Nawal M. Al Musayeib*, Sabrin R.M. Ibrahim*, Musarat Amina, Gadah A. Al Hamoud and Gamal A. Mohamed

Curviflorside and curviflorin, new naphthalene glycoside and flavanol from *Plicosepalus* curviflorus

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Abstract: The naphthalene glycosidecurviflorside [1,5-dihydroxy-8-methoxynaphthalene-2-*O*-β-D-xylopyranoside] (**3**) and the flavanol curviflorin [(+)-catechin-7-*O-3"*,4"-dihydroxybenzoate] (**4**), along with two known flavonoids: (+)-catechin (**1**) and quercetin (**2**) were isolated from the shoots of *Plicosepalu scurviflorus* Benth. (Loranthaceae) growing in Saudi Arabia and the chemical structures were elucidated by 2D-NMR spectroscopy.

Keywords: curviflorin; curviflorside; flavonoids; naphthalene; *Plicosepalus curviflorus*.

1 Introduction

Naphthalenes are reported from fungi, plants, insects, and liverworts. Naphthalenes possess a wide range of bioactivities: antimicrobial, antioxidant, cytotoxic, anti-inflammatory, anti-platelet aggregation, and antiprotozoal [1]. Several naphthalene-containing drugs are available, such as nafacillin, naftifine, tolnaftate, and terbinafine,

*Corresponding authors: Nawal M. Al Musayeib, Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia, Phone: +966 598566807, E-mail: nawal.pharmacy@gmail.com; nalmusayeib@ksu.edu.sa; and Sabrin R.M. Ibrahim, Department of Pharmacognosy and Pharmaceutical Chemistry, College of Pharmacy, Taibah University, Al Madinah Al Munawwarah 30078, Saudi Arabia, Phone: +966 581183034, E-mail: sabrinshaur@gmail.com; sribrahim@taibahu.edu.sa; and Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt Musarat Amina and Gadah A. Al Hamoud: Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia

Gamal A. Mohamed: Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Saudi Arabia; and Department of Pharmacognosy, Faculty of pharmacy, Al-Azhar University, Assiut Branch, Assiut 71524, Egypt

which play a vital role in controlling microbial infection [2]. Flavan-3-ols are the most common group of flavonoids in dietare 3-ring phenolic compounds having multiple hydroxyl groups on the A, B, and C rings. They are considered functional ingredients of fruits, beverages, vegetables, food grains, dietary supplements, herbal remedies, and dairy products [3]. Many studies indicated that they exhibited several health beneficial effects by acting as antioxidant, anti-diabetic, anti-proliferative, neuro- and cardio-protective, antimicrobial, and antiviral agents [3-5]. Family Loranthaceae plays an important and complex role in the biological system in which species of this family live by interacting with insects, birds, and mammals [6]. This family comprises four genera: Phragmanthera, Oncocalyx, Tapinanthus, and Plicosepalus, which grow naturally in Saudi Arabia. These genera include six species, Plicosepalus acacia, Plicosepalus curviflorus, Phragmanthera austroarabica, Oncocalyx schimperi, Oncocalyx glabratus, and Tapinanthus globiferus spread in the north, west, and south of the Saudi Arabia [7]. Earlier investigations on genus *Plicosepalus* reported various biological activities such as antioxidant [8–10], antihepatotoxic [11], anti-diabetic [12-14], antiviral [10, 15], antimicrobial [8, 10, 16, 17], and cytotoxic [10, 18, 19] activities. A literature survey revealed that very little phytochemical work has been carried out on the genus Plicosepalus. Flavonoids [8, 14, 19], phenolic acids [5], triterpenes, sterols [14], and sesquiterpene lactones [20] have been isolated from different Plicosepalus species. Flavane gallates, triterpenes, and sterols have been isolated from P. curviflorus Benth. [8, 15]. P. curviflorus is a parasitic plant which is generally known as "Enam ElTalh" (in Arabic). It is found in north East Africa, East Africa, Yemen, and Saudi Arabia [21]. The stems of *P. curviflorus* are used for the treatment of cancer in Yemen [10] and traditionally used in Saudi Arabia for increasing lactation in cattle [21]. Moreover, P. curviflorus is used for the treatment of diabetes in Saudi Arabia folk medicine [22, 23]. The present work reports the isolation and structural elucidation of two new compounds (3 and 4), along with two known ones (Figure 1) from the shoots of *P. curviflorus*. Their structures were assigned by

Figure 1: Structure of isolated compounds 1-4.

extensive spectroscopic methods as well as comparison with the literature.

