Xanthone and lignan glycosides from the aerial parts of *Polygonum bellardii* all growing in Egypt

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ABSTRACT

A new long chain fatty alcohol acetate identified as 17-hydroxypentacosanyl acetate, **(1)** together with a new xanthone identified as 1,8-Dihydroxy-3,6-dimethoxy-xanthone-5-*O*-[α-L-rhamnopyranosyl-(1′′→2′)]-β-D-glucopyranoside **(3)**, as well as two new lignans identified as (+)-Lyoniresinol-3a-*O*-[α-L-rhamnopyranosyl-(1′′′→6′′)]-β-D-glucopyranoside **(4)** and (+)-Isolariciresinol-3a-*O*-[α-L-rhamnopyranosyl-(1′′′→2′′)-α-L-rhamnopyranosyl-(1′′′′→6′′)]-β-D-glucopyranoside **(5)**, in addition to β-sitosterol-3-*O*-acetate **(2)** were isolated from the methanolic extract of the aerial parts of *Polygonum bellardii* growing in Egypt. Their structures were elucidated on the basis of different chemical and spectroscopic evidences. The total extract and its fractions, in addition to compounds **(3, 4 and 5)** showed significant antioxidant potential by DPPH• scavenging activity technique.

Key words: Antioxidant, lignans, *Polygonaceae, Polyonum bellardii*, xanthone

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INTRODUCTION

Polygonum bellardii (Polygonaceae) grows in sandy places around Alexandria, Mandara, Abukir, Damietta, Tel-El-Kebir, Assiut, Ekhmim, Dendera, Farafra and Kharge, known locally as Maksus-El-Gariyla, Qardab or Qordob.[1] Many species of the genus *Polygonum* had been reported to exhibit a variety of interesting biological activities as: Laxative, expectorant, diuretic, tonic, anti-aging, anti-inflammatory, antibacterial, anti-allergic, anthelmintic, astringent, haemostatic and demulcent.[2-5] Moreover, some other species were used in treatment of skin burns, gallstone, hepatitis, osteomyelitis, nephritis, cystitis, suppurative dermatitis, gonorrhea, chronic gastritis, duodenal ulcers, gout, haemorroids and dental diseases.[6-9] A variety of chemical constituents such as flavonoids,[10,11] anthraquinones,[12] sesquiterpenoids,[13] lignans,[14] coumarins[15] and stilbenes[16] have been reported from the genus *Polygonum*.

Generally Polyphenolic compounds, including phenols, phenolic acids, flavonoids, tannins and lignans have been shown to be potent antioxidants because of their radical scavenging activity and their ability to form complex with heavy metal ions.[17,18] The former chemical and biological information about the genus *Polygonum* motivated the investigation of *Polygonum bellardii* All. growing in Egypt for its chemical composition and antioxidant activity.

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MATERIAL AND METHODS

General

The UV was measured on Ultrospec 1000, UV/visible Spectrometer, Pharmacia Biotech (England). FAB-MS was measured on JEOL JMS 600 Hz (Japan). IR was measured on Shimadzu Infra-red-470 spectrophotometer (Japan). NMR analysis (^{1}H-NMR, ^{13}C-NMR, HMBC) were measured on JEOL TNM-LA 600 MHz FT- NMR spectrometer (Japan), Varian mercury 400 MHz Spectrometer (Oxford) using TMS as internal standard. Column chromatography was carried on silica gel (70-230, mesh, Merck), Sephadex LH-20 (20-100 μm, Sigma-Aldrich chemicals) and RP-18 (30-50 μm), TLC was carried on aluminium plates covered with a 0.25 mm silica (kieselgel 60 GF_{254}, Merck, Germany). The plates were examined under UV light (at 365 and 254 nm) and visualized by spraying with 20% *v/v* H_2SO_4 in EtOH, allowed to dry at room temperature followed by heating at 110-140^{0}C for 1-2 min. The following solvent systems were used for TLC:
I- n-Hexane-EtOAc (9:1 v/v)
II- *n*-Hexane-EtOAc (8:2 v/v)

III- $CHCl_3$-MeOH-H_2O (8: 2:0.2 v/v)
IV- $CHCl_3$-MeOH-H_2O (7.5: 2.5:0.3 v/v)
V Toluene-$(CH_3)_2C$=O-HCHO (3:6:1 v/v)
VI- MeOH-H_2O (6: 4 v/v)

Plant material

The aerial parts of *Polygonum bellardii* All. were collected during flowering stage in April 2007 from Assiut governorate and kindly identified and authenticated by Prof. Dr. Moamen M. Zarea, Professor of Plant Taxonomy, Department of Botany, Faculty of Science, Assiut University, Assiut, Egypt. A voucher specimen was kept in the Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt.

Authentic sugar materials

D-glucose, L-rhamnose, L-arabinose and L-galactose [obtained from El-Nasr Pharmaceutical and Chemical Co., Egypt (ADWIC) and Sigma-Aldrich chemicals].

Antioxidant materials

2,2-Diphenyl-1-picryl hydrazyl (DPPH), ascorbic acid and quercetin as an antioxidant standard (all were obtained from Sigma-Aldrich Chemicals Co.) and Digital Ultrasonic Cleaner, MTI Corporation, USA for mixing samples.

Extraction and isolation

The air-dried powdered aerial parts (2 Kg) of *Polygonum bellardii* All. were extracted by maceration and percolation with methanol till complete exhaustion (1:4 ratio) [four times each 8 L; overnight]. The combined methanolic extracts were concentrated under reduced pressure till constant weight to give a syrupy residue (150 g). The extract was subjected to successive solvent fractionation on VLC with *n*-hexane, chloroform, ethyl acetate, *n*-butanol and methanol till complete exhaustion in each case to give *n*-hexane (20 g), chloroform (25 g), ethyl acetate (20 g), *n*-butanol (10 g) and methanol (50 g).

