

Article

Antioxidant, Anti-Glycation and Anti-Inflammatory Activities of Phenolic Constituents from *Cordia sinensis*

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Abstract: Nine compounds have been isolated from the ethyl acetate soluble fraction of *C. sinensis*, namely protocathechuic acid (**1**), *trans*-caffeic acid (**2**), methyl rosmarinate (**3**), rosmarinic acid (**4**), kaempferide-3-*O*- β -D-glucopyranoside (**5**), kaempferol-3-*O*- β -D-glucopyranoside (**6**), quercetin-3-*O*- β -D-glucopyranoside (**7**), kaempferide-3-*O*- α -L-rhamnopyranosyl (1 \rightarrow 6)- β -D-glucopyranoside (**8**) and kaempferol-3-*O*- α -L-rhamnopyranosyl (1 \rightarrow 6)- β -D-glucopyranoside (**9**), all reported for the first time from this species. The structures of these compounds were deduced on the basis of spectroscopic studies, including 1D and 2D NMR techniques. Compounds **1–9** were investigated for biological activity and showed significant anti-inflammatory activity in the carrageen induced rat paw edema test. The antioxidant activities of isolated compounds **1–9** were evaluated by the DPPH radical scavenging test, and compounds **1**, **2**, **4** and **7–9** exhibited marked scavenging activity compared to the standard BHA. These compounds were further studied for their anti-glycation properties and some compounds showed significant anti-glycation inhibitory activity. The purity of compounds **2–5**, **8** and **9** was confirmed by HPLC. The implications of these results for the chemotaxonomic studies of the genus *Cordia* have also been discussed.

Keywords: *Cordia sinensis*; antioxidant; anti-inflammatory; anti-glycation

1. Introduction

The genus *Cordia* belongs to the family Boraginaceae, with some 300 species distributed worldwide, mostly in the warmer regions of the World [1]. According to a literature survey, several uses in traditional medicine have been reported for different *Cordia* species [2-6]. The ethnopharmacological and chemotaxonomic importance of the genus *Cordia* led us to investigate the chemical constituents of one of its species, namely *Cordia sinensis*, which is a medicinal plant found widespread in the drier parts of Saudi Arabia, Africa and India [7]. The bark of *C. sinensis* is used for stomach disorders and for chest pains [8]. A literature survey revealed that very little phytochemical work has so far been carried out on *C. sinensis*. A methanolic extract of this plant showed strong toxicity in the brine shrimp lethality test and on subsequent fractionation, the major toxicity was observed in the ethyl acetate soluble sub-fraction. Further pharmacological screening of this fraction revealed potent antioxidant activity. In this paper we report the isolation of nine known compounds isolated for the first time from this plant: protocatechuic acid (**1**) [9], *trans*-caffeic acid (**2**) [10], methyl rosmarinate (**3**) [11], rosmarinic acid (**4**) [11], kaempferide-3-*O*- β -D-glucopyranoside (**5**) [12], kaempferol-3-*O*- β -D-glucopyranoside (**6**) [13], quercetin-3-*O*- β -D-glucopyranoside (**7**) [14], kaempferide-3-*O*- α -L-rhamnopyranosyl (1 \rightarrow 6)- β -D-glucopyranoside (**8**) [13] and kaempferol-3-*O*- α -L-rhamnopyranosyl (1 \rightarrow 6)- β -D-glucopyranoside (**9**) [13] (Figure 1). This study was undertaken to investigate the antioxidant potential of these compounds using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay. *In vivo* anti-inflammatory activity for all of the compounds was also determined by the carrageen induced rat paw edema test. Finally, these compounds were studied for their anti-glycation properties. The purity of compounds **2–5**, **8** and **9** was also confirmed by analytical HPLC.

2. Results and Discussion

2.1. Compounds and Their Biological Activities

The MeOH extract of the aerial part of *C. sinensis* was divided into different sub-fractions soluble in *n*-hexane, EtOAc and *n*-BuOH. The EtOAc soluble sub-fraction was subjected to a series of column chromatography fractionations to afford compounds **1–9**. Their structures were established by MS spectrometry, UV, IR as well as NMR spectroscopy and comparison with literature data.

The antioxidant activity of compounds **1–9** was measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method and the results are summarized in Table 1. Compounds **1–4**, **7–9** showed significant free radical scavenging activity in this assay. Among these, rosmarinic acid (**4**), protocatechuic acid (**1**), *trans*-caffeic acid (**2**) and quercetin-3-*O*- β -D-glucopyranoside (**7**) were found to have high antioxidant potentials, with IC₅₀ = 13.5, 14.1, 16.3 and 19.1 μ M, respectively, compared to the standard BHA (IC₅₀ = 44.3 μ M). The antioxidant activity of flavonoids depends upon the arrangement of functional groups about the nuclear structure [15]. Accordingly, the *ortho*-dihydroxy (catechol) structure in compounds **1**, **2**, **4** plays an important role in their antioxidative function as in flavonoid **7**, and this can further explain the weak scavenging activity (IC₅₀ = 53.4 μ M) of kaempferol-3-*O*- β -D-glucopyranoside (**6**) which lacks the B-ring catechol system [16]. On the other hand, the reduced antioxidant activity of kaempferide-3-*O*- β -D-glucopyranoside (**5**) (IC₅₀ = 55.5 μ M) could be attributed

