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Triterpenoid Saponins from the Stem Bark of Xeromphis Nilotica (Rubiaceae)

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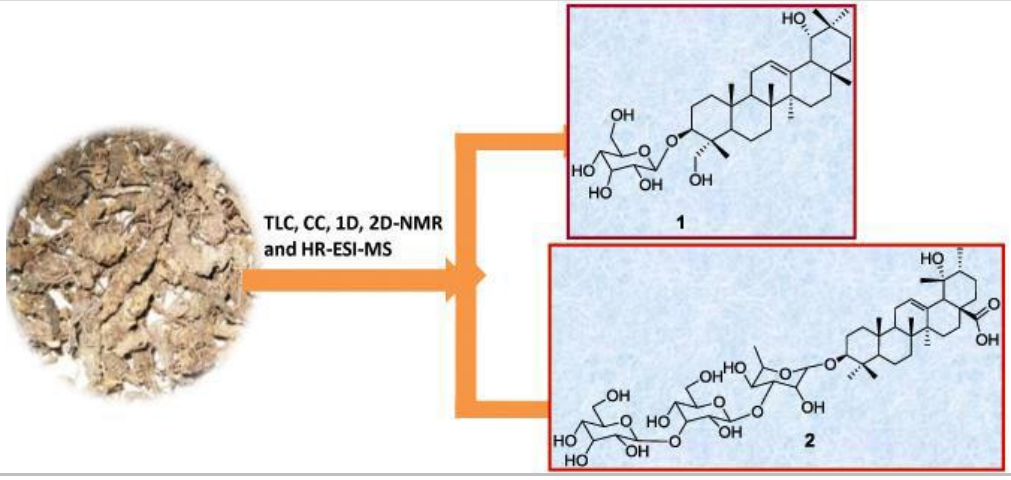
ABSTRACT

Two new triterpenoid saponins, named, 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-glucopyranosyl}-pomolic acid were isolated from the stem bark of *Xeromphis nilotica* through extensive chromatographic technique [TLC and CC] and their structures were elucidated on the basis of their spectral data [1D, 2D NMR and HR-ESI-MS] and chemical evidence.

GRAPHICAL ABSTRACT



1. Introduction

Xeromphis nilotica (Rubiaceae), is a folk medicine plant distributed in tropical and subtropical regions, it is use in the traditional medicinal systems of Sudan for its antispasmodic, antidysenteric, anti-inflammatory, immunomodulatory,and antifertility properties. [1-6] Chemical investigation of *X. nilotica* led to the isolation of triterpenes and saponins, iridoid glucosides, coumarin glucosides, and norneolignans. [7-10] Our previous investigations on this plant had led to the isolation and structural elucidated of coumarin glycosides and triterpene saponines. [11] In continuation of our studies on the chemical diversity of Sudanese *X. nilotica*, two new triterpenoid saponins3-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-glucopyranosyl}-pomolic acid, together were obtained from the n-BuOH extract of its stem bark (**Fig. 1**). This paper deals with the isolation and structure elucidation of those triterpenoid saponins.

2. Results and Discussion

Compound **1**, was obtained as a white powder amorphous 43 mg , the high resolution electrospry ionization mass spectrometry (HR-ESI-MS), showed peak at m/z 639.2464 $[M+NH_4]^+$ which were correspond to the molecular formula $C_{36}H_{60}O_8$. The ^{1}H-NMR spectrum of compound **1** showed seven tertiary methyl groups δ 0.80 (s, 3H), 0.84 (s, 3H), 0.89 (s, 3H), 0.93 (s, 3H), 0.94 (s, 3H), 1.04 (s, 3H) and 1.15 (s, 3H), represent methyl groups Me-28, 24, 25, 29, 30, 26 and 27 respectively, one olefinic proton (δ 5.22, bar, s) and two signals at δ 2.84 (1H, m , H-18), 3.15 (3H, dd, J = 4.2, 10.0Hz, H-3) were observed in the ^{1}H nuclear magnetic resonance (NMR) spectrum. ^{13}C-NMR data of the aglycone part of **1** confirmed the presence of seven methyl carbons (δ 32.35, 14.50, 15.56, 33.47, 22.45, 16.32 and 25.54 ppm)