The chloroform-soluble fraction (10g) was chromatographed on silica gel column chromatography, eluted with *n*-hexane and *n*-hexane-ethyl acetate gradients. Fractions 100ml, each were collected. Similar fractions were pooled together, concentrated under reduced pressure to give 6 sub-fractions labeled PCH1-PCH6. A part of sub-fraction PCH3 (500mg), was rechromatographed on silica gel column chromatography and eluted with *n*-hexane, followed by *n*-hexane-ethyl acetate gradients. The fractions eluted with *n*-hexane- ethyl acetate (9:1) afforded compound **1** (35 mg), whereas fractions eluted with *n*-hexane-ethyl acetate (8:2) afforded compound **2** (30 mg).

The ethyl acetate-soluble fraction (10 g) was chromatographed on silica gel column, eluted with $CHCl_3$ followed by $CHCl_3$-MeOH gradiently; eluates, 100 ml each were collected. Related fractions were grouped together and concentrated under reduced pressure to give 5 sub-fractions labeled PE1-PE5. Fraction PE4 (150mg) was chromatographed on Sephadex LH-20 using $CHCl_3$-MeOH (1:1). Fractions 8-12 were pooled together, concentrated under reduced pressure and chromatographed on RP-18 column using MeOH-H_2O (6:4), where fractions 5-8 afforded compound **3 (**40 mg).

The *n*-butanol soluble fraction (5 g) was chromatographed on silica gel column chromatography eluted with $CHCl_3$ followed by $CHCl_3$-MeOH gradients; fractions 100 ml, each were collected. Similar fractions were grouped and concentrated under reduced pressure to give 3 sub-fractions labeled PN1-PN3.

Sub-fraction PN2 (200 mg) was chromatographed on Sephadex LH-20 using $CHCl_3$-MeOH (1:1). Fractions 10- 15 were collected, concentrated under reduced pressure and rechromatographed on RP-18 using MeOH-H_2O (1:1), afforded compound 4 (50 mg). Furthermore fractions 19-25 rechromatographed on RP-18 using MeOH-H_2O (1:1), afforded compound 5 (35 mg).

Compound1 [17-hydroxypentacosanyl acetate]: Obtained as white amorphous powder from $CHCl_3$, R_f = 0.5 (system, I), $[\alpha]_D^{20}$ +10.3 (MeOH, c.003). The IR spectrum (KBr) showed broad absorption band at 3410 cm^{-1} (OH stretching), in addition to other bands at 2995 cm^{-1} (CH stretching), 1722 cm^{-1} (C = O stretching), 1450 cm^{-1} (CH_2 bending) and 1217 cm^{-1}, 1066 and 989 (C-O stretching). EI mass spectrum showed the molecular ion peak at *m/z* 426 $[M]^+$ (15%), other peaks at *m/z* 411 $[M\text{-}CH_3]^+$ (3.8%), 408 $[M\text{-}H_2O]^+$ (2.7%), 367 $[M\text{-}CH_3COO]^+$ (10%), 313 $[M\text{-}CH_3(CH_2)_7]^+$ (2.7%), 283 $[M\text{-}CH_3(CH_2)_7CHOH]^+$ (3.3%), 269 $[M\text{-}CH_3(CH_2)_7COO]^+$ (1.4%), 157 $[M\text{-}CH_3(CH_2)_{16}CHOH]^+$ (2.6%), 143 $[M\text{-}CH_3(CH_2)_{16}COO]^+$ (63%), 113 $[M\text{-}CH_3(CH_2)_{16}COOCHOH]^+$ (58.2%), 59 $[M\text{-}CH_3(CH_2)_{23}CHOH]^+$ (4%). Selected ^{1}H-NMR spectral data (CD_3OD, 600 MHz): δ_H 0.94 (3H, t, H_3-25), 1.28-1.62 (30 H, m, CH_2 residues), 2.30 (2H, m, H_2-2), 2.29 (3H, s, CH_3-C=O), 3.98 (1H, m, H-17), and 4.06 (2H, t, H_2-1). Selected ^{13}C-NMR spectral data (CD_3OD, 150 MHz): δ_C 13.94 (C-25), 23.66 (C-24), 26.66 (C-2), 29.89-30.29 (CH_2 residue), 31.77 (C-23), 35.03 (C-16 and C-18), 65.14 (C-1), and 69.00 (C-17) in addition to δ_C 20.11, and 175.61(CH_3CO).

Compound 2 [β-sitosterol acetate]: Obtained as white amorphous powder from $CHCl_3$, R_f = 0.6 (system, II).

Positive FAB-MS showed peak at *m/z* 457 $[M+H]^+$ and 415 $[(M+1)\text{-acetyl}]^+$. ^{1}H-NMR spectral data (CD_3OD, 600 MHz): δ_H 0.72 (3H, s, CH_3-18), 0.85 (3H, d, *J*=6.8 Hz, CH_3-26), 0.88 (3H, d, *J*=7.5 Hz, CH_3-27), 0.90 (3H, d, *J*=7.5 Hz, CH_3-29), 1.00 (3H, d, *J*=6.2 Hz, CH_3-21), 1.05 (3 H, s, CH_3-19), 1.24-2.03 (m, other CH and CH_2), 2.05 (3H, s, CO-CH_3), 2.32 (1H, br.s, H-4), 3.45 (1 H, m, H-3), and 5.37 (1 H, br s, H-6). ^{13}C-NMR (CD_3OD, 150 MHz): δ_C 37.29 (C-1), 30.62 (C-2), 77.79 (C-3), 39.67 (C-4), 142.86 (C-5), 122.76 (C-6), 32.92 (C-7), 32.92 (C-8), 49.42 (C-9), 37.29 (C-10), 21.63 (C-11), 39.67 (C-12), 42.13 (C-13), 58.34 (C-14), 25.92 (C-15), 30.26 (C-16), 57.56 (C-17), 12.23 (C-18), 19.32 (C-19), 37.29 (C-20), 19.32 (C-21), 34.98 (C- 22), 25.92 (C-23), 47.43 (C-24), 30.26 (C-25), 20.07 (C-26), 21.63 (C-27), 24.02 (C-28), 14.36 (C-29), 20.07 (CO-CH_3) and 175.20 (CO-CH_3).