to 4'-*O*-methylation that perturbs ring planarity through steric effects. The antioxidant activities of kaempferol-3-*O*- α -L-rhamnopyranosyl (1 \rightarrow 6)- β -D-glucopyranoside **9** (IC_{50} = 39.5 μ M) and kaempferide-3-*O*- α -L-rhamnopyranosyl (1 \rightarrow 6)- β -D-glucopyranoside **8** (IC_{50} = 42.4 μ M), were comparable to that of BHA [17].

Figure 1. Structures of compounds 1–9.

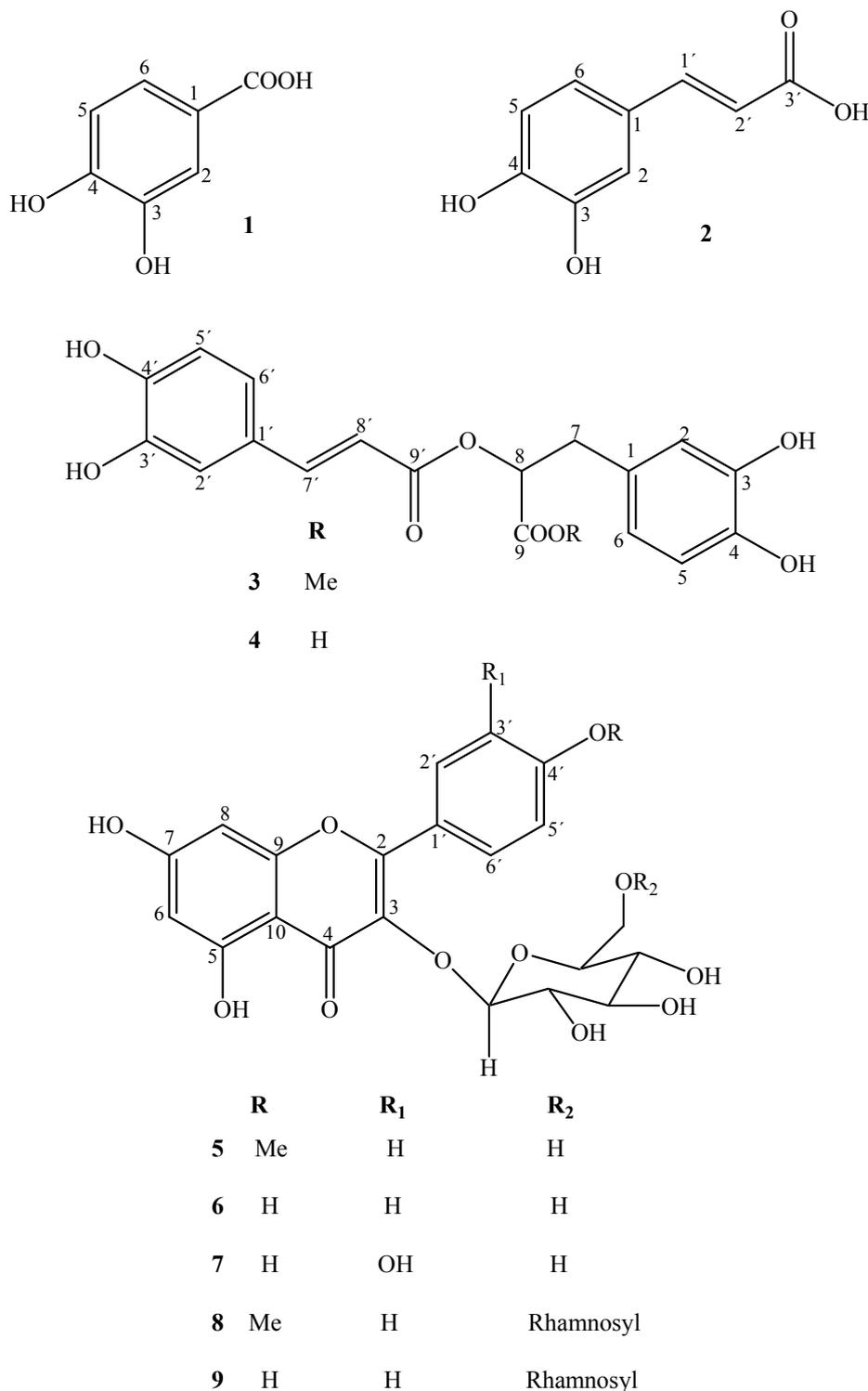


Table 1. IC₅₀ (μM) values of compounds 1–9 in the DPPH antioxidant assay.

