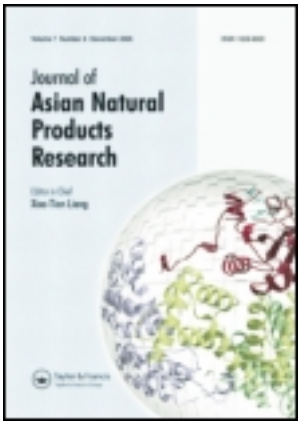

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Antioxidant and urease inhibitory *C*-glycosylflavonoids from *Celtis africana*

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Two new *C*-glycosylflavonoids celtisides A (**1**) and B (**2**) have been isolated from *n*-butanol-soluble fraction of *Celtis africana*, along with five known *C*-glycosylflavonoids vitexin (**3**), orientin (**4**), isoswertiajaponin (**5**), isoswertisin (**6**), and 2″-*O*-rhamnosyl vitexin (**7**) reported for the first time from this species. Their structures were assigned from 1D and 2D NMR spectra. These compounds were investigated for biological activities and showed significant antioxidant and urease inhibitory activities.

Keywords: *Celtis africana*; *C*-glycosylflavones; antioxidant; urease enzyme inhibition

1. Introduction

The genus *Celtis* belonging to the family Ulmaceae comprises 70 species. One of these is *Celtis africana* Burm.f., which is a medicinal plant. The sun-dried bark and roots are powdered and infused in water or milk and taken orally every day by the patient for treatment of cancer in South Africa [1]. The leaves of *C. africana* are also used as an African traditional human and veterinary medicine for the treatment of indigestion and edema [2,3]. Shifting of literature has revealed that no phytochemical work has been carried out on *C. africana*. Its ethanolic extract showed antioxidant and urease inhibitory activities, and on subsequent fractionation the major activity was located in the *n*-butanol-soluble fraction. This prompted us to carry out bioassay-directed isolation studies on the *n*-butanol-soluble fraction of *C. africana*. As a result, we herein report the isolation and structure elucidation of two new *C*-glycosylflavonoids named celtisides A (**1**) and B (**2**) along with five known *C*-glycosylflavonoids vitexin (**3**), orientin (**4**), isoswertiajaponin (**5**) [4], isoswertisin (**6**) [5], and 2″-*O*-rhamnosyl vitexin (**7**) [6] (Figure 1). The *n*-butanol-soluble fraction and isolated glycosides **1**–**7** were evaluated for their antioxidant activity using DPPH free radical scavenging assay [7]. Compounds **1**–**7** were also screened for their urease inhibitory activity using Jack bean urease [8].

2. Results and discussion

The active *n*-butanol-soluble sub-fraction of the ethanol extract of *C. africana* was subjected to column chromatography on Sephadex LH-20 to obtain five known and two new flavonoid glycosides, named celtisides A (**1**) and B (**2**).

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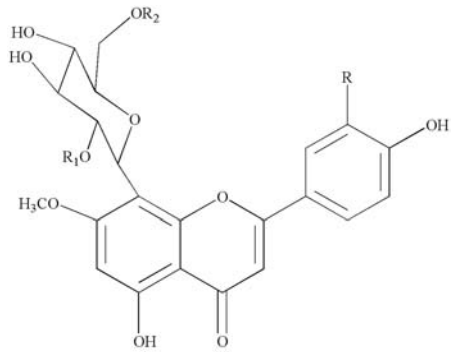

Figure 1. Structures of compounds **1** and **2**.

Celtiside A (**1**) was obtained as a yellow amorphous solid and gave a violet coloration with ferric chloride. The HR-FAB-MS of **1** gave $[M - H]^-$ peak at *m/z* 591.1695 corresponding to the molecular formula $C_{28}H_{31}O_{14}$. It further showed prominent fragments at *m/z* 446 and 297 due to the successive losses of sugar units. The IR spectrum showed the presence of hydroxyl groups (3370 cm^{-1}), conjugated carbonyl (1680 cm^{-1}), and aromatic moiety (1545 and 1490 cm^{-1}). The UV spectrum exhibited characteristic absorption maxima of a flavone at 330 and 274 nm.