Compound 3 [1,8-Dihydroxy-3,6-dimethoxyxanthone-5-*O*-[α-L-rhamnopyranosyl-(1"→2')]-β-D-glucopyranoside]: Obtained as yellowish needles from MeOH (m.p. 177-180), R_f = 0.40 (system, III), $[\alpha]_D^{20}$ -9.8 (MeOH, c.001). Positive FAB-MS showed peak at *m/z* 613 $[M+1]^+$, 467 $[(M+1)\text{-rhamnose}]^+$ and 305 $[(M+1)\text{-(rhamnose + glucose)}]^+$. ^{1}H-NMR spectral data (DMSO-d_6, 600 MHz) was listed in Table 1. ^{13}C-NMR spectral data (DMSO-d_6, 150 MHz) was listed in Table 2.

Compound 4 [Lyoniresinol-3a-*O*-[α-L-rhamnopyranosyl-(1"'→6")]-β-D-glucopyranoside]: Obtained as a white amorphous powder from MeOH, R_f = 0.40 (system, IV), $[\alpha]_D^{20}$ +9.6 (MeOH, c.05). Positive FAB-MS showed peak at *m/z* 729 $[M+1]^+$ and other peaks at 583 $[(M+1)\text{-rhamnose}]^+$ and 421 $[(M+1)\text{-(glucose + rhamnose)}]^+$. The ^{1}H-NMR spectral data (DMSO-d_6, 400 MHz) was listed in Table 3. ^{13}C-NMR spectral data (DMSO-d_6, 100 MHz) was listed in Table 4.

Compound 5 [(+)-Isolariciresinol-3a-*O*-[α-L-rhamnopyranosyl-(1"'→2")-α-L-rhamnopyranosyl-(1""→6")]-β-D-glucopyranoside]: Obtained as white powder from MeOH, R_f = 0.35 (system, IV), $[\alpha]_D^{20}$ +11 (MeOH, c. 02). Positive FAB-MS showed peak at *m/z* 815 $[M+1]^+$ and other peaks at 669 $[(M+1)\text{- rhamnose}]^+$, 521 $[(M+1)\text{-two rhamnose units}]^+$, 361 $[(M+1)\text{- (glucose + two rhamnose units)}]^+$. The ^{1}H-NMR spectral data (CD_3OD, 600 MHz) was listed in Table 3 and ^{13}C NMR spectral data (CD_3OD, 150 MHz) was listed in Table 4.

DPPH radical-scavenging assay

Firstly, free radical scavenging activity of the total methanolic extract, its fractions in addition to compounds **3, 4** and **5** isolated from the aerial parts of *Polygonum bellardii* All. was carried out against stable DPPH• with a rapid TLC screening method using 0.2% DPPH• in methanol. Thirty minutes after spraying, the active compounds appear as yellow spots against purple background.[19]

Table 1: ^{1}H-NMR spectral data of compound 3 (DMSO-δ_6, 600 MHz)

Chemical shift (δ) ppm	No. of protons, Multiplicity	Coupling constant (Hz)	Assignment
1.19	3H, d	6.0	H-6"
3.77	3H, s	-	3-OCH_3
3.91	3H, s	-	6-OCH_3
4.47-5.32	16H, m	-	Sugar protons
5.17	1H, br.s	-	H-1"
5.35	1H, d	7.4	H-1'
6.37	1H.,s	-	H-7
6.55	1H, br.s	-	H-2
6.67	1H, br.s	-	H-4
11.72	1H, s	-	1-OH
11.87	1H, s		8-OH

Table 2: ^{13}C-NMR spectral data of compound 3 (DMSO-δ_6, 150 MHz.

Carbon No.	Chemical shift	Carbon No.	Chemical shift
1	161.71	Glucose	
2	97.59	1'	99.01
3	164.10	2'	76.38
4	94.82	3'	77.03
5	128.40	4'	69.67
6	160.10	5'	77.10
7	95.60	6'	60.53
8	157.47		
9	182.86	Rhamnose	
4a	157.02	1"	100.53
4b	148.45	2"	70.41
8a	101.08	3"	70.48
8b	102.18	4"	71.85
3-OCH_3	56.66	5"	68.38
6-OCH_3	60.99	6"	18.07

In a second experiment, DPPH• scavenging activity was measured by spectrophotometric method.[20] Briefly, 1ml of a wide range of concentrations (25-200 μg/ml) of test sample (in methanol) was added to 1 ml of 200 μM of methanolic solution of DPPH•. The mixture was mixed by Digital Ultrasonic Cleaner (sonicator) for 1 min and then left to stand at room temperature for 30 min in dark. When DPPH• reacts with an antioxidant compound, which can donate hydrogen, it is reduced and the changes in colour (from deep-violet to light-yellow) were measured at 517nm on a UV/visible light spectrophotometer. Absorption of blank sample containing the same amount of methanol and DPPH• solution was prepared and measured daily. The experiment was carried out in triplicate, using ascorbic acid and quercetin as a positive control standards.[21] The percentage reduction of the DPPH• was calculated by the following formula: %Inhibition (scavenging) = $[(A_B\text{-}A_A)/A_B]\times100$ Where: A_B-

Table 3: ^{1}H-NMR spectral data of compound 4 and 5 (DMSO-d_6, 400 and 600 MHz)

