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Termiglaucescin, a new polyhydroxy triterpene glucoside from *Terminalia glaucescens* with antioxidant and anti-inflammatory potential

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Abstract: Termiglaucescin (1), a new triterpene glucoside, has been isolated from the ethyl acetate extract of the root bark of *Terminalia glaucescens* Planch. ex Benth, together with 11 known compounds, β -D-glucopyranosyl $2\alpha,3\beta,6\beta$ -trihydroxy-23-galloylolean-12-en-28-oate (2), arjunglucoside I (3), sericoside (4), arjungenin (5), sericic acid (6), arjunetin (7), chebuloside II (8), 3,3',4-tri-*O*-methylelagic acid (9), 3,3'-di-*O*-methylelagic acid (10), β -sitosterol (11) and stigmasterol (12). Compounds 2, 3, 7, 8 and 9 are reported from the plant for the first time. The structures of the isolated compounds were characterized by spectroscopic data interpretations, especially 1D and 2D NMR. The triterpenic isolates showed potent antioxidant and anti-inflammatory activities.

Keywords: anti-inflammatory; antioxidant; Combretaceae; *Terminalia glaucescens*; triterpene glucosides.

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1 Introduction

The genus *Terminalia* (Combretaceae) consists of 200 species distributed in the tropics and subtropics [1, 2]. About 30 species of this genus are found in Africa [1]. *Terminalia glaucescens* Planch. ex Benth is used in Central Africa for the treatment of dysentery and other microbial infections. Leaves of this plant are reported to be useful in the last phase of AIDS [3]. The extracts of leaves and roots of *T. glaucescens* have been found to be highly active against both *Candida* species and dermatophytes such as *Trichophyton* spp. [4]. *T. glaucescens* is used as chewing sticks in some countries of Western Africa, and extracts made from the sticks (the stem bark and wood) showed a wide spectrum of antibacterial activity against different bacterial species including methicillin-resistant *Staphylococcus aureus* and dentally relevant bacteria [5–7]. The antioxidant and anti-hyperglycemia effects of methanol-methylene chloride extract in streptozotocin-induced diabetes is also in agreement with the use of *T. glaucescens* for diabetes treatment in Cameroon [8, 9]. A number of terpenoids (arjunic acid, arjungenin, sericoside, frideline, glaucinoic and glaucescic acid), phytosterols (β -sitosterol and stigmasterol) and ellagic acids have previously been isolated from the stem bark and roots of *T. glaucescens* [10–12], but the identification of active principles and the mechanism of therapeutic activity of this species have not so far been established.

In the present study, we have isolated one new oleanane type triterpene glucoside named as termiglaucescin (1) from the ethyl acetate sub-fraction of the root bark of *T. glaucescens* along with 11 known compounds (2–12). The isolated triterpenes (1–8) showed significant antioxidant and anti-inflammatory activities (Figure 1). The potent activities of these compounds supplemented the previous literature reports and medicinal uses of this plant in the treatment of diabetes and dental caries.