Compounds	DPPH Scavenging Activity IC ₅₀ ^a [μM]
1	16.3 ± 0.19
2	14.1 ± 0.14
3	22.7 ± 0.17
4	13.5 ± 0.21
5	55.5 ± 0.12
6	53.4 ± 0.88
7	19.1 ± 0.90
8	42.4 ± 0.91
9	39.5 ± 0.85
BHA^b	44.3 ± 0.09

^a Values ± SEM (standard mean error of three assays); ^b Standard DPPH scavenging activity.

The oxidation process is believed to play an important role in advanced glycation as endproducts (AGEs) formation [18]. On the basis of a literature search, several natural phenolic compounds known to possess antioxidative properties, such as curcumin, rutin, garcinol and arbutin have been found to have strong antiglycation activity [19,20]. We report here for the first time the anti-glycation properties of the plant phenolics 1–9, obtained from *C. sinensis* (Table 2).

Table 2. Anti-glycation activity of compounds 1–9.

Compounds	% Inhibition
1	68.0
2	69.2
3	88.4
4	87.3
5	76.5
6	74.0
7	71.2
8	80.7
9	79.0
Rutin^a	86.0

^a Used as Standard.

Moreover, evaluation of the anti-inflammatory potential of the isolated compounds on carrageen-induced rat paw edema showed significant the potent anti-inflammatory activity of compounds 5 and 6 (62.4% and 59.6%, respectively). These flavonol glycosides were evidently more active than diclofenac sodium, which showed 57.6% inhibition of carrageen-induced rat paw edema, while compound 1 (55.0%) showed an effect comparable to that of the reference compound. The other tested compounds 2, 7, 8 and 9 displayed lower percentages of inhibition in the 38.4–51.2% range and were therefore less active than diclofenac sodium in this assay (Table 3) [21].

Table 3. Anti-inflammatory potential of compounds 1–9 in carrageen induced paw edema of rats.

Group (3 rats in each)	Treatment 100 mg/kg	Edema Volume ($V_c = V_f - V_0$)	Percent Inhibition (%)
1	Cage-1 control	0	
2	Diclofenac Sodium	0.22 ± 0.05	57.6
3	1	0.23 ± 0.04	55.0
4	2	0.26 ± 0.19	50.0
5	3	-	-
6	4	-	-
7	5	0.24 ± 0.13	62.4
8	6	0.21 ± 0.11	59.6
9	7	0.25 ± 0.08	51.2
10	8	0.35 ± 0.21	43.5
11	9	0.32 ± 0.07	38.4

2.2. Chemotaxonomic Significance

The genus *Cordia* is known for the presence of a variety of secondary metabolites. Previous investigations led to the isolation of cytotoxic monoterpenes from the roots of *C. curassavica* and *C. globosa* [3,4], meroterpenoid naphthoquinones from the roots of *C. linnaei* [6], anti-inflammatory sesquiterpenes from *C. trichotoma* and *C. verbenacea* [5,22], abietane type diterpenes from *C. latifolia* [23], triterpenes from *C. spinescens*, *C. multispicata* and from *C. verbenacea* [24–26], saponins from *C. piauiensis* [27–29], antifungal and larvicidal phenylpropanoid derivatives from the root bark of *C. alliodora* [2], essential oils from *C. cylindrostachya* [30], flavonoids from *C. dichotoma*, *C. obliqua* and from the flowers of *C. dentate* [31–33], flavonones from the aerial parts of *C. globosa*, *C. obliqua*, *C. francisci*, *C. martinicensis*, *C. myxa* and *C. serratifolia* [34], pyrrolizidine alkaloids from *C. myxa* and glutarimide alkaloids from *C. globifera* [35,36], lignan from *C. rufescens* [37], rosmarinic acid from *C. americana* and different aromatic compounds from *C. latifolia* and *C. dentate* [38–40]. The present study reported the isolation of compounds 1–9 from the aerial parts of *C. sinensis*. This adds kaempferol type flavonoid glycosides 6–9 to the list of genus *Cordia*, while all of the other compounds were isolated for the first time from the plant *C. sinensis*. The isolation and identification of these compounds from *C. sinensis* represents a contribution to the phytochemical analysis of the components of the plant and it may be useful in further chemotaxonomic studies on the genus *Cordia*.