The ^{1}H NMR spectrum displayed a signal at δ 13.30 for a chelated hydroxyl group and one singlet at δ 3.85 (s) for the methoxyl protons. A C-6 or C-8 substituted flavonoid skeleton was suggested by the appearance of one-proton singlet at δ 6.87, typical for the H-3 of flavones. Two signals of ring B with AA′BB′ pattern at δ 8.05 (d, $J = 9.0$ Hz) and 6.90 (d, $J = 9.0$ Hz) indicated 4′-substituted ring B, and another one-proton singlet at δ 6.50 suggesting that ring A was trisubstituted. Apart from the aromatic protons, two anomeric protons were observed at δ 4.61 (d, $J = 9.1$ Hz) and 4.57 (br s), suggesting the occurrence of two sugar units with β and α configurations, respectively. Further signals of the hexose moiety were observed between δ 4.25 and 3.18. The ^{1}H NMR spectrum further showed a methyl doublet at δ 0.49 (d, $J = 6.0$ Hz), suggesting that one of the sugar residue is rhamnose. The ^{13}C NMR spectrum of **1** showed 28 signals comprising 2 methyl, 1 methylene, 16 methine, and 9 quaternary carbons. The signals at δ 162.0, 102.6, 182.5, and 105.8 were typical of C-2, C-3, C-4, and C-10 of a flavone moiety [9]. Apart from further peaks of the aromatic carbons, the spectrum showed the signals of two anomeric carbons at δ 74.5 and 101.9, oxymethine carbons ranging between δ 70.1 and 79.0, oxymethylene carbons at δ 65.8 and a methyl group at δ 17.7. These data and resistance to hydrolysis suggested that celtiside A (**1**) is a C-8 diglycoside of flavonoid with L-rhamnose at the terminal position. The downfield shift of the oxymethylene carbon of the glucose moiety at δ 65.8 indicated the 1 → 6 linkage between L-rhamnose and D-glucose. The point of attachment of the sugar moiety was shown to be C-8 through HMBC correlations (Figure 2). It was also

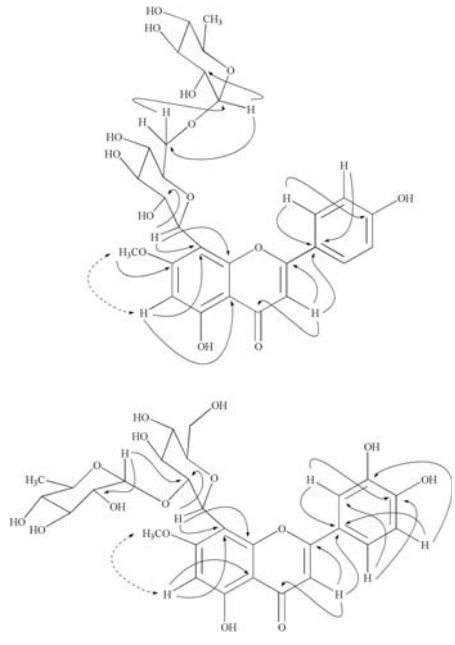

Figure 2. Important HMBC (→) and NOESY (↔) correlations of compounds **1** and **2**.

confirmed by the ^{13}C NMR chemical shifts of C-8 (δ 104.3) and C-6 (δ 94.5), which were similar to the data reported for C-8 glycosylflavones [10]. The position of the methoxy group on ring A was confirmed by the HMBC experiment in which OCH_3 proton (δ 3.85) showed a ^3J correlation to C-7 (δ 163.2). The position of the methoxyl group was further confirmed by NOE difference experiments, and the irradiation of the methoxyl proton at δ 3.85 (3H, s) resulted in the enhancement of H-6 (δ 6.50). On the basis of these evidences, the structure of celtiside A (**1**) could be assigned as isoswertisin 8-C-[α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside].