2 Materials and methods

2.1 General

Optical rotations were measured on a Perkin-Elmer Model 341 LC polarimeter (Perkin-Elmer, Waltham, MA, USA). Infrared (IR) spectra were measured with a Shimadzu Infrared-400 spectrophotometer (Shimadzu, Kyoto, Japan). The UV spectra were carried out in MeOH using a Perkin-Elmer Lambda 25 UV/VIS spectrophotometer (Perkin-Elmer, Waltham, MA, USA). Electrospray ionization mass spectrometry (ESIMS) spectra were recorded on a Finnigan MAT TSQ-7000 triple stage quadrupole mass spectrometer (Thermo Finnigan, Bremen, Germany). High-resolution electrospray ionization mass spectrometry (HRESIMS) spectra were obtained using an LTQ Orbitrap mass spectrometer (Thermo Fisher, Waltham, MA, USA). 1D and 2D nuclear magnetic resonance (NMR) spectra were measured on Bruker DRX 700 spectrometers (Bruker, Rheinstetten, Germany). Vacuum liquid chromatography (VLC) was performed using silica gel 60 (0.04-0.063 mm, Merck, Darmstadt, Germany). Column chromatographic separations were performed on silica gel 60 (0.04–0.063 mm, Merck, Darmstadt, Germany). Thin-layer chromatography (TLC) analyses were conducted on pre-coated silica gel F254 aluminum sheets (Merck, Darmstadt, Germany). Compounds were detected by spraying the sheets with p-anisaldehyde/

H₂SO₄ reagent (Sigma-Aldrich Chemical Co., Taufkirchen, Germany) followed by heating at 110°C for 1–2 min.

2.2 Plant material

Shoots of *P. curviflorus* were collected in March 2013 from Abha, Saudi Arabia. The plant was identified by Dr. Mohamed Yousef, Prof. of Pharmacognosy, College of Pharmacy, King Saud University, Saudi Arabia. A voucher specimen (PC-3-2010) was deposited at the Department's herbarium.

2.3 Extraction and isolation

The air-dried powdered shoots (500 g) were extracted with MeOH (2×3 L, each) using soxhlet apparatus for 8 h at room temperature. The combined extracts were concentrated under reduced pressure to afford a dark green residue (14.0 g). The latter was suspended in distilled water (150 mL) and partitioned between *n*-hexane (3×500 mL), EtOAc (3×500 mL), and *n*-BuOH (3×500 mL) successively. Each fraction was concentrated to give n-hexane (4.1 g), EtOAc (2.9 g), n-BuOH (1.8 g), and aqueous (4.6 g) fractions. The EtOAc fraction (2.9 g) was subjected to VLC using CHCl₂: MeOH gradient to afford five subfractions: PC-1 to PC-5. Subfraction PC-2 (232 mg) was chromatographed over silica gel column (50 g \times 50 \times 2 cm) using *n*-hexane: EtOAc gradients to get 1 (11.6 mg, yellow amorphous powder). Subfraction PC-2 (345 mg) was similarly treated as subfraction PC-1 to give 2 (34.7 mg, yellow amorphous powder).

Subfraction PC-3 (541 mg) was chromatographed over sephadex LH-20 column (100 g \times 50 \times 3 cm) using MeOH as an eluent to give two major subfractions: PC-3A (261 mg) and PC-3B (207 mg). Subfraction PC-3A was subjected to RP, column (50 g×50×2 cm) using MeOH: H,O gradient to afford 3 (4.6 mg, white amorphous powder). Subfraction PC-3B (207 mg) was similarly treated as subfraction PC-3A to give 4 (6.2 mg, yellow amorphous powder).