3. Experimental

3.1. General

The UV and IR spectra were recorded on Hitachi-UV-3200 and JASCO 320-A spectrometers, respectively. The ^1H -, ^{13}C -NMR and 2D-NMR spectra were recorded on a Bruker AMX-400 spectrometer with tetramethylsilane (TMS) as an internal standard. Chemical shifts are in given ppm (δ), relative to tetramethylsilane as an internal standard and scalar coupling constants (J) are reported in Hertz. FAB and HRFABMS (neg. ion mode, matrix: glycerol) were registered on a JEOL JMS-HX110 mass spectrometer. Thin layer chromatography (TLC) was performed on precoated silica

gel F₂₅₄ plates (E. Merck, Darmstadt, Germany); the detection was done at 254 nm and by spraying with ceric sulphate reagent. All chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA). Analytical HPLC was performed with Shimadzu HPLC equipment comprising an LC-10 ATVP pump, SPD-M.OA AVP photodiode-array (PDA) detector set at 271 nm, and a Rheodyne 7010 SIL-10 ADVP injector equipped with a 50- μ L sample loop. Compounds were injected on a 250 mm \times 4.0 mm, 5 μ m particle size LiChrospher 100 RP-18 column from Merck (Darmstadt, Germany). The mobile phase was a 1:1 mixture of acetonitrile (ACN) and 0.05% aqueous trifluoroacetic acid at a flow rate of 1.0 mL min⁻¹. Before use the mobile phase was filtered and degassed.

3.2. Plant Material

The whole plant of *Cordia sinensis* (Boraginaceae) was collected from Riyadh (Saudi Arabia) and identified by Dr. M. Atiqur Rahman, Plant Taxonomist, College of Pharmacy, King Saud University. A voucher specimen No.30 was deposited in the herbarium of the Department of Pharmacognosy, King Saud University, Riyadh, Saudi Arabia.

3.3. Extraction and Isolation

The shade dried plant material (1.5 kg) was extracted with methanol (6.0 L, thrice) at room temperature. The combined methanolic extract was evaporated under reduced pressure to give a thick gummy mass (70 g) that was suspended in water and successively extracted with *n*-hexane, ethyl acetate and *n*-butanol to afford the corresponding sub-fractions. The ethyl acetate soluble sub-fraction (15 g) was subjected to column chromatography eluting with CHCl₃, CHCl₃-MeOH and MeOH in increasing order of polarity to obtain five fractions I–V. Fraction I obtained from CHCl₃-MeOH (9.9:0.1) was further purified by column chromatography eluting with CHCl₃-MeOH (9.7:0.3) to afford compounds **1** (10 mg) and **2** (25 mg) from the top and the tail fractions, respectively. Fraction II obtained from CHCl₃-MeOH (9.8:0.2) was a mixture of two components, which were separated by column chromatography using the solvent system CHCl₃-MeOH (9.5:0.5) to afford compounds **3** (18 mg) and **4** (25 mg) from the top and the tail fractions, respectively. Fraction III obtained from CHCl₃-MeOH (9.5:0.5) was further purified by Sephadex LH-20 column chromatography eluting with H₂O-MeOH (8.0:2.0) to afford compounds **5** (19 mg) and **6** (10 mg). Fraction IV obtained from CHCl₃-MeOH (9.3:0.7) was quite pure and rechromatography using the solvent system CHCl₃-MeOH (9.0:1.0) gave compound **7** (11 mg). Fraction V obtained from CHCl₃-MeOH (9.0:1.0) was further purified by Sephadex LH-20 column chromatography eluting with H₂O-MeOH (8.5:1.5) to afford compounds **8** (20 mg) and **9** (20 mg). Compounds **1**–**9** were identified through comparison of their physical and spectral data with those reported in the literature. The absolute configuration of the sugar moieties in compounds **5**–**9** were determined by acid hydrolysis and the separated sugars were identified as L-rhamnose and D-glucose through co-TLC with authentic samples and the sign of the optical rotation $[\alpha]_{\text{D}}^{25} = +7.8$ to $+7.9$ for L-rhamnose and $[\alpha]_{\text{D}}^{25} = +52.0$ to $+52.2$ for D-glucose, respectively.

3.4. HPLC Analysis of the Purity of Compounds 2–5, 8 and 9

Compounds 2–5, 8 and 9 (each 2.0 mg) was dissolved in Millipore water (HPLC grade, 5 mL) and the solution was filtered through a 0.45 μm Millipore filter. Compound 2 was detected at R_t 9.061 min, compound 3 was detected at R_t 20.569 min, compound 4 was detected at R_t 27.256 min, compound 5 was detected at R_t 22.542 min, compound 8 was detected at R_t 36.937 min, while compound 9 appeared at R_t 39.181 min (Figure 2). Compounds 1, 6 and 7 were not isolated in sufficient amounts, so the purity of these compounds was not checked by HPLC.

Figure 2. HPLC chromatograms of compounds 2–5, 8, 9.