Celtiside B (**2**) was obtained as a yellow amorphous powder. The molecular formula was determined as $C_{28}H_{31}O_{15}$ by HR-FAB-MS, showing the $[M - H]^-$ peak at *m/z* 607.1650, which differed from that of celtiside **1** by 16 mass units. The UV and IR spectra were very similar to those of **1**. In FAB-MS, the prominent fragment at *m/z* 461 resulted from the loss of rhamnose moiety. The most significant difference between the ^1H NMR spectra of **1** and **2** was the replacement of the AA′BB′ spin system by ABX type pattern [δ 7.45 (d, $J = 2.0$ Hz, H-2′), δ 6.89 (d, $J = 8.5$ Hz, H-5′), δ 7.50 (dd, $J = 8.5$ Hz, 2.0, H-6′)] which suggested that ring B is substituted by two hydroxyl groups [11]. The other major difference was observed in the interglycoside linkage between rhamnose and glucose sugar moieties. It was authenticated by the upfield shift of oxymethylene carbon (δ 61.0) as well as oxymethylene protons (δ 3.55 and 3.74) of the glucose with subsequent deshielding of both proton (δ 4.02) and carbon (δ 75.1) signals of C-2″, which is in conformity of rhamnose attachment at C-2″ of the glucose moiety. The interglycoside connection pattern could also be confirmed by comparing its ^{13}C NMR spectral data to those of another compound which has the same (1 → 2) sugar linkage as compound **2** [12]. It was further confirmed by HMBC correlation of H-2″ with anomeric carbon of the rhamnose sugar (δ 100.4). Signals of the two sugar moieties were completely assigned from the HMQC and HMBC spectra. On the basis of these cumulative evidences, the structure of celtiside B (**2**) could be assigned as 7-methoxy luteolin 8-*C*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside].

Compounds **1**–**7** were evaluated for their antioxidant activity as given in Table 1. All the isolated flavonoid glycosides showed high to moderate antioxidant activity. It was also observed that the antioxidant activity of the *n*-butanol-soluble fraction is more pronounced than the isolated compounds; this may be due to the result of some kind of synergism or interaction among compounds.

The inhibitory activity of **1**–**7** against urease was determined by the method used by Weatherburn [8]. The data indicated that compounds **3**–**6** were potent urease inhibitors with IC_{50} values 35, 28, 38, and 43 μM, respectively, as against IC_{50} value of 21.5 μM observed for thiourea used as a positive control (Table 2). The compounds **1**, **2**, and **7** did not show pronounced activity. So, it is evident that the urease inhibitory activity is decreased by the incorporation of additional sugar moiety,

Table 1. IC_{50} (μM) values of **1**–**7** in antioxidant assay.

	DPPH scavenging activity
Sr. no.	IC_{50}[a] (μM)
n-Butanol-soluble fraction	40.5 ± 0.13
1	85.5 ± 0.12
2	75.3 ± 0.13
3	48.4 ± 0.11
4	43.2 ± 0.19
5	49.5 ± 0.14
6	54.5 ± 0.19
7	83.2 ± 0.12
Butylated hydroxyanisole[b]	44.3 ± 0.09

Notes: [a]Values ± standard mean error (SEM) of three assays.
[b]Standard DPPH scavenging activity.

Table 2. *In vitro* quantitative inhibition of urease by compounds **1**–**7**.

Compounds	$IC_{50} \pm SEM$ (μM)[a]
1	99 ± 0.12
2	95 ± 0.20
3	35 ± 0.18
4	28 ± 0.15
5	38 ± 0.25
6	43 ± 0.12
7	92 ± 0.15
Thiourea	21.5 ± 0.12

Note: [a]SEM ($n = 3$–5).

as in the case of compounds **1**, **2**, and **7** (flavonoid diglycoside).

