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Extracts of Triterpenoid Saponins from Xeromphis nilotica Stem Bark (Rubaceae Family)

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ABSTRACT

The authors have introduced two novel triterpenoid saponins, 3-O-β-D-glucopyranosyl (1→3). 3-O-{O-α-L-rhamno-pyranosyl-(1→3) and 23-diol 1 are the compounds in question. The compound is named "-O-[-O-β-D-glucopyranosyl-(1→3)" The stem bark of Xeromphis nilotica was used to isolate -β-D-gluco-pyranosyl}-pomolic acid, which was then further studied using spectrum data from 1D and 2D nuclear magnetic resonance and HR-ESI-MS, as well as chemical evidence. The compounds were then identified based on their structures.

Keywords:
Xeromphis nilotica
Triterpene saponines
Structural elucidation
Chemical evidence

1. Introduction

Traditional medicine in Sudan makes use of the antispasmodic, antidysenteric, anti-inflammatory, immunomodulatory, and antifertility qualities of the Rubiaceae plant, eromphis nilotica. The plant is native to tropical and subtropical climates. Iridoid glucosides, coumarin glucosides, norneolignans, saponins, and triterpenes were isolated by chemical research of X. nilotica (1–5). 6-9 Isolation and structural elucidation of coumarin glycosides and triterpene saponines were outcomes of our earlier research on this plant. 10 The chemical diversity of Sudanese X. nilotica is being further studied, and two novel triterpenoid saponins, 3-O-β-D-gluco-pyranosyl (1→3), have been identified. The compounds 3O-{O-α-L- rhamno-pyranosyl-(1→3) and 19-Olean-12-ene-1, 23-diol-1 The compound is named "-O-[-O-β-D-glucopyranosyl-(1→3)" The n-BuOH extract of its stem bark was used to generate -β- D-glucopyranosyl-pomolic acid and other compounds (Fig. 1). Isolation and structural elucidation of those triterpenoid saponins are the main topics of this work.

2. Results and discussion

High resolution electrospray ionization mass spectrometry (HR-ESI-MS) revealed a peak at m/z 639.2464 [M+NH4]+, which corresponded to the chemical formula C36H60O8, when compound 1, an amorphous white powder, was measured to be 43 mg. The 1H nuclear magnetic resonance (NMR) spectrum of compound 1 revealed seven tertiary methyl groups, denoted as δ0.01 (s, 3H), 0.84 (s, 3H), 1.89 (s, 3H), 0.93 (s, 3H), 0.94 (s, 3H), 1.04 (s, 3H), and 1.15 (s, 3H), which stand for methyl groups Me-28, 24, 25, 29, 30, 26, and 27 respectively. Additionally, there was one olefinic proton (δ5.22, bars) and two signals at δ2.84 (1H, m, H-18) and 3.15 (3H, dd, J = 4.2, 10.0Hz, H-3). The presence of seven methyl carbons (δ 32.35, 14.50, 15.56, 33.47, 22.45, 16.32 and 25.54 ppm) representing methyl groups Me-28, 24, 25, 29, 30, 26 and 27, respectively, two olefinic carbons (δ 122.18 and 143.06 ppm) and three oxygenated carbons (δ 62.87, 72.08 and 89.36 ppm) were confirmed by the 13C-NMR data of the aglycone portion of 1. They allocated six of the thirty-six carbons to the oligosaccharide groups. One sugar anomeric proton was seen at δ 4.30 (d, J = 7.7 Hz) in the 1H and 13C NMR spectra shown in Table 1, along with carbon at δ 105.29ppm. HMBC provided additional evidence regarding the anomeric proton H-1' of glucose at δ 4.30 ppm having a cross beak with the aglycone at δ 89.36 ppm, confirming that it is glucose linked to the aglycone on C-3. Additionally, there was an interaction between C-13 (δ 143.06) and H-18 (δ 2.84) and H-12 at (δ 5.23), as well as a cross beak from C-23 with H-3 and H3, C-24. With the use of acid hydrolysis and paper chromatography (PC), the monosaccharide was determined to be glucose by comparing its retention factor (Rf) value to that of D-glucose. 11 The high values of the H-1' coupling