Proton No	Chemical shift	Multiplicity	Coupling constant (Hz)	Chemical shift	Multiplicity	Coupling constant (Hz)
	4			5		
1	2.87	2H, m	-	3.21	2H, m	-
2	1.92	1H, m	-	1.84	1H, m	-
2a	3.41	2H, m	-	3.62	1H, dd	10.8, 4.5
-	-	-	-	3.65	1H, dd	10.8, 6.1
3	2.71	1H, m	-	2.72	1H, m	-
3a	4.06	1H, dd	11, 6	3.73	1H, dd	9.9, 3.1
-	4.15	1H, dd	11, 4.2	3.75	1H, dd	9.9, 4.6
4	4.26	1H, d	6	4.42	1H, d	5.8
5	-	-	-	6.56	1H, s	-
8	6.53	1H, s	-	6.53	1H, s	-
2’	6.29	1H, s	-	6.54	1H, d	2.1
5’	-	-	-	6.66	1H, d	7.9
6’	6.29	1H, s	-	6.49	1H, d	7.9,2.1
5-OCH_3	3.69	3H, s	-	-	-	-
7-OCH_3	3.75	3H, s	-	3.70	3H, s	-
3’-OCH_3	3.63	3H, s	-	3.67	3H, s	-
5’-OCH_3	3.63	3H, s	-	-	-	-
1”	4.60	1H, d	7.6	4.18	1H, d	7.9
1”’	6.08	1H, s	-	6.07	1H, s	-
1””	-	-	-	6.07	1 H, s	-
6”’	1.11	1H, d	6.5	1.19	1H, d	6.5
6””	-	-	-	1.09	1H, d	6.5

Table 4: ^{13}C-NMR spectral data of compounds 4 and 5 (DMSO-δ_6, 100 and 150 MHz)

Carbon No Aglycone	Chemical shift		Carbon No Sugar part	Chemical shift	
	4	5		4	5
1	35.91	33.56	Glucose		
2	41.10	40.07	1”	103.87	
2a	65.94	66.96	2”	73.41	102.24
3	48.70	48.31	3”	76.80	74.91
3a	70.75	70.09	4”	69.04	77.91
4	44.68	45.47	5”	77.29	68.01
5	146.50	117.14	6”	64.04	78.23
6	137.35	145.22	Rhamnose	100.86	65.36
7	147.06	147.23	1”’	69.74	101.92
8	106.70	113.54	2”’	70.86	71.64
9	128.59	128.90	3”’	70.99	72.51
10	124.90	134.00	4”’	69.55	73.91
1’	137.83	138.04	5”’	17.99	70.09
2’	105.65	116.08	6”’	-	17.90
3’	147.60	149.14	Rhamnose	-	101.22
4’	133.44	146.07	1””	-	71.19
5’	147.60	117.13	2””	-	72.29
6’	105.98	123.16	3””	-	73.84
5-OCH_3	58.90	-	4””	-	70.09
7-OCH_3	55,76	56.39	5””		17.89
3’-OCH_3	56.12	-	6””		
5’-OCH_3	56.12	56.39			

absorption of blank sample (t = 0 min) and A_A-absorption of tested extract solution (t = 30 min).

RESULTS AND DISCUSSION

Compound 1

The low resolution EI-MS showed the molecular ion peak $[M]^+$ at m/z 426, together with IR, ^{1}H-NMR and ^{13}C-NMR spectral data confirmed the molecular formula to be $C_{27}H_{54}O_3$. Careful investigation of ^{1}H-NMR and ^{13}C-NMR spectral data of compound **1** demonstrated the presence of long chain aliphatic ester moiety. EI-MS showed a peak at 408 $[M-H_2O]^+$ (2.7%) indicated the presence of hydroxyl group, confirmed from IR band at 3410. Promising abundant peaks

at *m/z* 143 [M-CH_3 $(CH_2)_{16}COO]^+$ (63%) and 113 [M-$CH_3(CH_2)_{16}COOCHOH]^+$ (58.2%) indicated the presence of hydroxyl group at C-17. Additionally, it revealed the presence of an ester methyl moiety at *m/z* 59 (4%). The ^{1}H-NMR showed a triplet methyl at δ_H 0.94 (3H, t) attributed to terminal methyl at C-25 together with another methyl signal at δ_H 2.63 (3H, s) belonging to methyl protons of the acetyl group. It also showed a signal at δ_H 3.98 (1H, m) assigned to oxygenated methine group. Moreover, it revealed the presence of a triplet signal at δ_H 4.06 (2H, t, H-1) and a multiplet signal at δ_H 2.30 (2H, m, H-2). Moreover, a cluster of CH_2 signals at δ_H 1.28-1.6 (30 H, m) indicative for long chain aliphatic moiety, which was obvious from ^{13}C-NMR signals at δ_C 29.89-30.29.[22,23] The ^{13}C-NMR spectrum displayed two signals at δ_C 175.61 and 20.11, assigned to acetyl group. The oxygenated methine carbon appeared at δ_C 69.00, while the terminal methyl appeared at δ_C 13.94. Therefore, compound **1** was assigned as17-hydroxypentacosanyl acetate,[22,23] a new natural product [Figure 1-3, Tables 1-4].