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represent 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). Six of 36 carbons were assigned to the oligosaccharide moieties. The ^{1}H and ^{13}C NMR spectra of **Table 1** showed one sugar anomeric proton at δ 4.30 (d, J = 7.7 Hz) and carbon at δ 105.29ppm. HMBC showed further supporting information about the anomeric proton H-1' of glucose at δ 4.30 ppm has cross beak with aglycone at δ 89.36ppm which confirmed to be glucose linked to the aglycone on C-3, and interaction of C-13 (δ 143.06) with H-18 (δ 2.84) and H-12 at (δ 5.23), the cross beak from C-23 with H-3 and H_3-24, **Fig 2.** The monosaccharide was identified as glucose by comparing the retention factor (Rf) value with that of D-glucose using the paper chromatography (PC) after acid hydrolysis. [12] The β configuration of the anomeric proton of the glucose units was deduced from the large values of the coupling constant of the H-1' [13]. According to above information compound **1,** established as 3-O-β-D-glucopyranosyl (1→3)-Olean-12-ene- 19, 23-diol.

Compound **2** was obtained as an amorphous powder. The high resolution electrospry ionization mass spectrometry (HR-ESI-MS), showed peaks at m/z 965.4366 [M+Na]+ which was correspond to the molecular formula $C_{48}H_{78}O_{19}$. The ^{1}H-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 two protons on C-18, one signal at *δ* 3.19 (1H, dd, J = 11.6, 4.5 Hz, H-3). The corresponding signals at *δ* 16.33, 15.59, 14.50, 32.74, 25.19, 23.37 and 27.10 (seven methyl carbons), tow signals at *δ* 128.04 and 139.40 ppm (an olefinic carbons) from the signals of the ^{13}C NMR spectrum the anomeric carbons (resonances at *δ* 101.87, 103.65, 104.22). Acid hydrolysis suggested that the monosaccharides of this compound are D-glucose and L-rhamnose, which were identified by TLC analysis of it Rf value. According to the characteristic proton signal at *δ* 1.24 (3H, d, J = 6.0 Hz) in the ^{1}H NMR spectrum, **2** could be deduced to contain one L-rhamnose and two D-glucose residues. The appearance of a characteristic signal at 2.49 ppm (1H, s, H-18) in the ^{1}H-NMR spectrum and the resonance of a carbon supporting a hydroxyl group at 72.16 ppm (C-19) in the ^{13}C-NMR, proved that compound **2** is a 19-O- substituted urs-12-en-28-oic acid derivative. The resonance of the hydroxyl group at C-19 supported the 19-α-OH stereochemistry. The interpretation of ^{1}H and ^{13}C NMR spectral data of the aglycone part of molecule **2** were assigned using values reported for 3 –hydroxy-12-en-19-ol-28-oic acid (pomolic acid), which were good agreement**.** The β configuration of the anomeric position of the glucosyl units was deduced from the large values of the coupling constant of the H-1' and 1"(*J*=7.9 Hz and J=7.9 Hz) respectively, the downfield shifts of C-3' (86.54) and C-3" (82.52) of the first and second glucosyl moieties suggested the points of linkage of the trisaccharidic chain [14, 15]. the key HMBC spectrum of compound **2**, the anomeric proton H-1'" of terminal sugar at δ 5.17ppm has cross beaks with C-3" of intermediate glucose at δC-82.52 ppm and the anomeric proton H-1" of intermediate glucose at δ 4.56ppm was connected with C-3'of first glucose at δ 86.54 then observed the anomeric proton H-1' of first glucose at δ 4.37 connected with C-3 of aglycoe part at δ C-89.30ppm respectively **Fig 2.** On the basis of this evidence, the structure of compound **2** elucidated as 3-O-{O-α-L-rhamno-pyranosyl-(1→3)-O-[-O-β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl}-pomolic acid.