Curviflorside (3): White amorphous powder (4.6 mg). $[\alpha]_{\rm n}$: 52.3 (c 0.06, MeOH). UV (MeOH) $\lambda_{\rm max}$ (log ε): 237 (5.36), 258 (4.04), 297 (3.78) nm. IR (KBr) v_{max}: 3425, 2934, 1625, 1518, 1070 cm⁻¹. NMR data (DMSO- d_6 , 700 and 175 MHz), see Table 1. HRESIMS m/z 339.1075 (calcd for $C_{16}H_{19}O_{8}$, $[M+H]^+$, 339.1080).

Curviflorin (4): Yellow amorphous powder (6.2 mg). [α]_D: + 15.3 (c 0.5, MeOH). UV (MeOH) λ _{max} (log ε): 218 (4.86), 277 (2.96) nm. IR (KBr) v_{max}: 3356, 1732, 1614, 1519, 1463 cm⁻¹. NMR data (DMSO- d_{ϵ} , 700 and 175 MHz), see Table 2. HRESIMS m/z 427.1033 (calcd for $C_{22}H_{19}O_{o}$, $[M+H]^+$, 427.1029).

3 Results and discussion

Compound 3 was obtained as white amorphous powder, and its molecular formula was defined as C₁₆H₁₈O₈ by HRESIMS pseudo-molecular ion peak at m/z 339.1075 $[M+H]^+$ (calcd for $C_{16}H_{19}O_8$, 339.1080), requiring eight degrees of unsaturation (see Supplementary Material, Figure S17). Seven of degrees of unsaturation were

Table 1: NMR spectral data of compound 3 (DMSO-d_c, 700 and 175 MHz).

Position	$\delta_{_{\! H}}$ [multiplicity, / (Hz)]	$\delta_{_{C}}$ (multiplicity)	нмвс
1	_	152.6 C	_
2	_	140.1 C	-
3	7.55 d (8.2)	112.0 CH	1, 10
4	7.70 d (8.2)	112.8 CH	1, 2, 3, 5
5	-	149.0 C	-
6	7.47 d (8.5)	110.7 CH	8, 10
7	7.47 d (8.5)	109.1 CH	5, 9
8	-	148.9 C	-
9	_	108.1 C	-
10	-	123.5 C	-
1'	5.00 d (7.5)	103.3 CH	2
2′	3.41 m	73.5 CH	
3′	3.25 m	76.0 CH	
4'	3.43 m	69.8 CH	
5′	3.86 m	66.1 CH ₂	
	3.38 m	-	
8-OCH ₃	4.05 s	61.4 CH ₃	8

Table 2: NMR spectral data of compound 4 (DMSO- d_{s} , 700 and

Position	$\delta_{\rm H}$ [multiplicity, / (Hz)]	$\delta_{\rm c}$ (multiplicity)	НМВС
2	4.64 d (7.2)	81.6 CH	3, 4, 9, 1', 2', 6'
3	3.94 m	66.4 CH	2, 5, 10, 1'
4	2.67 dd (16.1, 5.2) 2.45 dd (16.1, 8.2)	28.1 CH ₂	2, 3, 5, 10
5	-	155.6 C	-
6	6.13 d (2.0)	100.6 CH	5, 7, 8, 10, 7"
7	_	150.7 C	_
8	6.21 d (2.0)	101.2 CH	6, 7, 9, 10, 7"
9	_	156.6 C	-
10	_	106.2 C	-
1'	_	130.7 C	-
2′	6.75 brs	114.9 CH	2, 4', 6'
3′	-	145.4 C	-
4'	-	146.1 C	-
5′	6.68 d (7.8)	115.6 CH	1', 3', 4'
6′	6.58 brd (7.8)	118.9 CH	2, 1', 2', 4'
1"	_	139.7 C	_
2"	7.09 d (2.2)	109.5 CH	1", 4", 6", 7"
3″	_	145.9 C	_
4"	-	146.1 C	-
5"	6.95 d (8.2)	108.0 CH	1", 3", 6"
6"	7.06 dd (8.2, 2.2)	109.5 CH	1", 2", 4", 7"
7"	-	164.9 C	-
3-0H	5.75 brs	_	_
5-OH	9.75 s	-	-
3"-OH	8.87 s	_	_
4"-OH	9.38 s	_	-