Compound 2

Chemical investigation of compound 2 indicated its steroidal nature.[24] ^{1}H-NMR spectrum of compound **2** showed six methyl signals at δ_H 0.72, 0.85, 0.88, 0.90, 1.00 and 1.05 each (3H, s) assigned to CH_3-18, 26, 27, 29, 21 and 19 respectively in addition to an extra downfield

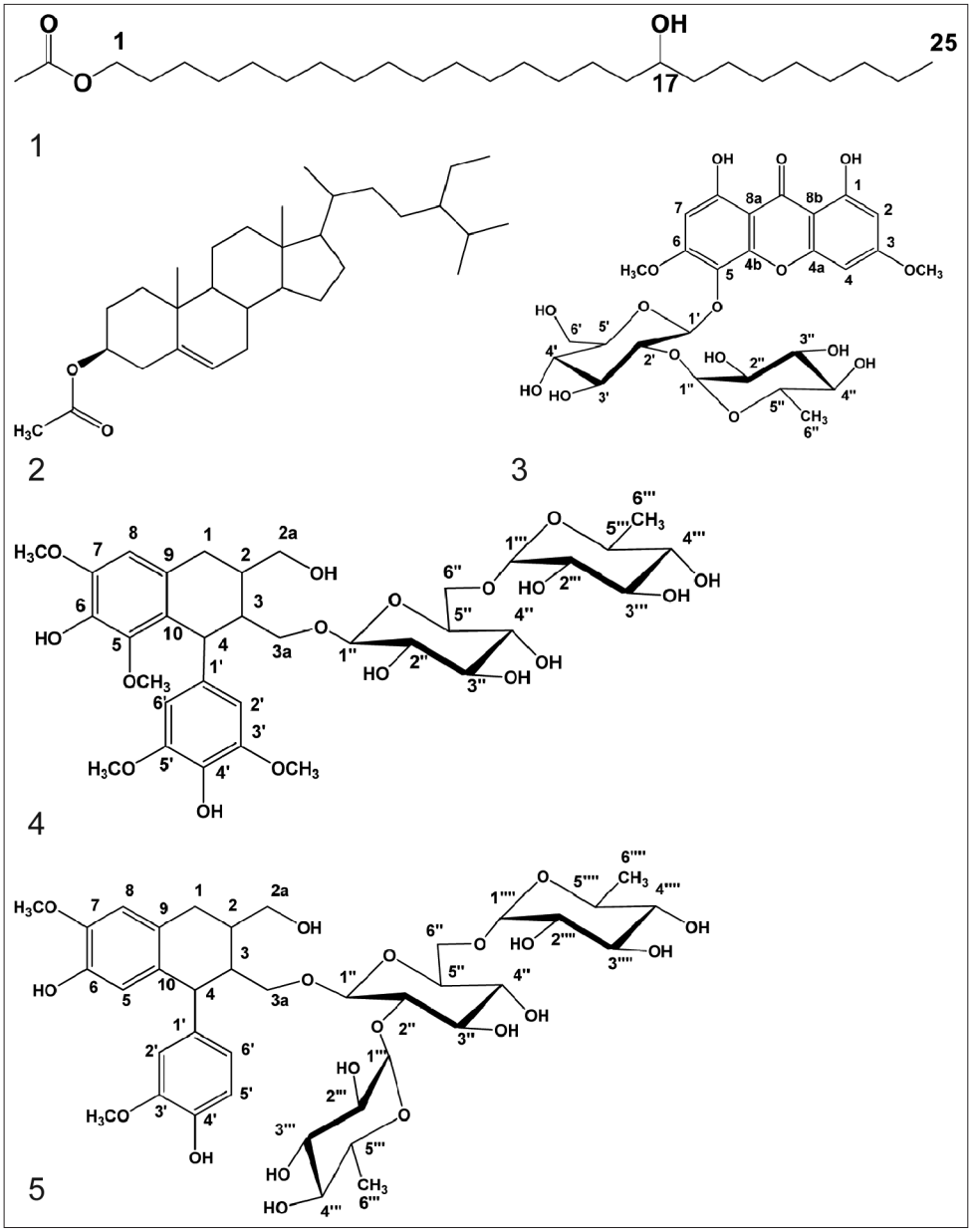

Figure 1: Structures of the isolated compounds **1- 5**

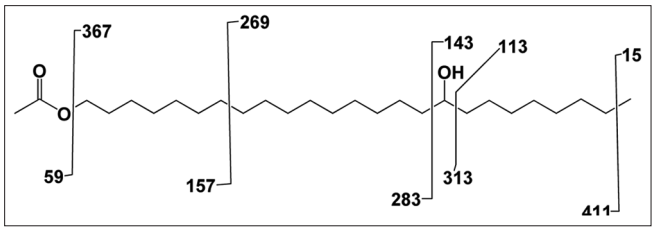

Figure 2: Significant fragments of compound **1**

shifted oxygenated methyl signal at δ_H 2.05 (3H, s) allocated to acetyl group. It also showed signals at δ_H 2.32 (1H, br. s, H-4), 3.45 (1 H, m, H-3) and 5.37 (IH, br s, H-6). The ^{1}H-NMR together with ^{13}C-NMR data concluded the existence of acetylated β-sitosterol moiety, this was confirmed from ^{13}C-NMR signals at δ_C 20.07 and 175.35 assigned to acetate moiety. The localization of acetyl group at C-3 was obvious from the downfield shift of C-3 at δ_C 77.79 upon comparison with non-substituted β-sitosterol.[25] From the previous data and upon comparison with literature,[26,27] compound **2** was identified as β-sitosterol-3-*O*-acetate, previously isolated from *Prunella vulgaris*[26] and firstly reported from the genus *Polygonum*.