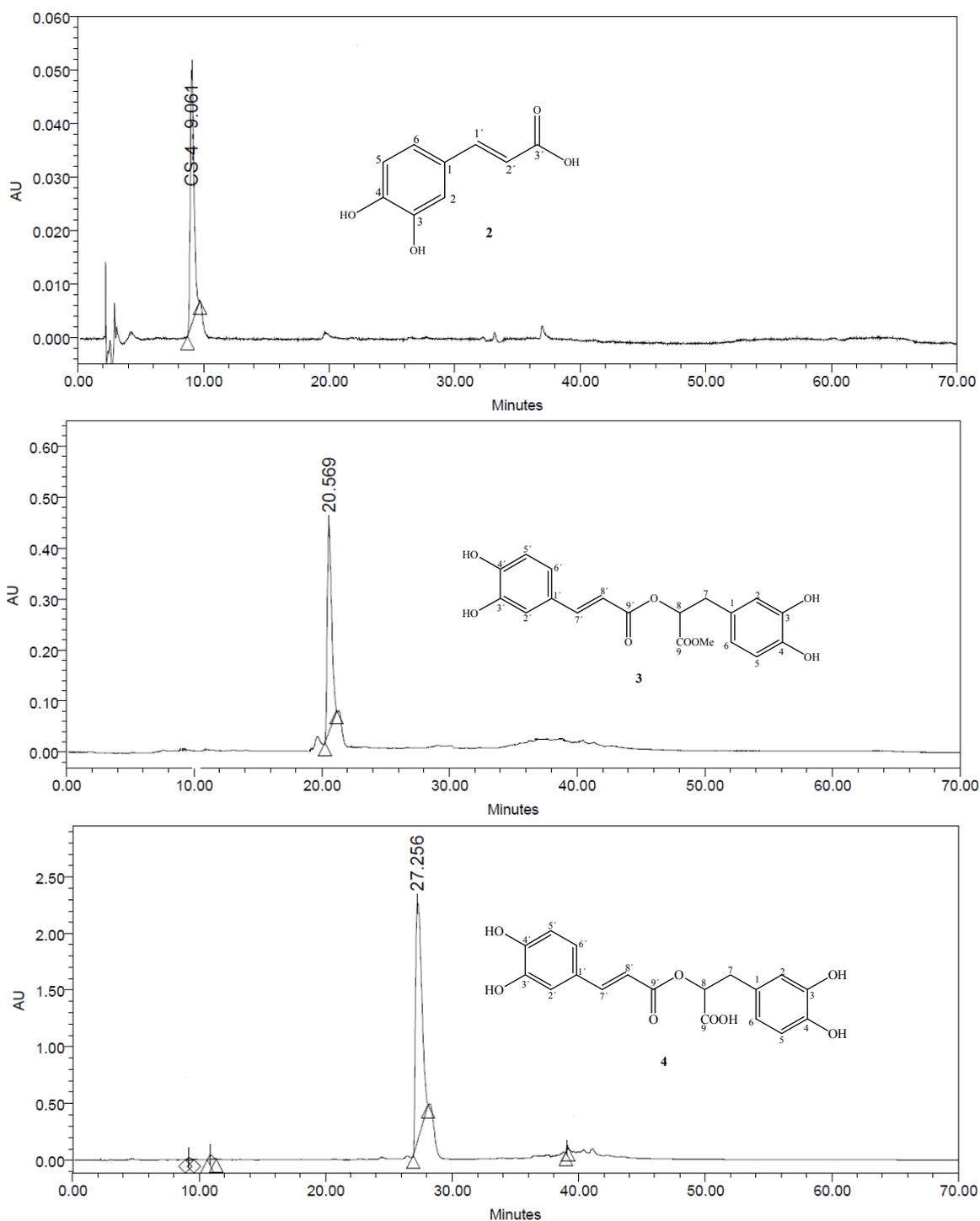
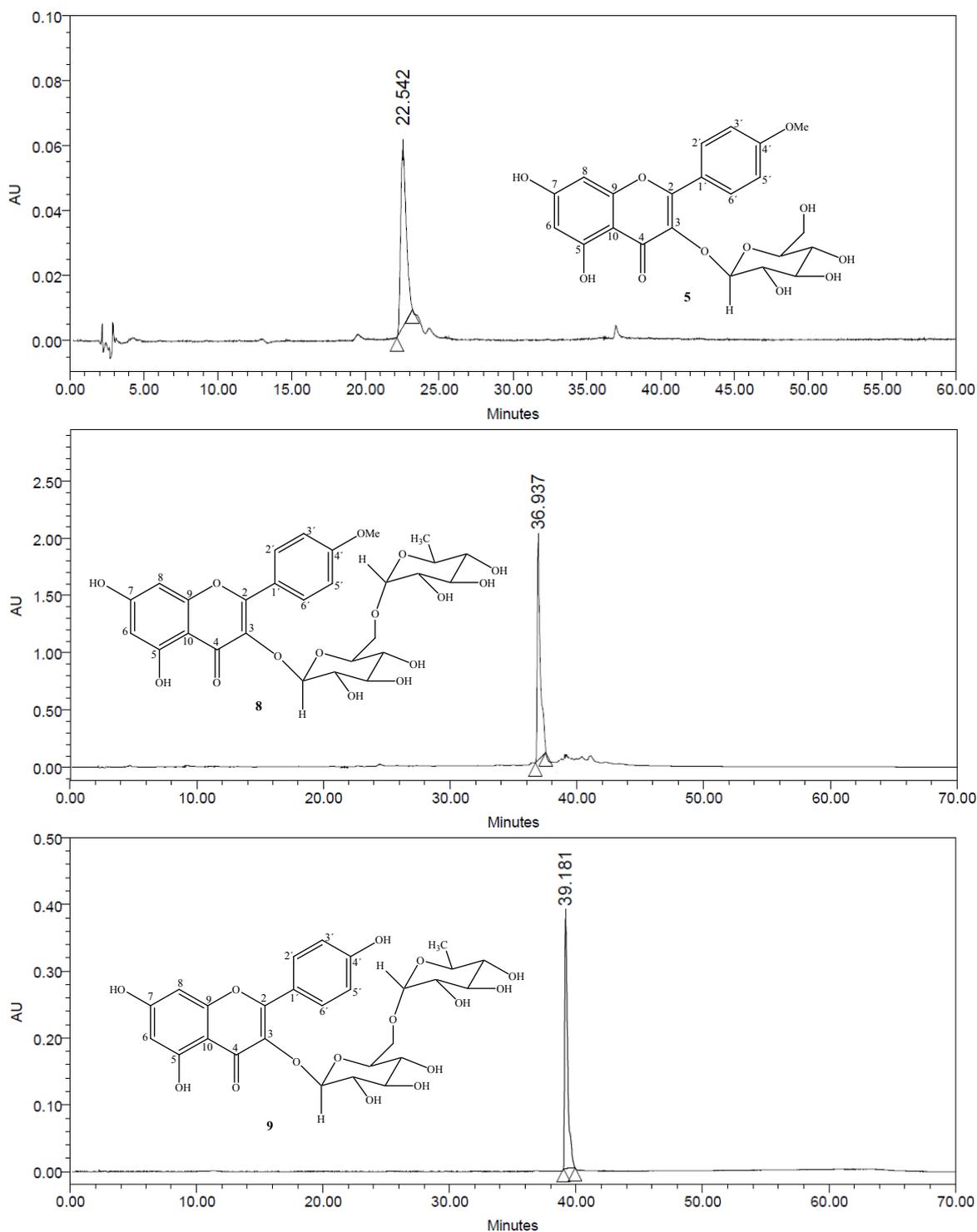