attributed to the naphthalene moiety and one for sugar moiety. The ESIMS revealed a fragment ion peak at m/z190 [(M+H)-149]+, indicating the loss of a pentose moiety. Its IR spectrum exhibited absorption bands for hydroxyl (3425 cm⁻¹) and aromatic (1625 and 1518 cm⁻¹) functionalities. The ¹³C NMR and heteronuclear single quantum coherence (HSQC) data showed resonances for 16 carbon signals: 10 carbon signals in the range δ_c 108.1–152.6 for a naphthalene ring, five oxygen-bonded aliphatic carbons in the range δ_{c} 66.1–103.3 for pentose moiety, and methoxy group (δ_c 61.4) (see Supplementary Material, Figures S12 and S15). The ¹H NMR spectrum of **3** exhibited two pairs of *ortho*-coupled aromatic protons at δ_{H} 7.55 (d, J=8.2 Hz, H-3), 7.70 (d, J=8.2 Hz, H-4), and 7.47 (2H, d, J=8.5 Hz, H-6, 7), correlating to the carbons at δ_c 112.0, 112.8, 110.7, and 109.1, respectively, in the HSQC spectrum characteristic for a tetra-substituted naphthalene moiety (Table 1). The heteronuclear multiple bond correlations (HMBC) of H-3 to C-1 and C-10; H-4 to C-1, C-2, C-3, and C-5; H-6 to C-8 and C-10; and H-7 to C-5 and C-9 confirmed the presence of such moiety (Figures 2 and S16). Moreover, the ¹H NMR spectrum showed characteristic signals of a sugar moiety

Figure 2: Some key HMBC correlations of 3 and 4.

between δ_{H} 3.25 and 5.00, including an anomeric proton signal at δ_{H} 5.00 (d, J = 7.5 Hz, H-1′). The sugar was assigned as xylopyranose according to 13C NMR data (Table 1), where signals belonging to the sugar moiety were three oxymethines at δ_c 69.8 (C-4'), 73.5 (C-2'), and 76.0 (C-3'), one oxymethylene at δ_c 66.1 (C-5'), and one anomeric oxymethine at δ_c 103.3 (C-1') [1, 24]. The configuration at the anomeric center of the xylopyranosyl moiety (C-1') was determined to be β based on the coupling constant value $(J_{y,y}=7.5 \text{ Hz})$. The sugar moiety was placed at C-2 on the basis of the ³J HMBC correlation of the anomeric proton to C-2 (δ_c 140.1). Moreover, signals for methoxy group at δ_{μ} 4.05/ δ_{c} 61.4 were observed. Its attachment at C-8 was established by the HMBC cross peak of the methoxy group to C-8 (δ_c 148.9). On the basis of these data, compound **3** was identified as 1,5-dihydroxy-8-methoxynaphthalene-2-*O*-β-D-xylopyranoside. The trivial name curviflorside was given to it.

Compound 4 was obtained as yellow amorphous powder and gave positive tests for flavonoids [25, 26]. The HRESIMS spectrum of 4 gave a pseudo-molecular ion peak at m/z 427.1033 [M+H]+, corresponding to the molecular formula C₂₂H₁₀O₀. The ESIMS spectrum showed a significant fragment ion peak at m/z 290 [M + H-(3,4-dihydroxybenzoyl)]+. Its UV spectrum displayed absorption bands at 218 and 277 nm, which are characteristic for the presence of a flavan-3-ol moiety in 4 [27]. Its IR spectrum exhibited absorption bands for hydroxyl (3356 cm⁻¹), ester carbonyl (1732 cm⁻¹), and phenyl (1614, 1519, and 1463 cm⁻¹) moieties. The ¹³C, distortionless enhancement by polarization transfer, and HSQC spectra of 4 showed the presence of 22 carbons, consisting of methylene, 8 aromatic methines, 2 oxymethines at δ_c 81.6 (C-2) and 66.4 (C-3), and 11 quaternary carbons, including