Table 1. ^{13}C NMR data of compounds 1 and 2 (100 MHz, MeOH-d_4, δ, 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

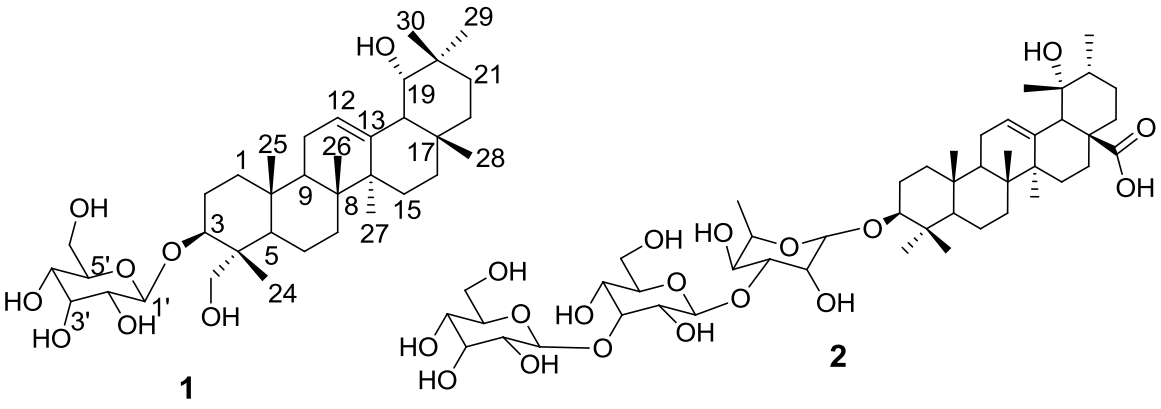

Fig 1. Structures of compound **1 and 2**

4. Experimental or Martials and methods

4.1. Instrumentation and Materials

NMR Spectra were recorded on a Bruker-DRX-400-NMR, (1H at 400Hz and ^{13}C at 100Hz) spectrometer (Bruker Biospin Inc., Germany) and chemical shift values are given on a δ (ppm) scale with TMS as internal standard. 2D-NMR experiment was performed using standard Bruker micro-program (XWIN-NMR version 2.6 software. HR-EI-MS experiments were performed using a micro-mass–QTOF micro instrument, with an electro-spray ionization source (eV= 70 V, 80°C) (Waters Ltd., England). Column chromatography was carried out on silica gel (Merck kiesel gel 300-400 mesh, Qingdao Haiyang Chemical Group Company, China), TLCs were carried out on GF254 silica gel plates (Merck, Qingdao Haiyang Chemical Group Company, China). All solvents were of commercial grade and used after further purification by simple distillation.

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.

The stem barks of *Xermophis nilotica* were air-dried for four weeks and grinded in to powder then the stem barks powder (1.5 kg) was extracted three times with 95% EtOH at room temperature (each 7 days × 3 L). The filtrates were combined together and concentrated in vacuum (Rotary evaporation) for removal of the organic solvent and dried a total of 355g of ethanolic extracts were dissolved in 2L of hot distilled water and then prepared by successive partition with petroleum ether (40-60°C) (F_I), chloroform (F_{II}),ethyl acetate (F_{III}) and n-butanol (F_{1V}). Each partition step was repeated three times to ensure complete extraction in each case. (Fraction I) was rejected since it was rich on fatty substances, Fr. II was evaporated to yield 40 g , which were chromatographed on 800 g of silica gel (Merck kiesel gel 300–400 mech) used ($CHCl_3$:MeOH) gradual, first eluted with $CHCl_3$ pure then with $CHCl_3$:MeOH (10:1–1:1) and in the last eluted with MeOH pure, thirty three fractions were collected, then checked by Thin Layer Chromatography (TLC) used the $CHCl_3$:MeOH (10:1, 10:2, 10:3) as mobile phases, fractions showing similar on TLC were combined together to provided four fractions (i, ii, iii, and v). Fraction III was loaded on a silica gel column, elution was carried out with EtOAc by increasing polarity with addition of MeOH, yielding subfractions A to G. Subfraction E was further subjected to column chromatography (CC) using again EtOAc and MeOH mixtures with increasing polarities to give two major fractions E-1 and E-2. 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.