Figure 2. Cont.



3.5. Spectral Data

Protocatechuic acid (**1**). White crystals; m.p. 199–201 °C; HREIMS: $m/z = 154.0258$ (calc. for $C_7H_6O_4$, 154.0266). 1H -NMR (CD_3OD , 500 MHz): δ 6.80 (d, 1H, $J = 8.1$ Hz, 5-H), 7.30 (dd, 1H, $J = 2.0, 8.1$ Hz, 6-H), 7.46 (d, 1H, $J = 2.0$ Hz, 2-H); ^{13}C -NMR (CD_3OD , 125 MHz): δ 167.5 (COOH), 150.1 (C-4), 145.0 (C-3), 122.0 (C-6), 121.8 (C-1), 116.7 (C-2), 115.3 (C-5).

trans-Caffeic acid (**2**). White solid; m.p. 129–130 °C; HREIMS: $m/z = 180.0391$ (calc. for $C_9H_8O_4$, 180.0423). 1H -NMR (CD_3OD , 500 MHz): δ 7.51 (1H, d, $J = 15.9$ Hz, H-1'), 6.23 (1H, d, $J = 15.9$ Hz, H-2'), 7.14 (1H, d, $J = 1.8$ Hz, H-2), 7.02 (1H, dd, $J = 1.8, 8.1$ Hz, H-6), 6.85 (1H, d, $J = 8.1$ Hz, H-5); ^{13}C -NMR (CD_3OD , 125 MHz): δ 125.2 (C-1), 115.9 (C-2), 117.0 (C-5), 122.0 (C-6), 149.6 (C-4), 147.5 (C-3), 175.0 (C-1'), 123.5 (C-2'), 156.0 (C-3').

Methyl rosmarinate (**3**). Yellow amorphous powder; m.p. 164–166 °C; HRFABMS: $m/z = 375.1072$ (calc. for $C_{19}H_{19}O_8$, 375.1080). 1H -NMR (CD_3OD , 500 MHz): δ 7.55 (1H, d, $J = 15.5$ Hz, H-7'), 7.04 (1H, d, $J = 2.0$ Hz, H-2'), 6.95 (H, dd, $J = 8.5, 2.0$ Hz, H-6'), 6.78 (1H, d, $J = 8.5$ Hz, H-5'), 6.70 (1H, d, $J = 2.0$ Hz, H-2), 6.69 (1H, d, $J = 8.0$ Hz, H-5), 6.57 (1H, dd, $J = 8.0, 2.0$ Hz, H-6), 6.26 (1H, d, $J = 15.5$ Hz, H-8'), 5.19 (1H, dd, $J = 7.5, 5.0$ Hz, H-8), 3.70 (3H, s, OCH_3), 3.06 (1H, dd, $J = 14.5, 5.5$ Hz, H-7a), 3.00 (1H, dd, $J = 14.5, 5.5$ Hz, H-7b); ^{13}C -NMR (CD_3OD , 125 MHz): δ 172.34 (C-9), 168.50 (C-9'), 150.10 (C-4'), 148.14 (C-7'), 147.03 (C-3'), 146.37 (C-3), 145.55 (C-4), 128.89 (C-1), 127.67 (C-1'), 123.38 (C-6'), 121.92 (C-6), 117.67 (C-2), 116.66 (C-5'), 116.45 (C-5), 115.34 (C-2'), 114.23 (C-8'), 74.82 (C-8), 52.82 (OCH_3), C-38.05 (C-7).