constant [14] were used to determine the β configuration of the anomeric proton of the glucose units. Compound 1, as mentioned before, is 3-O-β-D-glucopyranosyl (1→3)- Olean-12- ene-19, 23-diol.An amorphous powder was produced as Compound 2. The HR-ESI-MS analysis revealed peaks at m/z 981.9786 [M+Na]+, which might be interpreted as the chemical formula C48H78O19. The 1H-NMR spectrums revealed signals due to seven methyl protons (δ 0.79, s, 0.81, 84 s, 0.95, s, 1.05 s, 1.16 s, and 1.23 (d, J=6.2 Hz) which represent H-26, H-24, H-25, H-29, H-27, H- 30 and H-23 respectively , an olefinic proton (δ 5.26,m , H-12), three anomeric protons δ (4.37, d, J=7.9 Hz; 4.56, d, J=7.9 Hz and 5.17, d, J=7.6Hz), a multiplet of one proton at δ 2.49 (1H, m, H- 18) which indicating to tow protons on C-18, one signal at δ 3.19 (1H, dd, J = 11.6, 4.5 Hz, H-3). The 13C NMR signals for the anomeric carbons (resonances at δ 101.87, 103.65, 104.22) correlate to seven methyl carbons, two olefinic carbons, and two signals at δ 12.8.04 and 139.40 ppm, respectively. The compound's monosaccharides, as revealed by TLC examination of its Rf value, are D-glucose and L-rhamnose, as indicated by acid hydrolysis. It may be inferred that 2 contains one L-rhamnose based on the distinctive proton signal at δ1.24 (3H, d, J = 6.0 Hz) in the 1H NMR spectrum. two residues of D-glucose. The presence of a distinct signal at 2.49 ppm (1H, s, H-18) in the 1H-NMR spectrum and the resonance of a carbon atom supporting a hydroxyl group at 72.16 ppm (C-19) in the 13C-NMR analysis confirmed that compound 2 is a derivative of urs-12-en-28-oic acid with a 19-O substitution. The 19-α-OH stereochemistry was confirmed by the hydroxyl group's resonance at C-19. Using values provided for 3-hydroxy-12-en-19-ol-28-oic acid (pomolic acid), the 1H and 13C NMR spectrum data of the aglycone half of molecule 2 were interpreted, and there was excellent agreement. 12 It was determined from the high values of the coupling constant that the glucosyl units' anomeric positions were arranged in the β configuration.

The locations of linking of the trisaccharidic chain were indicated by the downfield shifts of C-3' (86.54) and C-3" (82.52) of the first and second glucosyl moieties, respectively, of the H-1' and 1" (J=7.9 Hz and J=7.9 Hz). pages 13–14 In compound 2's key HMBC spectrum, the anomeric proton H-1'" of terminal sugar at 5.17 ppm intersects with C-3" of intermediate glucose at 82.52 ppm. Similarly, the anomeric proton H-1" of intermediate glucose at 4.56 ppm is linked to C-3' of first glucose at 86.54 ppm. Finally, the anomeric proton H-1' of first glucose at 4.37 ppm is linked to C-3 of the glycoside part at 89.30 ppm, as shown in Figure 2. The data presented here led to the following deduced structure for compound 2: 3-O-{O-α-L-rhamno-pyranosyl-(1→3)-O-[-O-β- D-glucopyranosyl-(1→3)] pomolic acid -β-D-lucopyranosyl.