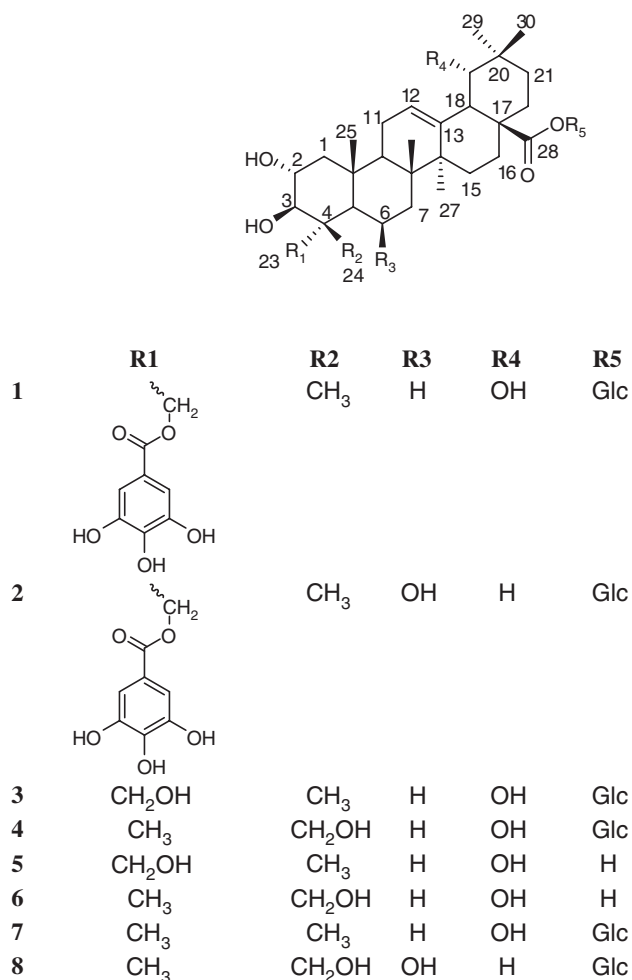


Figure 1: Structures of isolated triterpenes from *Terminalia glaucescens*.

2 Results and discussion

2.1 Structural elucidation

Termiglaucescin (**1**) was isolated as a colorless amorphous solid which gave similar color reactions as those of **1**. The molecular ion peak at m/z 817.2631 $[M-1]^-$ in the high resolution-fast atom bombardment-mass spectroscopy (HR-FAB-MS) was consistent with the molecular formula $C_{43}H_{62}O_{15}$. Absorption bands at 3417, 1696 and 1614 cm^{-1} in the IR spectrum suggested the presence of hydroxyl, ester and olefinic functionalities. The 1H -NMR spectrum of **1** displayed signals for six tertiary methyl groups (δ_H 0.73, 0.82, 0.92, 0.93, 1.05 and 1.16), an olefin proton (δ_H 5.31, br t), three oxygenated methines (δ_H 3.74, 3.33 and 3.25) and an oxygenated methylene (δ_H 4.03, 4.19, each d, $J=11.0$ Hz), together with six other oxygenated methine and methylene protons ascribable to a sugar unit (Table 1). The ^{13}C -NMR spectrum showed 43 signals including 6 primary, 10 secondary, 14

tertiary, and 13 quaternary carbons (Table 1). The olefinic carbons at δ_C 124.8 and 144.6 were typical of oleanane-type triterpenes [13]. Comparison of NMR spectral data with those of 28-*O*- β -D-glucopyranosyl 2 α ,3 β ,6 β -trihydroxy-23-galloylolean-12-dien-28-oate (**2**) previously isolated from *Combretum molle* revealed a downfield shift of C-19 (δ_C 82.5) and an upfield shift of C-6 (δ_C 19.5) indicative of the absence of hydroxyl group at C-6 and its presence at C-19. Full assignments of proton and carbon resonances of the aglycone were achieved by analysis of the heteronuclear single quantum correlation (HSQC), correlation spectroscopy (COSY) and heteronuclear multiple quantum correlation (HMBC) experiments (Table 1). Moreover, the presence of one singlet of two protons at δ_H 7.08 (H-2'', H-6'') on the 1H NMR spectrum and carbon atoms of one carbonyl at δ_C 168.3 (C-7''), one aromatic quaternary carbon at δ_C 121.6 (H-1''), two aromatic methines at δ_C 110.1 (C-2'', C-6'') and three quaternary oxygenated carbons at δ_C 139.9 (C-4'') and 146.6 (C-3'', C-5'') on the ^{13}C NMR spectrum evidenced the presence of the galloyl group in compound **1** [14]. The galloyl moiety could be assigned to C-23 on the basis of HMBC experiments in which the oxymethylene protons showed cross peak correlation with δ C-3 (δ_C 78.7), C-5 (δ_C 49.5), C-24 (δ_C 13.7) and C-7'' (δ_C 168.3). The sugar moiety was determined as β -D-glucopyranoside on the basis of the characteristic $J_{1,2}$ coupling constant of its anomeric proton ($J=8.0$ Hz) and typical 1H and ^{13}C NMR shifts [15]. The position of the glucose moiety was confirmed by correlation of the anomeric proton with C-28 (δ 178.0).

The stereochemistry at different stereocenters was established through nuclear Overhauser effect spectroscopy (NOESY) spectrum, which showed correlation of the oxymethines proton at δ_H 3.74 (H-2) with the angular methyl groups at δ_H 0.82 (H-24) and 1.05 (H-25) and the proton at δ_H 3.33 (H-3) with the oxymethylene protons at δ_H 4.08 and 4.19 (H-23). The proton at δ_H 3.03 (H-18) and δ_H 3.25 (H-19) also showed through space coupling with the methyl at δ_H 0.93 (H-30). On the basis of these evidence and comparison of NMR spectral data with related triterpenes, the structure of termiglaucescin (**1**) was assigned as 28-*O*- β -D-glucopyranosyl 2 α ,3 β ,19 α -trihydroxy-23-galloylolean-12-dien-28-oate.