Rosmarinic acid (**4**). Yellow amorphous powder; m.p. 172–174 °C; HRFABMS: $m/z = 361.0875$ (calc. for $C_{18}H_{17}O_8$, 361.0923). 1H -NMR (CD_3OD , 500 MHz): δ 7.51 (1H, d, $J = 15.5$ Hz, H-7'), 7.03 (1H, d, $J = 2.0$ Hz, H-2'), 6.91 (1H, dd, $J = 8.0, 2.0$ Hz, H-6'), 6.77 (1H, d, $J = 8.0$ Hz, H-5'), 6.72 (1H, d, $J = 2.0$ Hz, H-2), 6.68 (1H, d, $J = 8.0$ Hz, H-5), 6.63 (1H, dd, $J = 8.0, 2.0$ Hz, H-6), 6.27 (1H, d, $J = 15.5$ Hz, H-8'), 5.09 (1H, dd, $J = 10.0, 3.5$ Hz, H-8), 3.10 (1H, dd, $J = 14.5, 3.5$ Hz, H-7a), 2.94 (1H, dd, $J = 14.5, 10.0$ Hz, H-7b); ^{13}C -NMR (CD_3OD , 125 MHz): δ 177.64 (C-9), 169.24 (C-9'), 149.50 (C-4'), 146.85 (C-3'), 146.79 (C-7'), 146.08 (C-3), 144.93 (C-4), 131.29 (C-1), 128.12 (C-1'), 123.04 (C-6'), 121.89 (C-6), 117.63 (C-2), 116.60 (C-5'), 116.34 (C-5), 115.77 (C-8'), 115.27 (C-2'), 77.79 (C-8), 38.93 (C-7).

Kaempferide-3-O- β -D-glucopyranoside (**5**). Yellow amorphous powder; m.p. 165–167 °C; HRFABMS: $m/z = 461.1072$ (calc. for $C_{22}H_{21}O_{11}$, 461.1084). 1H -NMR: δ ($DMSO-d_6$, 500 MHz,) 7.90 (2H, d, $J = 8.4$ Hz, H-2', 6'), 6.87 (2H, d, $J = 8.4$ Hz, H-3', 5'), 6.41 (1H, brs, H-8), 6.23 (1H, brs, H-6), 5.30 (1H, d, $J = 7.5$ Hz, H-1''), 3.2–4.3 (6H, sugar), 3.85 (3H, s, OCH_3). ^{13}C -NMR ($DMSO-d_6$, 125 MHz): δ 156.9 (C-2), 133.5 (C-3), 177.6 (C-4), 161.4 (C-5), 98.8 (C-6), 164.6 (C-7), 94.2 (C-8), 156.7 (C-9), 103.9 (C-10), 121.5 (C-1'), 131.4 (C-2', C-6'), 116.7 (C-3', C-5'), 157.5 (C-4'), 101.5 (C-1''), 74.3 (C-2''), 76.5 (C-3''), 70.3 (C-4''), 76.0 (C-5''), 62.2 (C-6''), 56.7 (OCH_3).

Kaempferol-3-O- β -D-glucopyranoside (**6**). Yellow amorphous powder; m.p. 176–178 °C; HRFABMS: $m/z = 447.0897$ (calc. for $C_{21}H_{19}O_{11}$, 447.0927). 1H -NMR ($DMSO-d_6$, 500 MHz): δ 7.91 (2H, d, $J = 8.3$ Hz, H-2', 6'), 6.86 (2H, d, $J = 8.2$ Hz, H-3', 5'), 6.38 (1H, brs, H-8), 6.21 (1H, brs, H-6), 5.32 (1H, d, $J = 7.5$ Hz, H-1''), 3.2–4.3 (6H, sugar). ^{13}C -NMR ($DMSO-d_6$, 125 MHz): δ 156.8 (C-2), 133.6 (C-3), 177.8 (C-4), 161.6 (C-5), 98.9 (C-6), 164.5 (C-7), 94.0 (C-8), 156.6 (C-9), 104.2 (C-10), 121.3 (C-1'), 131.0 (C-2', C-6'), 115.5 (C-3', C-5'), 160.0 (C-4'), 101.4 (C-1''), 74.5 (C-2''), 76.7 (C-3''), 70.1 (C-4''), 75.9 (C-5''), 62.3 (C-6'').