Compound 3

The ^{1}H and ^{13}C-NMR measurements of compound **3** indicated the presence of xanthone glycoside moiety.[28] Positive FAB-MS spectrum showed the quasi-molecular ion peak at *m/z* 613 $[M+1]^+$, together with ^{1}H-, ^{13}C-NMR spectral data confirmed the molecular formula to be $C_{27}H_{32}O_{16}$. ^{1}H-NMR spectrum of compound **3** showed two downfield shifted singlet signals at δ_H 11.72, 11.87 each (1H, s) ascribed to chelated 1,8-OH groups respectively. The singlet signal at δ_H 6.37 and broad singlet signals at δ_H 6.55 and 6.67 were assigned to isolated aromatic protons H-7, H-2 and H-4 respectively,[29-32] two intense signals appeared at δ_H 3.91, 3.77 each (3H,s) indicating two aromatic methoxy groups. Moreover, the ^{1}H-NMR showed signals at δ_H 5.35 (1H d, J = 7.4 Hz) and 5.17 (1H, s) assigned to couple of anomeric sugar protons, indicative for the existence of bioside moiety. The previous ^{1}H-NMR data together with diagnostic ^{13}C-NMR signals listed in Table 2 were conclusive for the presence of pentasubstituted 1,8-dihydroxy bioside moiety. The site of sugar attachment at C-5 was obvious from ^{13}C-NMR downfield shifted signals at δ_C 128.4 allocated to C-5 upon comparison with C-5 unsubstituted 1,8 dihydroxyxanthone series.[28-32] ^{13}C-NMR displayed pair of diagnostic signals at δ_C 99.01, 100.53 attributed to two anomeric sugar carbons.[28-32] The aforementioned data associated with ^{13}C-NMR prominent signals at δ_C 99.01, 76.38, 77.03, 69.67, 77.10 and 60.53 deduced the occurrence of C-2 substituted glucose moiety. The position of the intraglycosidic linkage was approved from ^{13}C-NMR downfield shifted signal at

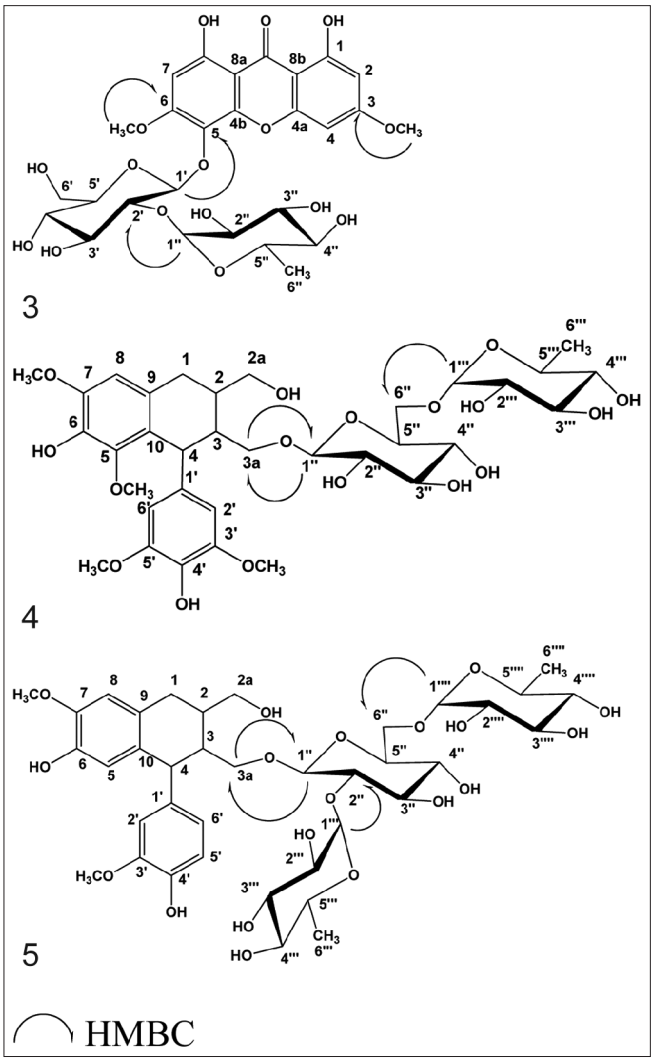

Figure 3: Significant HMBC correlations of compounds **3-5**

δ_C 76.38 belonging to C-2 of glucose.[28-32] Other ^{13}C-NMR signals at δ_C 100.53, 70.41, 70.48, 71.85, 68.38 and 18.07 in addition to characteristic doublet methyl group at δ_H 1.19 (3H, d, J=6 Hz) in ^{1}H-NMR were ascribed to rhamnosyl moiety.[28-32] The partial and complete acid hydrolysis of compound **3** in parallel with investigation of the hydrolysate by paper chromatography (system IV) revealed the presence of glucose and rhamnose sugars. On the other hand, HMBC measurements showed a significant correlation between the anomeric proton of glucose unit H-1' (δ_H 5.35) and C-5 (δ_C 128.40) of the aglycone part, supported our hypothesis about position of attachment of the sugar part to C-5 of the xanthone aglycone. It also showed an important correlation between anomeric proton of rhamnosyl moiety H-1" (d_H 5.17) and C-2' (δ_C 76.38) of glucose moiety, confirming the site of attachment of rhamnose to glucose at C-2'. Furthermore the HMBC experiment showed cross linkage between protons of two methoxy groups at C-3 (δ_H 3.77) and C-6 (δ_H 3.91) and carbons C-3 δ_C 164.10) and C-6 (δ_C 160.10)

respectively, supporting the presence of 3,6-dimethoxylated xanthone moiety. From the previously mentioned chromatographic, spectral and results of acid hydrolysis data, compound **3** was identified as 1,8-Dihydroxy-3,6-dimethoxy-xanthone-5-*O*-[α-L-rhamnopyranosyl-(1"→2')]-β-D-glucopyranosid, a new natural product.