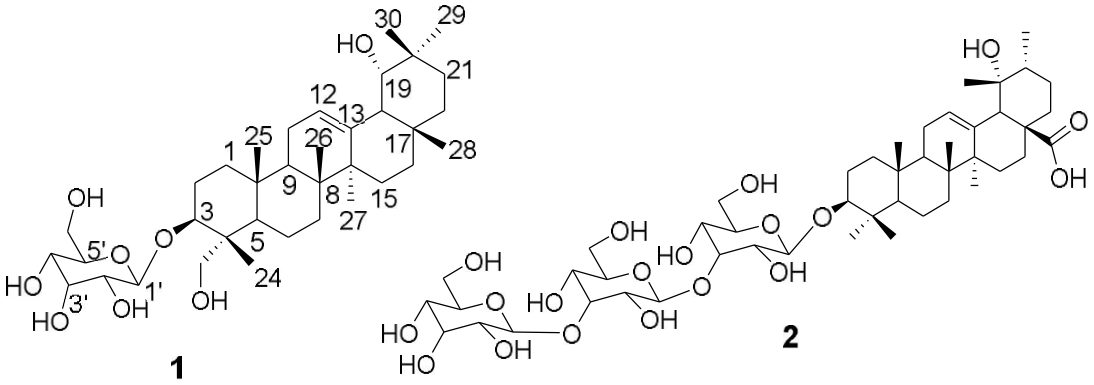

Fig. 1 Structures of compound **1** and **2**

3. Conclusion

From the above findings, as 3-O-β-D-glucopyranosyl (1→3)- Olean-12- ene- 19, 23-diol **1** and 3-O-{O-α-L-rhamno-pyranosyl- (1→3)-O-[-O-β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosy- l}-pomolic acid **2** were isolated from The stem barks of *Xermophis Nilotica*. We suggest these two compounds are first time isolated from natural sources.

4. Experimental or Martials and methods

4.1. Instrumentation and Materials

The chemical shift values are shown on a δ (ppm) scale using TMS as the internal standard, and the NMR spectra were captured using a Bruker-DRX-400-NMR spectrometer (1H at 400Hz and 13C at 100Hz) manufactured by Bruker Biospin Inc. in Germany. The XWIN-NMR version 2.6 software, a standard micro-program from Bruker, was used to

conduct the 2D-NMR experiment. An electro-spray ionization source (eV= 70 V, 80°C) from Waters Ltd., England, was used in the HR-EI-MS studies. The equipment used was a micro-mass-QTOF micro. Silica gel (300-400 mesh, Merck kiesel gel, Qingdao Haiyang Chemical Group Company, China) was used for column chromatography. A set of GF254 silica gel plates manufactured by the Chinese pharmaceutical company Qingdao Haiyang Chemical Group was used for the TLC analyses. The solvents were distilled to remove impurities before use, and they were all of commercial quality.

4.2. Plant material

The stem barks of *Xermophis Nilotica* were collected in august 2014 from Zalingei area, central Darfur state –Sudan, the plant was authenticated by prof:G,A,Yagoub, department of botany ,faculty of agriculture , University of Zalingei. Voucher specimens, (No. 20141013) have been deposited in the herbarium of author's laboratory.

4.3. Extraction and Isolation

After four weeks of air drying, the stem barks of Xermophis nilotica were ground into a powder. The resulting 1.5 kg of powder was then extracted three times at room temperature with 95% EtOH, each time using three liters. The organic solvent was removed from 355g of ethanolic extracts by combining them and then concentrating them in a vacuum (Rotary evaporation). After drying, the mixture was prepared by successively partitioning with petroleum ether (FI), chloroform (FII), ethyl acetate (FIII), and n-butanol (F1V). In each instance, we made care to repeat each partition process three times to guarantee thorough extraction. (Fraction I) was discarded due to its high fatty substance content. Fraction II was evaporated to obtain 40 g, which were then chromatographed on 800 g of silica gel using a gradual (CHCl3:MeOH) gradient. The first elution was with pure CHCl3, followed by CHCl3:MeOH (10:1-1:1), and finally, the last elution was with pure MeOH. Thirty-three fractions were collected. Then, they were examined using Thin Layer Chromatography (TLC) with CHCl3:MeOH (10:1, 10:2, 10:3) as mobile phases. Fractions that showed similar results on TLC were combined to produce four fractions: i, ɕɖ, , and v). Subfractions A–G were obtained by loading fraction III onto a silica gel column and then eluting the mixture with EtOAc by raising its polarity with the addition of MeOH. Part E underwent further column chromatography (CC) with increasing polarity EtOAc and MeOH mixtures to yield E-1 and E-2.