Quercetin 3-O-β-D-glucopyranoside (7). Yellow amorphous powder; m.p. 229–231 °C; HRFABMS: $m/z = 463.0758$ (calc. for $C_{21}H_{19}O_{12}$, 463.0876). 1H -NMR (DMSO- d_6 , 500 MHz): δ 6.10 (1H, d, $J = 1.9$ Hz, H-6), 6.26 (1H, d, $J = 1.9$ Hz, H-8), 6.85 (1H, d, $J = 7.5$ Hz, H-5'), 7.57 (1H, dd, $J = 2.0$, 7.5 Hz, H-6'), 7.70 (1H, d, $J = 2.0$ Hz, H-2'), 5.10 (1H, d, $J = 7.7$ Hz, H-1''), 3.30–3.80 (6H, m, H-2'', H-3'', H-4'', H-5'', H-6''); ^{13}C -NMR (DMSO- d_6 , 125 MHz): δ 158.1 (C-2), 134.9 (C-3), 179.2 (C-4), 162.8 (C-5), 100.9 (C-6), 166.8 (C-7), 94.7 (C-8), 158.8 (C-9), 105.0 (C-10), 123.2 (C-1'), 116.4 (C-2'), 146.1 (C-3'), 149.8 (C-4'), 117.6 (C-5'), 122.8 (C-6'), 101.5 (C-1''), 74.5 (C-2''), 76.6 (C-3''), 70.3 (C-4''), 77.2 (C-5''), 62.1 (C-6'').

Kaempferide-3-O-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside (8). Yellow amorphous powder; m.p. 202–204 °C; HRFABMS: $m/z = 607.1578$ (calc. for $C_{28}H_{31}O_{15}$, 607.1663). 1H -NMR (DMSO- d_6 , 500 MHz): δ 7.95 (2H, d, $J = 8.5$ Hz, H-2', 6'), 6.87 (2H, d, $J = 8.5$ Hz, H-3', 5'), 6.44 (1H, br.s, H-8), 6.25 (1H, br.s, H-6), 5.30 (1H, d, $J = 7.4$ Hz, H-1''), 4.38 (1H, br.s, H-1'''), 2.9–4.5 (10H, m, sugar), 3.82 (3H, s, OCH₃), 1.12 (3H, d, $J = 6.2$ Hz, CH₃). ^{13}C -NMR (DMSO- d_6 , 125 MHz): δ 156.4 (C-2), 133.1 (C-3), 177.2 (C-4), 161.1 (C-5), 98.7 (C-6), 164.0 (C-7), 93.7 (C-8), 156.7 (C-9), 103.7 (C-10), 120.7 (C-1'), 131.3 (C-2', C-6'), 116.5 (C-3', C-5'), 158.6 (C-4'), 101.2 (C-1''), 74.0 (C-2''), 76.2 (C-3''), 69.9 (C-4''), 75.8 (C-5''), 66.7 (C-6''), 100.6 (C-1'''), 70.1 (C-2'''), 70.6 (C-3'''), 71.7 (C-4'''), 68.3 (C-5'''), 56.9 (OCH₃), 17.5 (C-6''').

Kaempferol-3-O-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside (9). Yellow amorphous powder; m.p. 219–221 °C; HRFABMS: $m/z = 593.1397$ (calc. for $C_{27}H_{29}O_{15}$, 593.1506). 1H -NMR (DMSO- d_6 , 500 MHz): δ 7.97 (2H, d, $J = 8.6$ Hz, H-2', 6'), 6.89 (2H, d, $J = 8.6$ Hz, H-3', 5'), 6.42 (1H, brs, H-8), 6.24 (1H, brs, H-6), 5.32 (1H, d, $J = 7.6$ Hz, H-1''), 4.39 (1H, brs, H-1'''), 3.0–4.4 (10H, m, sugar), 1.14 (3H, d, $J = 6.5$ Hz, CH₃). ^{13}C -NMR (DMSO- d_6 , 125 MHz): δ 156.9 (C-2), 133.5 (C-3), 177.0 (C-4), 161.4 (C-5), 99.2 (C-6), 164.4 (C-7), 93.9 (C-8), 156.5 (C-9), 103.4 (C-10), 121.8 (C-1'), 130.5 (C-2', C-6'), 115.3 (C-3', C-5'), 160.1 (C-4'), 101.5 (C-1''), 74.3 (C-2''), 76.5 (C-3''), 70.0 (C-4''), 75.4 (C-5''), 66.5 (C-6''), 100.7 (C-1'''), 70.2 (C-2'''), 70.6 (C-3'''), 71.7 (C-4'''), 68.1 (C-5'''), 17.5 (C-6''').

4. Conclusions

Compounds **1–9** were isolated from the first time from the aerial parts of *C. sinensis* and showed significant biological activities. Furthermore the purity of compounds was also checked by HPLC and the data reported here should contribute to the phytochemical inventory of the species.

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Conflict of Interest

The authors declare no conflict of interest.

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