Among the isolated compounds, β -sitosterol, stigmasterol, arjungenin, sericoside and β -D-glucopyranosyl 2 α ,3 β ,6 β -trihydroxy-23-galloylolean-12-en-28-oate have previously been isolated from the roots and leaves of *T. glaucescens* [10–12], *Combretum racemosum* [16] and the stem bark of *C. molle* [17]. This reveals close taxonomical similarity between two species of Combretaceae family. *T. glaucescens* extracts have previously been evaluated for their antioxidant activity and showed promising activities [8, 9]. Moreover,

Table 1: ^1H and ^{13}C NMR data of compound **1** in methanol- d_4 .

1			
Position	δ_c	δ_H (J in Hz)	HMBC correlations
1	47.1	0.98 ^a	
		1.94 dd (12.5, 4.0)	C-2, C-5, C-10
2	69.3	3.74 m	C-3
3	78.7	3.33 d (7.0)	
4	44.1		
5	49.5	1.36 ^a	
6	19.5	1.44 ^a	
7	33.3	1.23 ^a	
		1.39 ^a	
8	40.9		
9	48.5	1.82 t	C-8, C-10, C-11, C-25
10	39.1		
11	24.9	1.99 ^a	C-12, C-13
12	124.8	5.31 br t	
13	144.6		
14	42.7		
15	29.5	0.96 ^a	
		1.72 ^a	
16	28.4	1.66 ^a	
		2.26 m	C-28
17	47.9		
18	45.1	3.03 br s	C-12, C-13, C-19, C-28
19	82.5	3.25 d (3.5)	C-18, C-20, C-29, C-30
20	35.9		
21	29.3	1.02 ^a	
		1.29 ^a	
22	33.5	1.66 ^a	
		1.76 ^a	
23	66.8	4.03 d (11.0)	C-3, C-4, C-5, C-24, C-7''
		4.19 d (11.0)	
24	13.7	0.82 s	C-3, C-4, C-5, C-23
25	17.8	1.05 s	C-9, C-10
26	17.4	0.73 s	C-6, C-8, C-9, C-14
27	25.2	1.16 s	C-8, C-13, C-14, C-15
28	178.6		
29	25.2	0.93 s	C-19, C-20, C-30
30	28.5	0.92 s	C-10, C-20, C-29
glc			
1'	95.8	5.35 d (8.0)	C-28
2'	73.9	3.30 ^a	
3'	78.1	3.42 d (9.5)	
4'	71.1	3.36 ^a	
5'	78.7	3.38 ^a	
6'	62.4	3.66 dd (12.5, 4.0)	
		3.80 dd (12.5, 4.0)	
Gal			
1''	121.6		
2''	110.1	7.08 s	C-1'', C-3'', C-4'', C-6'', C-7''
3''	146.6		
4''	139.9		
5''	146.6		
6''	110.1	7.01 s	C-1'', C-2'', C-4'', C-5'', C-7''
7''	168.3		

^aOverlapped, assignment determined by COSY, HSQC and HMBC.

arjungenin and β -D-glucopyranosyl 2 α ,3 β ,6 β -trihydroxy-23-galloylolean-12-en-28-oate have previously been reported as potent anti-inflammatory compounds against carrageenan-induced paw edema in rats [17]. In general, the results obtained (Table 2) showed that all the isolated triterpenes exhibit significant antioxidant and potent anti-inflammatory activities. The isolated compounds were subsequently assessed for 15-LOX (lipoxygenase) inhibitory activity and showed promising activity. They also showed potential scavenging ability against 1,1-diphenyl-2-picryl-hydrazil (DPPH) radicals. While being the active inhibitor of LOX, these compounds were also analyzed for anti-inflammatory prospective. From the results, it was concluded that all compounds exhibited potential inhibition of membrane lyses and albumin denaturation. These results are in good agreement with previous reported results. Thus, the potent antioxidant and anti-inflammatory activities of different extracts from *T. glaucescens* could be attributed to these compounds. Exposure of red blood cell (RBC) to injurious substances such as hypotonic medium and phenylhydrazine results in lysis of its membrane accompanied by hemolysis and oxidation of hemoglobin [18]. The hemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Such injury to RBC membrane will further render the cell more susceptible to secondary damage through free radical-induced lipid peroxidation [19]. Since our result showed a marked attenuation of heat-induced protein denaturation by compounds, these compounds can be useful in treatment of diseases such as rheumatic disorders, cataract and Alzheimer's diseases [20], and all the tested compounds presented their strong potential to be developed as anti-inflammatory drug.