Table 1. ^{13}C NMR Data of Compounds **1** and **2** (100 MHz, MeOH-d$_4$, □ , ppm)

C atom	1	3	C atom	1	3
1	38.72	38.34	25	15.56	14.50
2	27.11	27.38	26	16.32	16.33
3	89.36	89.30	27	25.54	25.19
4	41.44	39.16	28	32.35	180.31
5	55.56	55.59	29	33.47	32.74
6	17.90	18.02	30	22.45	23.37
7	32.59	33.48	1□	105.29	104.87
8	39.15	39.67	2□	74.24	73.63
9	47.61	48.13	3□	76.85	86.54
10	38.22	38.73	4□	70.24	68.71
11	23.02	24.97	5□	76.24	75.90
12	122.18	128.04	6□	61.68	62.85
13	143.06	139.40	1□ □		103.65
14	41.24	41.66	2□ □		72.54
15	27.39	29.31	3□ □		82.52
16	24.96	25.20	4□ □		70.81
17	46.63	46.24	5□ □		76.65
18	45.75	53.71	6□ □		61.18
19	72.12	72.16	1□ □ □		101.22
20	30.18	41.48	2□ □ □		72.71
21	34.22	30.12	3□ □ □		74.66
22	36.36	36.43	4□ □ □		70.92
23	62.27	27.10	5□ □ □		68.70
24	14.51	15.59	6□ □ □		16.45

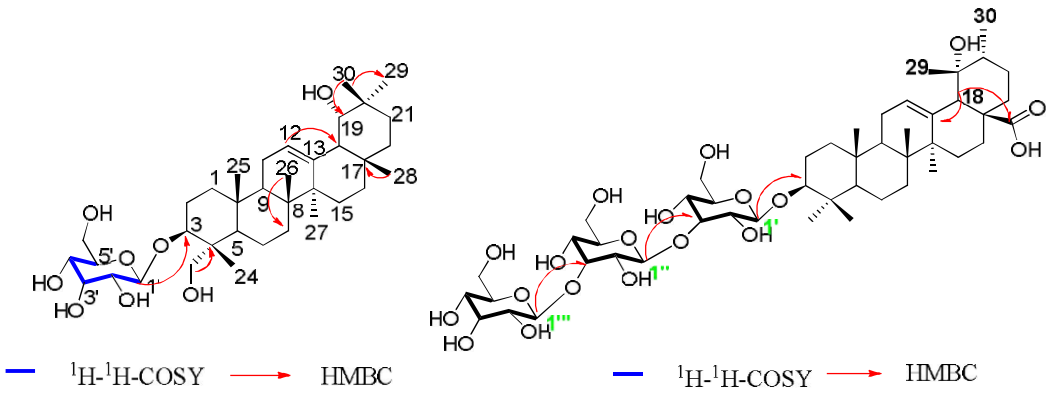

Fig. 2 The key ^{1}H-^{1}H-COSY and HMBC correlations observed in compound **1** and

Fraction E-1 was re-chromatographied over silica to obtain compound **1**. The n-butanol (F1V) was subjected to Polyamide and was eluted with increasing polarities of a MeOH: H_2O (5:1 - 1:1) mixture to obtain subfractions A to F. re-Chromatography of sub-fraction **C**over silica gel using EtOAc: MeOH: H_2O (8:2:1) as mobile phase afforded compound **2** as white powder.