Compound 4

Its positive FAB-MS showed the quasi-molecular ion peak at *m/z* 729 $[M+1]^+$, together with ^{1}H-, ^{13}C-NMR spectral data confirmed the molecular formula to be $C_{34}H_{48}O_{17}$. The detailed analysis of ^{1}H and ^{13}C-NMR spectra of compound **4** deduced the occurrence of glycosylated (+)-lyoniresinol lignan moiety;[33,37] However the ^{1}H-NMR spectrum of compound 4 displayed signals at δ_H 4.15 (1H, dd, *J*=11, 6 Hz), 4.06 (1H, dd, *J*=11, 4.2 Hz) assigned for primary alcoholic methylene protons H-3a, in addition to signal at δ_H 2.87 (2H, m) assigned to methylene protons, H-1. The signals appeared at δ_H 3.69 (3H, s), 3.75 (3H, s) and 3.63 (6H, s) could be assigned to four methoxy groups attached to C-5, C-7 and C-3´, C-5´ respectively.[33-37] Moreover, ^{1}H-NMR showed the oxygenated methylene protons (H-2a) as a multiplet signal at δ_H 3.41 (2H, m). The aliphatic methine protons H-2, H-3 and H-4 appeared at δ_H 1.92 (1H, br.s), 2.71 (1H, m) and 4.26 (1H, d, *J*=6 Hz) respectively while the aromatic protons appeared at δ_H 6.53, (1H, s, H-8) and 6.29 (2H, s, H-2' and H-6'). The ^{13}C- NMR spectrum of compound **4** [Table 4] illustrated the presence of 34 carbon atoms, of them 12 carbons belonging to sugar moiety and the rest 22 carbons indicated an aryl-tetralin-type lignin.[33-37] Concerning the sugar moiety, ^{1}H-NMR spectrum showed the presence of two anomeric protons, one of them appeared at δ_H 4.60 (1H, d, *J* = 7.6 Hz), the other proton noticed at δ_H 6.08 (1H, s). The ^{1}H-NMR data in conjunction with ^{13}C-NMR signals at δ_C 103.87, 100.86, indicating the existence of two anomeric carbons, in addition to the other signals at δ_C73.41, 76.80, 69.04, 77.29 and 64.04 pointed out the incidence of C-6" substituted glucose. This was further supported from C-6" downfield shifted signal at δ_C 64.04 in comparison with non-substituted glucose moiety.[33-37] The appearance of a characteristic doublet at δ_H 1.11 (3H, δ, *J*= 6.5 Hz) in addition to other carbon signals at δ_C 69.74, 70.86, 70.99, 69.55 and 17.99, indicated the presence of rhamnose moiety attached to C-6" of glucose. The sugar linkage at C-3a was determined mainly by the comparison of the ^{1}H,^{13}C-NMR data of compound **4** with (+)-lyoniresinol, where two protons at C-3a (δ_H 4.06, 4.15) of compound **4** showed obvious downfield shift signal upon comparison with corresponding protons at C-3a (δ_H 3.48) of (+)-lyoniresinol (about 0.6 ppm) and C-3a shifted from δ_C 64.20 to 70.75 when compared with (+)-lyoniresinol.[33,38] The optical rotation of Compound **4** is +9.6 (MeOH, c.05) compared with (+)-lyoniresinol with optical rotation +13.3 (MeOH, c.32),[38] indicated that, compound **4** is (+)-lyoniresinol derivative. Investigation of the HMBC spectrum revealed the presence of significant correlations between; methylene protons H-3a (δ_H 4.15, 4.06) of the aglycone and C-1" (δ_C 103.87) of glucose moiety, confirming the position of sugar linkage at C-3a. The HMBC spectrum showed diagnostic correlation between H-1'" (δ_H 6.08) of rhamnose and C-6" (δ_C 64.04) of glucose, confirming the attachment of rhamnose to C-6" of glucose unit. From the above chemical, chromatographic and spectral data,[33-38] compound **4** was identified as (+)-Lyoniresinol-3a-*O*-[α-L-rhamnopyranosyl-(1'"→6")]-β-D-glucopyranoside,[33-38] a new natural product.

Compound 5

The positive FAB-MS spectrum showed the quasi-molecular ion peak at *m/z* 815 $[M+1]^+$, together with ^{1}H-, ^{13}C-NMR spectral data confirmed the molecular formula to be $C_{38}H_{54}O_{19}$. The ^{1}H-NMR together with ^{13}C- NMR spectral data illustrated the presence of 38-carbon atoms, among them, 18 carbons for two aromatic rings and six aliphatic carbon exhibiting the existence of an aryl-tetralin-type lignan, could be identified as (+)-isolariciresinol.[34-37] Remaining 18 carbons concluded the presence of substituted glucose moiety. The ^{1}H-NMR of compound **5** displayed signals at δ_H 3.62 (1H, dd, *J* = 10.8, 4.5) and 3.65 (1H, dd, *J* = 10.8, 6.1), H-2a; δ_H 3.21 (2H, m), H-1; δ_H 3.70 and 3.67 each (3H, s), two methoxy groups at C-7 and C-3´ respectively and δ_H 3.73 (1H, dd, *J*= 9.9, 3 Hz), 3.75 (1H, dd, *J*= 9.9, 4.6 Hz), H-3a. Moreover, it revealed the presence of the following signals: At δ_H 1.84 (1H, m, H-2), 2.72 (1H, br.s, H-3), 4.42 (1H, d, *J*= 5.8 Hz, H-4), 6.56 (1H, s, H-5), 6.54 (1H, s, H-8), 6.49 (1H, d, *J*=7.9, 2.1 Hz, H-6'), 6.66 (1H, d, *J*=7.9 Hz, H-5') and 6.54 (1H, d, *J*=2.1 Hz, H-2'). The anomeric protons appeared at δ_H 4.18 (1H, d, *J*= 7.9 Hz) and 6.07 (2H, s). The ^{1}H-NMR data in association with ^{13}C-NMR signals at δ_C 102.24, 101.92 and 101.22, indicating the occurrence of three anomeric carbons, in addition to the other ^{13}C-NMR signals at δ_C74.91, 77.91, 68.01, 78.23 and 65.36 declared the presence of C-2" and C-6" substituted glucose moiety,[37-40] This was further supported from C-2", C-6" downfield shift signals at δ_C 74.91 and 65.36 accordingly upon comparison with non-substituted glucose moiety.[38] The two doublet signals at δ_H 1.09 and 1.19 each (3H, d, *J*= 6.5 Hz), in addition to other ^{1}H and ^{13}C-NMR sugar signals [Tables 3 and 4] indicated the existence of two rhamnose moieties attached to C-2" and C-6" of glucose. The linkage of sugar was configured at C-3a mainly by the comparison of the ^{1}H and ^{13}C-NMR data of compound **5**, with those reported for (+)-isolariciresinol,