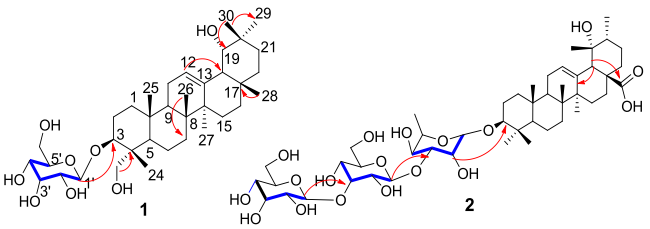

Figure 2. The key HMBC (⟶) and Cosy (—)correlations observed in compound **1** and **2**

2-4. 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* 639.2464 $[M + NH_4]^+$, correspond to the molecular formula $C_{36}H_{60}O_8$. ^1H NMR (400 MHz, MeOH-d_4, δ, 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′β), 3.66 (1H, dd, J = 11.9, 4.5, H-6′α), 3.65 (1H, d, J = 11.4, H-23β), 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-23α), 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-26), 0.94 (3H, s, H-30), 0.93 (3H, s, H-29), 0.89 (3H, s, H-25), 0.84 (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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References

1. Mariod, A.Adam, S.I. Abdelwahab, S. Elkheir, Y.M. Ahmed, P.N.M. Fauzi, C.S.. *Acta Scientiarum Polonorum Technologia Alimentaria.* **2012**, *11*, 249-58.
2. A. Nabeil. Antimicrobial, Doctoral dissertation, UOFK, **2015.**
3. B. Adzu. *International Journal of Biological and Chemical Sciences*, **2008**, *2*, 359-362.
4. J. Fotie, D.S. Bohle, M.L. Georges, E. Leimanis, G. Rukunga, A.E. Nkengfack.. *Journal of Natural Products*. **2006**, *69*, 62-67.
5. 2. S. Margaret, *Flowering Plants in West Africa*, Cambridge University Press, **1988.**
6. B. Adzu, M. B. Amizan, S. E. Okhale, *J. Ethnopharmacology***, 2014,** 158, 271.
7. A. K. Bashir, *Int. J. Pharmacognosy*, **1996**, 34, 202.
8. H. A. R. Farid, O. Kuner, E. Haslinger, C. Seger, *Monatshefte fur Chemie*, **2002**, 133, 1453.
9. I. Hedberg, O. Hedberg, P. J. Madati, K. E. Mshigeni, E. N. Mshiu, G. Samuelsson, *J. Ethnopharmacology*, **1983**, 9, 237.
10. S. C. Chabra, R. L. A. Mahunnah, E. N. Mshiu, *J. Ethnopharmcology*, **1991**, 33, 143.
11. I. Abdurrahman, Y. C. Xia, H. Y. Lai, *WJPR***, 2016**, 15, 292-300.
12. H. Jeffrey, *Phytochemical Methods a Guide to Modern Techniques of Plant Analysis*, Springer Science and Business Media, **1998.**
13. A. A. Magid, L. V. Nazabadioko, I. Renimel, D. Harakat, C. Moretti, C. Lavaud, *Phytochemistry*, **2006**, 67, 2098.
14. M. A. Ouyang, Y. Q. Liu, H. Q. Wang, C. R. Yang, *Phytochemistry*, **1998,** 49, 2485.
15. J. Li, X. Huang, X. H. Jiang, Q. F. Zhu, Y. Yang, G. C. Gao, Chem. Pharm. Bull., **2015**, 63, 389.

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