3. Experimental

3.1 General experimental procedures

The melting points were recorded on a Buchi melting point apparatus and are uncorrected. Optical rotation $[\alpha]_D^{25}$ was determined using a Jasco-DIP-360 digital polarimeter. The UV and IR spectra were recorded on Hitachi-UV-3200 and JASCO 320-A spectrometers, respectively. The ^1H, ^{13}C, and 2D NMR spectra were recorded on a Bruker AMX-400, 100 spectrometer. Chemical shifts are in ppm (δ), relative to tetramethylsilane as an internal standard and scalar coupling constants (J) reported in Hz. FAB and HR-FAB-MS: (neg. ion mode, matrix: glycerol) are performed on a JEOL JMS-HX110 mass spectrometer. Mass spectra (EI-MS) were measured in an electron impact mode on Finnigan MAT 12 and MAT 312 spectrometers and ions are given in m/z (%). Column chromatography was carried out on Sephadex LH-20. Preparative high-performance liquid chromatography (HPLC) was used for final purification on a recycling preparative HPLC (LC-908W-C-60, Japan Analytical Industry Co. Ltd, Tokyo, Japan) using a column of ODS-M-80 [4 μM (250 × 20 mm)]. Thin layer chromatography (TLC) was performed on precoated silica gel F_{254} plates (E. Merck, Darmstadt, Germany); the detection was done at 254 nm and by spraying with ceric sulfate reagent. All chemicals and urease were purchased from Sigma Chemical Company (St Louis, MO, USA).

3.2 Plant material

The aerial parts of *C. africana* (2.5 kg) were collected from Riyadh (Saudi Arabia) and air-dried. The identity of the plant was verified by Dr M. Atiqur Rahman, Plant Taxonomist, College of Pharmacy, King Saud University. A voucher specimen (No. 44) has been deposited in the herbarium of the Department of Pharmacognosy, King Saud University.

3.3 Extraction and isolation

The aerial parts of *C. africana* (2.5 kg) were shade-dried, ground, and extracted at room temperature with EtOH:H_2O (8:2, thrice). The combined ethanol extract (100 g) was divided into *n*-hexane (25 g), $CHCl_3$ (20 g), *n*-BuOH (30 g), and water (20 g)-soluble sub-fractions. A part of *n*-BuOH-soluble fraction (25 g) was dissolved in water and loaded on Sephadex LH-20 column and the elution was successively carried out with water and mixtures of H_2O and MeOH in decreasing order of polarity, leading to four major sub-fractions I–IV. The sub-fraction I (22 mg) obtained from MeOH–H_2O (1:9) showed one major spot on TLC along with little impurities and further purified by HPLC with flow rate 1.0 ml/min eluted with MeOH–H_2O (1.3:8.7), which delivered compound (**2**, 15 mg). The sub-fraction II (46 mg) obtained from MeOH–H_2O (1.5:8.5) was a binary mixture which was separated through HPLC with flow rate 0.5 ml/min using MeOH–H_2O (2:8) as eluant, to afford (**1**, 12 mg) and (**7**, 25 mg). The purification of sub-fraction III (58 mg) was carried out on HPLC with flow rate 0.8 ml/min using MeOH–H_2O (3:7) as eluant to afford

Table 3. ^{1}H and ^{13}C NMR spectral data of **1** and **2** in DMSO-d_6.

	1		**2**	
Position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
2	–	162.0	–	162.4
3	6.87 (s)	102.6	6.85 (s)	103.3
4	–	182.5	–	182.3
5	–	160.7	–	160.5
6	6.50 (s)	94.5	6.47 (s)	95.2
7	–	163.2	–	162.8
8	–	104.3	–	104.6
9	–	155.0	–	154.8
10	–	105.8	–	105.7
1′	–	121.0	–	121.9
2′	8.05 (d, 9.0)	129.1	7.45 (d, 2.0)	113.1
3′	6.90 (d, 9.0)	115.7	–	145.6
4′	–	161.3	–	149.5
5′	6.90 (d, 9.0)	115.7	6.89 (d, 8.5)	116.0
6′	8.05 (d, 9.0)	129.1	7.50 (dd, 8.5, 2.0)	118.9
5-OH	13.30	–	13.33	–
1″	4.61 (d, 9.1)	74.5	4.78 (d, 9.3)	71.5
2″	3.42 (m)	71.4	4.02 (t, 9.3)	75.1
3″	3.36 (m)	79.0	3.51 (m)	79.8
4″	3.18(m)	71.9	3.41 (m)	70.4
5″	3.48 (m)	73.5	3.23 (m)	81.9
6″	3.78 (dd, 12.2, 4.6)	65.8	3.55 (dd, 12.1, 4.8)	61.0
	3.95 (m)		3.74 (dd, 12.1, 2.8)	
1‴	4.57 (br s)	101.9	5.12 (br s)	100.4
2‴	4.25 (m)	72.0	3.76 (m)	70.3
3‴	3.41 (m)	71.2	3.01 (m)	70.2
4‴	3.21(m)	71.6	2.88 (m)	71.4
5‴	3.97 (m)	70.1	3.01 (m)	68.3
6‴	0.49 (d, 6.0)	17.7	0.46 (d, 6.0)	17.5
7-OMe	3.85 (s)	56.4	3.83 (s)	56.5