3.4. Acid Hydrolysis of the Saponins

25 mg of each saponin was added to a solution of 10% HOAc:EtOH (10 ml). The mixture was refluxed for 6 h and then concentrated under reduced pressure, the residue was diluted with H_2O (5 ml), the resulting precipitate was collected and chromatographed on a Si gel column (35 g, Si gel)with elution with 10% MeOH in $CHCl_3$ afforded the aglycones. The aqueous phase was neutralised by $NaHCO_3$ and analyzed for sugars using PC, the solvent system used was: n-BuOH–HOAc–H_2O, (4:1:5 upper layer).[12]

Compound 1, obtained as a white powder amorphous. UV 365 nm showed plue color, deep purble color after spraying 7% H_2SO_4 reagent. HR-ESI-MS, showed peaks at *m/z* ٦٣٩.2464 [M + NH_4]$^+$, correspond to the molecular formula $C_{36}H_{60}O_8$. ^{1}H NMR (400 MHz, MeOH-d_4, □, ppm, J/Hz): 5.23 (1H, br.s, H-12),
4.30 (1H, d, J = 7.7, H-1□), 3.83 (1H, dd, J = 11.9, 4.6, H-6□□),
3.6 ٦(1H, dd, J = 11.9, 4.5, H-6□□), 3.65 (1H, d, J = 11.4, H-23□),
3.64 (1H, m, H-3□), 3.32 (1H, m, H-5□), 3.29 (1H, m, H-4□), 3.25
(1H, m, H-2□), 3.23 (1H, d, J = 11.4, H-23□), 3.15 (1H, dd, J = 4.2, 10.0, H-3), 2.84 (1H, m, H-18), 1.15 (3H, s, H-27), 1.04 (3H,
s, H-2٦), 0.9 ٤(3H, s, H-30), ٠.93 (3H, s, H-29), 0.89 (3H, s, H-
25), 0.8 ٤(3H, s, H-24), 0.80(3H, s, H-28). ^{13}C NMR (100 MHz,
MeOH-d_4), see **Table 1.**

Compound 2 was obtained as white powder amorphous. The high resolution electrospry ionization mass spectrometry (HR- ESI-MS), showed peaks at *m/z* 981.9781[M + Na]$^+$ correspond to formula $C_{48}H_{78}O_{19,}$ ^{1}H-NMR (400MHz, MeOH-d4) δ 2.53 (1H, s, H-18), 5.19 (1H, m, H-12), 5.15 (1H, d, J=7.6Hz H-1'''), 4.57
(1H, d, *J* = 7.8 Hz, H-1''), 4.36 (1H, d, *J* = 7.8, Hz, H-1'), 3.98
(1H, dd, *J* = 7.8, 6.2 Hz, H-6''β), 3.94 (1H, dd, *J* = 7.1, 1.7, Hz,
H-6'''β), 3.87 (1H, t, *J* = 10.2 Hz, H-6'β), 3.83 (1H, m, H-
6'''α),3.70, (1H, d, H-6''α), 3.66 (1H, m, H-6'α), 3.64(1H, m, H-
2''') 3.58,(1H, m, H-4'''), 3.50-3.53 (1H, t, *J* = 8.8 Hz, H-3', 3'',
3'''), 3.35-3.38 (3H, m, H-5', 5'', 5'''), 3.34 (1H,m, H-2'), 3.29 (m, H-2''), 3.26(1H, dd, *J* = 8.6, 5.9 Hz, H-2'''), 3.17 (1H, dd, *J* =
11.6, 4.5 Hz, H-3), 2.88 (1H, m, H-18), 1.23 (1H, d, *J* = 6.2 Hz,
H-23), 1.13 (3H, s, H-26), 1.04 (3H, s, H-25), 0.93 (3H, s, H-
30), 0.87 (3H, s, H-29), 0.83 (3H, s, H-24). ^{13}C NMR (100

MHz, MeOH-d_4), see **Table 1.**

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