3 Experimental

3.1 General experimental procedure

Column chromatography (CC): silica gel (SiO₂; 250–400 mesh; E. Merk, D-Darmstadt). Thin-layer chromatography: SiO₂ F₂₅₄ plates (E. Merk, D-Darmstadt). Melting point: Buchi M-560 melting point. Optical rotations: Jasco DIP-360 digital polarimeter. UV Spectra: Hitachi UV-3200 spectrophotometer; λ_{max} (log ϵ) in nm. IR Spectra: Jasco 302-A spectrophotometer; in KBr; $\tilde{\nu}$ in cm⁻¹. NMR Spectra: Bruker 600 MHz instrument; δ in ppm rel. to Me₄Si as internal standard, J in Hz. EI-, and HR-FAB-MS: Joel JMS-HX-110 and JMS-DA-500 mass spectrometers with glycerol as matrix; in m/z (rel, %). All the reagents, devices and software were supplied through ICCBS Karachi, Pakistan.

Table 2: Antioxidant and anti-inflammatory activities of compounds (1–8).

Compound	Antioxidant		Anti-inflammatory	
	DPPH scavenging IC 50 μ M	LOX inhibition IC 50 μ M	% Inhibition of albumin denaturation	% Inhibition of hemolysis
1	24.0 $\pm$ 0.11	79.0 $\pm$ 1.10	88 $\pm$ 4.10	76.33 $\pm$ 7.91
2	101 $\pm$ 0.98	61 $\pm$ 2.23	55 $\pm$ 3.44	61.55 $\pm$ 3.22
3	61.32 $\pm$ 0.19	77.0 $\pm$ 3.50	71 $\pm$ 1.42	55.14 $\pm$ 3.51
4	102.4 $\pm$ 0.15	61 $\pm$ 2.11	44 $\pm$ 1.58	46.53 $\pm$ 2.52
5	56.7 $\pm$ 0.05	78.0 $\pm$ 4.22	68 $\pm$ 2.78	56.0 $\pm$ 3.10
6	46.11 $\pm$ 0.21	71.65 $\pm$ 2.15	79 $\pm$ 0.98	76.46 $\pm$ 2.89
7	37.32 $\pm$ 0.03	84.0 $\pm$ 2.5	76 $\pm$ 1.22	65.14 $\pm$ 4.58
8	83.11 $\pm$ 0.73	69.45 $\pm$ 2.41	65 $\pm$ 1.28	85.36 $\pm$ 2.85
BHA	44.2 $\pm$ 0.06	–	–	–
Baicalein	–	22.1 $\pm$ 0.03	–	–
Aspirin	–	–	78 $\pm$ 0.55	75.32 $\pm$ 3.51

3.2 Plant material

The root bark of *T. glaucescens* was collected in Ngaoundéré (Adamaoua region of Cameroon) in October 2015 and authenticated by Dr G. Achoundong of the Cameroon National Herbarium, Yaoundé, where specimens documenting the collection are deposited (22126/SRF/CAM).

3.3 Extraction and isolation

The dried and powdered roots bark of *T. glaucescens* (1.45 kg) was macerated for 24 h with 10 L of MeOH. The filtrate obtained was concentrated under reduced pressure to yield a dark residue (155 g). Part of this extract (133 g) was suspended in water (500 mL) and successively extracted with EtOAc and n-BuOH, yielding 45 and 88 g of extracts after evaporation to dryness, respectively. One part of the EtOAc extract (40 g) was subjected to silica gel (63–200 mm) CC, using a gradient of EtOAc in n-hexane, then with EtOAc–MeOH (90 : 10) to give six main fractions (A–F). Fraction A (n-hex–EtOAc 90 : 10) was subjected to CC using silica gel (32–63 mm) and eluted successively with 0%, 5%, 10%, 15% and 20% of EtOAc in n-hexane to afford compounds **9** (18.2 mg), **10** (13.7 mg), **11** (36.4 mg) and **12** (67.0 mg). Fraction C (n-hex–EtOAc 80 : 20–60 : 40) was chromatographed on a silica gel column with n-hex–EtOAc gradient system to yield compound **4** (124 mg), **5** (68 mg) and **6** (42 mg). Further purification of two sub-fractions of fraction C with successive silica gel and sephadex LH-20 CC and elution with DCM–MeOH gradient system gave compounds **2** (34 mg), **7** (18 mg) and **8** (11 mg). Other sub-fractions of C were subjected to reversed-phase high-performance liquid chromatography using a 10 $\times$ 250 nm Reliasil C18 column, eluting with an isocratic system on