one carbonyl at δ_c 164.9 (C-7") (see Supplementary Material, Figures S19 and S20). The ¹H NMR spectrum showed resonances for two meta-coupled protons at $\delta_{_{\rm H}}$ 6.13 (d, J=2.0 Hz, H-6) and 6.21 (d, J=2.0 Hz, H-8) (Table 2). These signals showed HSQC cross peaks to the carbons, resonating at δ_c 100.6 and 101.2, respectively, consistent with a 5,7-dioxygenated A ring of flavane [28]. The ¹H NMR and ¹H-¹H correlation spectroscopy (COSY) also displayed an ABX system for 1,3,4-tri-substituted ring B at $\delta_{\rm H}$ 6.75 (brs, H-2'), 6.68 (d, J = 7.8 Hz, H-5'), and 6.58 (brd, J=7.8 Hz, H-6') [29]. They showed HSQC cross peaks to carbon signals at δ_c 114.9, 115.6, and 118.9, respectively (Table 2). Moreover, two oxymethine protons at $\delta_{_{\rm H}}$ 4.64 (d, J = 7.2 Hz, H-2) and 3.94 (m, H-3) and methylene group at δ_{H} 2.67 (dd, J = 16.1, 5.2 Hz, H-4A) and 2.45 (dd, J = 16.1, 8.2 Hz, H-4B) were observed, suggesting that 4 contained a catechin moiety. This moiety was confirmed by the observed ¹H-¹H COSY cross peaks and HMBC correlations of H-2 to C-4, C-9, C-1', C-2', and C-6'; H-3 to C-5, C-10, and C-1'; H-4 to C-2, C-3, C-5, and C-10; H-6 and H-8 to C-7 and C-10; H-2' and H-6' to C-2 and C-4'; and H-5' to C-1' and C-3' (Figure 2). This was further secured by the ESIMS fragment ion peak at m/z 290 [M + H-(3,4-dihydroxybenzoyl)]+. The ¹HNMR spectrum of **4** also displayed three coupled proton signals for a *tri*-substituted phenyl moiety at δ_{H} 7.09 (d, J = 2.2 Hz, H-2"), 6.95 (d, J = 8.2 Hz, H-5"), and 7.06 (dd, J = 8.2, 2.2 Hz, H-6"), indicating the presence of a 3.4-dihydroxybenzovl moiety [30]. This was confirmed by 13 C NMR signals at δ_c 139.7 (C-1"), 109.5 (C-2"), 145.9 (C-3"), 146.1 (C-4"), 108.0 (C-5"), 109.5 (C-6"), and 164.9 (C-7") and further secured by the observed HMBC correlations (Figure 2). Moreover, the four singlet signals at δ_{μ} 5.75, 9.75, 8.87, and 9.38 were assigned to 3, 5, 3', and 4'-OH groups, respectively. The connectivity of 3,4-dihydroxybenzoyl moiety at C-7 was established by the HMBC correlations of H-6 and H-8 to C-7" at δ_c 164.9. Upon the hydrolysis of 4, catechin and 3,4-dihydroxybenzoic acid were identified by co-TLC alongside authentic samples. On the basis of the above evidences, the structure of 4 was assigned as (+)-catechin-7-0-3",4"dihydroxybenzoate and named curviflorin.

The known compounds were identified as (+)-catechin (1) [31] and quercetin (2) [32, 33] by comparing their NMR spectral and physical data with the literature.

4 Conclusions

Four compounds (1-4) were isolated and characterized from the shoots of P. curviflorus; two of them are new natural products (3 and 4). Their structures were determined on the basis of extensive spectroscopic data analysis.

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