Table 5: Antioxidant activity of total extract, fractions and isolated compounds (3-5) from the aerial parts of *Polygonum bellardii* All

Fraction or Compound	Concentrations µg/ml					IC_{50}
	10	25	50	100	200	
Ascorbic acid	47.1±2.03%	66.3±1.79%	86.9±3.12%	98.8±1.54%	99.6±3.10%	14.5±2.92
Quercetin	45.0±2.95%	65.0±2.88%	85.0±3.62%	97.3±0.91%	99.1±3.22%	15.1±2.25
Total extract	10.0±0.97%	19.8±1.24%	25.0±1.07%	42.9±2.14%	64.8±1.09%	135.4±2.05
n-Hexane F.	05.8±0.45%	13.8±1.21%	20.8±1.13%	35.9±3.14%	41.9±1.45%	253.4±1.91
Chloroform F.	15.8±3.20%	21.8±1.65%	36.8±1.88%	50.9±2.98%	67.9±0.78%	98.1±3.02
Ethyl acetate F.	29.9±3.12%	51.9±3.10%	68.2±3.51%	73.3±1.56%	82.5±3.21%	20.51±1.87
n-Butanol F.	26.8±2.66%	44.9±2.88%	60.1±2.43%	71.1±3.32%	77.3±1.88%	34.7±2.32
Compound **3***	18.2±1.56%	28.4±1.93%	41.1±0.99%	51.0±0.98%	66.1±0.87%	79.3±2.65
Compound **4****	22.8±1.99%	40.6±2.79%	54.8±1.76%	63.6±2.11%	75.3±3.08%	38.2±1.85
Compound **5*****	26.4±2.11%	48.5±3.01%	55.6±3.13%	67.3±3.09%	78.6±2.31%	35.4±2.46

*3: 1,8-Dihydroxy-3,6-dimethoxy-xanthone-5-O-[α-L-rhamnopyranosyl-(1"→2')]-β-D-glucop-yranosid, **4: (+)-Lyoniresinol-3a-O-[α-L-rhamnopyranosyl-(1'''→6")]-β-D-glucopyranoside, ***5: (+)-Isolariciresinol-3a-O-[α-L-rhamnopyranosyl-(1'''→2")-α-L-rhamnopyranosyl-(1''''→6")]-β-D-glucopyranoside

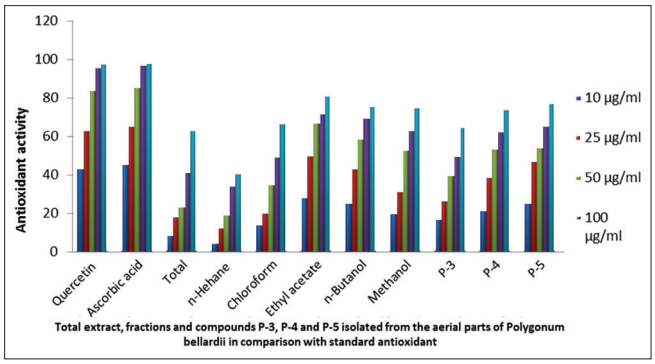

Figure 4: Antioxidant activity of the total extract, fractions and compounds **3, 4** and **5** isolated from the aerial parts of *Polygonum bellardii* All

however the methylene protons H-3a (δ_H 3.73, 3.75) was obviously downfield shifted.[39,40] The optical rotation of Compound **5** is +11 (MeOH, c 0.05) compared with (+)-isolariciresinol +34 (MeOH, c 0.10),[40] indicated that, compound **5** is (+)-isolariciresinol derivative. HMBC correlations showed significant cross linkage between the protons at C-3a (δ_H 3.73, 3.75) of the aglycone and anomeric carbon of glucose moiety C-1" (δ_C 102.24), confirming the attachment of sugar part at C-3a. The inter-glycosidic linkages between glucose and two rhamnose units were daignosed from HMBC correlations between the two anomeric protons of rhamnose units H-1''', H-1'''' (δ_H 6.07) and C-2" (δ_C 74.91) together with C-6" (δ_C 65.36) of glucose moiety which confirmed the site of attachment of rhamnose units to C-2" and 6" of glucose. From the above chemical, chromatographic and spectral data,[33-40] it could be concluded that compound **5** was identified as (+)-Isolariciresinol-3a-*O*-[α-L-rhamnopyranosyl-(1'''→2")-α-L-rhamnopyranosyl-(1''''→6")]-β-D-glucopyranoside, a new natural product.

Results of radical scavenging activity

The obtained results revealed that compounds **4, 5** in addition to ethyl acetate, *n*-butanol and methanol extracts displayed the strongest radical scavenging activity, while the weakest radical scavenging activity was observed with *n*-hexane extract. The radical scavenging activities of the extracts were correlated to the presence of high concentrations of flavonoids and phenolic compounds in the extracts which were isolated from *Polygonum bellardii* in our previous work.[10] The pure compounds showed lower activity than that of crude extracts (ethyl acetate and *n*-butanol) may be due to synergism (synchronization) of high flavonoid content in the plant (18.52 mg/kg) and other phenolic constituents present in crude extracts of the plant [Table 5, Figure 4].

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