orientin (**4**, 30 mg) and isoswertiajaponin (**5**, 14 mg). Sub-fraction IV (63 mg), eluted with MeOH–H_2O (2:8), was subjected to HPLC with flow rate 0.6 ml/min using MeOH–H_2O (3.5:6.5) to afford vitexin (**3**, 32 mg) and isoswertisin (**6**, 15 mg).

3.3.1 *Celtiside A (**1**)*

An amorphous powder, m.p. 208–210°C, $[\alpha]_D^{25}$ −74 ($c = 0.04$, MeOH). UV (MeOH) λ_{max} (log ε): 330 (4.35), 274 (4.30) nm. IR (KBr): 3370, 1680, 1545, and 1490 cm^{-1}. ^{1}H and ^{13}C NMR spectral data see Table 3. EI-MS *m/z* (rel. int.): 297 (32), 180 (20), 118 (100). HR-FAB-MS (− ve ion mode): *m/z* 591.1695 $[M - H]^-$ (calcd for $C_{28}H_{31}O_{14}$, 591.1713).

3.3.2 *Celtiside B (**2**)*

An amorphous powder, m.p. 219–221°C, $[\alpha]_D^{25}$ −35 ($c = 0.03$, MeOH). UV (MeOH) λ_{max} (log ε): 328 (4.32), 275 (4.28) nm. IR (KBr) ν_{max}: 3372, 1678, 1546, and 1490 cm^{-1}. ^{1}H and ^{13}C NMR spectral data see Table 3. EI-MS *m/z* (rel. int.): 313 (15), 180 (25), 134 (55). HR-FAB-MS (− ve ion mode): *m/z* 607.1650 $[M - H]^-$ (calcd for $C_{28}H_{31}O_{15}$, 607.1663).

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References

[1] K. Srinivas, D.S. Grierson, and A.J. Afolayan, *Curr. Sci.* **92**, 906 (2007).
[2] I.C. Verdoorn, *Celtis Africana: The Flowering Plants of Africa* (Botanical Research Institute, South Africa, 1956), Vol. 31, p. 1210.
[3] S. Krief, C.M. Hladik, and C. Haxaire, *J. Ethnopharmacol.* **101**, 1 (2005).
[4] X. Zhou, J.Y. Peng, G.R. Fan, and Y.T. Wu, *J. Chromatogr. A* **1092**, 216 (2005).
[5] M.D. Greca, M. Ferrara, A. Fiorentino, P. Monaco, and L. Previtera, *Phytochemistry* **49**, 1299 (1998).
[6] P. Elisa, R.B. Anna, D.F. Vincenzo, and I. Morelli, *Phytochemistry* **35**, 1381 (1994).
[7] I. Gulcin, H.A. Alici, and M. Cesur, *Chem. Pharm. Bull.* **53**, 281 (2005).
[8] M.W. Weatherburn, *Anal. Chem.* **39**, 971 (1967).
[9] A. Stochmal, A.M. Simonet, F.A. Macias, M.A. Oliveira, J.M. Abreu, R. Nash, and W. Oleszek, *Phytochemistry* **57**, 1223 (2001).
[10] G. Cheng, Y.J. Bai, Y.Y. Zhao, J. Tao, Y. Liu, G.Z. Tu, L.B. Ma, N. Liao, and X.J. Xu, *Tetrahedron* **56**, 8915 (2000).
[11] J.H. Zou, J.S. Yang, Y.S. Dong, L. Zhou, and G. Lin, *Phytochemistry* **66**, 1121 (2005).
[12] M. Kaltenhauser, E.P. Ellmerer, and C. Zidorn, *J. Serb. Chem. Soc.* **75**, 733 (2010).