74.2
3‴	3.37 t (9.4)	73.8	3.37 t (9.3)	73.8	3.23 t (8.8)	76.2	3.23 t (8.8)	76.2
4‴	4.58 t (9.6)	69.8	4.58 t (9.6)	69.8	3.10 t (9.1)	69.2	3.11 t (9.1)	69.2
5‴	3.18 dt (10.0, 2.3)	71.2	3.20 dt (9.9, 2.3)	71.2	3.05 m	73.8	3.07 m	73.7
6‴	3.31	61.4	3.32	61.4	3.75 dm (12.5)	62.6	3.72 dd (11.6, 2.4)	62.6
	3.98 dd (12.2, 3.0)		3.97 dd (12.3, 3.0)		4.10 dd (12.5, 3.0)		4.16 dd (11.6, 2.5)	
Ac at glcII-C-4								
1	1.94 s	19.3	1.94 s	19.3				
2	-	170.3	-	170.4				
Ac at glcII-C-6	acetyl		acetyl		feruloyl		sinapoyl	
1⁗	1.98 s	19.4	1.98 s	19.3	-	126.5	-	125.5
2⁗	-	171.3	-	171.3	7.21 s	110.2	6.93 s	105.6
3⁗					-	147.9	-	148.0
4⁗					-	149.1	-	138.0
5⁗					6.86 d (8.0)	115.0	-	148.0
6⁗					7.04 d (8.0)	123.0	6.93 s	105.6
7⁗					6.20 d (15.9)	114.0	6.25 d (15.8)	114.5
8⁗					7.53 d (15.9)	145.5	7.52 d (15.8)	145.5
9⁗					-	167.8	-	167.8
3⁗-*O*-CH₃					3.96 s	55.1	3.94 s	55.5
5⁗-*O*-CH₃							3.94 s	55.5

[a] Overlapped signals are reported without designated multiplicity.
[b] δ in ppm, *J* in parentheses in Hz.

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