MeOH–H₂O (75:25) at the flow rate of 0.5 mL/min led to the isolation of compounds **1** (R_t 22 min, 14 mg) and **3** (R_t 26 min, 16 mg).

3.4 In vitro anti-inflammatory activities

3.4.1 Inhibition of albumin denaturation

Method of Labu et al. [19] followed with minor modifications. The reaction mixture consists of test and 1% aqueous solution of bovine albumin fraction; pH of the reaction mixture was adjusted using small amount of HCl. The reaction mixture was incubated at 37°C for 20 min and then heated to 51°C for 20 min. after cooling the samples; the turbidity was measured at 660 nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

$$\% \text{ inhibition} = \frac{\text{Abs of cont} - \text{Abs of test}}{\text{Abs of cont}} \times 100$$

where Abs control is the absorbance without sample and Abs_{test} is the absorbance of test.

3.4.2 Membrane stabilization test

Preparation of RBCs suspension: fresh whole human blood (10 mL) was collected and transferred to the anti-coagulant-containing centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline.

Heat-induced hemolysis: The reaction mixture (2 mL) consisted of 1 mL of test sample (1 mg/mL) solution and 1 mL of 10% RBCs suspension; instead of test sample only, saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56°C for 30 min, and then tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min, and the absorbance of the released hemoglobin in the supernatant was read at 560 nm.

The experiment was performed in triplicates for all the test samples. The percentage membrane stability was estimated using the expression:

$$\text{Per Heat} = \frac{\text{OD of cont} - \text{OD of test}}{\text{OD of cont}} \times 100$$

The percentage of membrane stabilization or protection was calculated by using the following formula:

$$\text{Per prot} = 100 - \frac{\text{OD of cont} - \text{OD of test}}{\text{OD of cont}} \times 100$$

3.5 Antioxidant

3.5.1 DPPH radical scavenging assay

The free radical scavenging activity was carried out by DPPH [21]. The solution of DPPH (0.3 µM) was prepared in ethanol. The solution of each sample was prepared in methanol. Five microliters of solution of each sample (with concentration range 50–500 µg) was added to 95 µL of DPPH solution; the mixture was then dispersed in 96-well plates and placed for 30 min into the incubator at 37°C. Then absorbance was recorded at 515 nm by Elisa plate reader (Spectramax plus 384 Molecular Device, CA, USA), and percent radical scavenging activity was assessed in contrast to methanol-treated control. BHA (butylated hydroxyanisole) was used as standard.

3.5.2 Lipoygenase inhibition assay

One hundred and sixty microliters of 100 mM sodium phosphate buffer (pH 8.0) and 10 µL of sample in methanol (concentration range 50–500 µM) was added to each labeled well. Twenty microliters of LOX solution (enzyme 130 units per well) was added, mixed and incubated for 10 min at 25°C. The reaction was then initiated by the addition of 10 µL substrate solution (linoleic acid, 0.5 mM,

0.12% w/v tween 20 in the ratio of 1:2) to each well, and the absorbance was measured after 15 min at 234 nm. Baicalin was used as standard [22].

Termiglaucescin (28-O-β-D-glucopyranoside-2α,3β,19α-dihydroxy-23-galloylolean-12-dien-28-oate (1): Colorless amorphous solid, mp 234–236°C and $[\alpha]_D^{28} -92$ (c 0.001, MeOH). IR: OH (3417 cm⁻¹), carboxylic C=O (1696 cm⁻¹) and C=C (1614 cm⁻¹). ¹H- and ¹³C-NMR data: Table 1. HR-FAB-MS: [M-1]⁻ at *m/z* 817.2631 (calcd, for C₄₃H₆₂O₁₄ 817).

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