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In vitro Cytotoxic Pentacyclic Triterpenoids of *Newbouldia laevis*

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Abstract: Bioactivity directed fractionation afforded four cytotoxic constituents from the bioactive methanolic leaf extract (LD_{50} 30 μg mL^{-1}) of *Newbouldia laevis* (Bignoniaceae) collected from Ibadan, Nigeria. Four pentacyclic triterpenoids namely 2α, 3β, 19α-trihydroxy-12-ursen-28-oic acid, 3β, 19α-dihydroxy-12-ursen-28-oic acid, 3β-hydroxy-12-ursen-28-oic acid, 3β-hydroxy 12-oleanen-28-oic acid were found to be the cytotoxic principles. These compounds demonstrated significant cytotoxicity *in vitro* against ST-57 brain tumor transformed fibroblasts with LD_{50} ranging from 0.4 μg mL^{-1} (compound 1) to 4 μg mL^{-1} (compound 4). These ursene and oleanene type triterpenoids are encountered for the first time in this plant. The structures of these compounds were established by spectral data.

Key words: Bignoniaceae, fibroblasts, cytotoxicity, bioactivity, ursenoic acid, oleanenoic acid

INTRODUCTION

In a continuing collaborative search for naturally occurring medicinal agents from plants, the leaves of *Newbouldia laevis* (Beauv.) Seem. ex Bureau (Bignoniaceae) collected from Ibadan, Nigeria were selected for investigation. Previous phytochemical reports on this species were on the root, root bark, flowers and stem. *N. laevis* is a common tree of West African origin well known for its ethno medical uses. The leaves, bark, stem and roots are used in the treatment of conjunctivitis, arthritis, dysentery, enlarged spleen, heart burn, wounds, ear and stomach ache, sore feet, chest pain, epilepsy and children's convulsion (Burkill, 1985, 2000; Oliver-Bever, 1986; Iwu, 2000). The anti-inflammatory, antipyretic, analgesic and anticonvulsant properties of the stem bark and flowers have been studied (Olajide *et al*., 1997; Usman *et al*., 2008; Tanko *et al*., 2008a). Different parts of the plant has also been shown to exhibit antimicrobial, antimalarial, sedative, anti-inflammatory, antinociceptive, anti-diabetic, uterine contractile and antioxidant properties (Gafner *et al*., 1996; Amos *et al*., 2002; Eyong *et al*., 2005; Kuete *et al*., 2007; Usman and Osuji, 2007; Tanko *et al*., 2008a, b; Ogunlana and Ogunlana, 2008; Bafor and Sanni, 2009; Ainooson *et al*., 2009; Bafor *et al*., 2010; Oloyede *et al*., 2010).

Recent phytochemical analysis of the root, root bark and stem of this plant afforded alkaloids, quinoids, ceramides and phenylpropanoids amongst others (Adesanya *et al*., 1994; Houghton *et al*., 1994; Aladesanmi *et al*., 1998; Gafner *et al*., 1996, 1997, 1998; Gormann *et al*., 2003, 2006; Eyong *et al*., 2005, 2006; Kuete *et al*., 2007).

There was no extensive report on the isolation of compounds from the leaves (Usman and Osuji, 2007). Bioassay-guided fractionation of the leaf methanolic extract of *N. laevis* afforded four cytotoxic pentacyclic triterpenoids which are encountered for the first time in the plant.

This study therefore, reports the isolation of four triterperoids namely 2α, 3β, 19α-trihydroxy-12-ursen-28-oic acid or tormentic acid, 3β, 19α dihydroxy-12-ursen-28-oic acid or pomolic acid, 3β-hydroxy-12-ursen-28-oic acid or Bungeolic acid, 3β-hydroxy-12-oleanen-28-oic acid or Astrantiagenin-C as cytotoxic constituents of the leaf methanolic extract of *N. laevis* (Fig. 1).

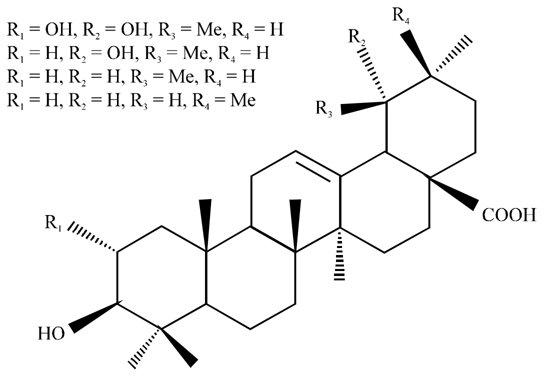

Fig. 1: Cytotoxic constituents of the leaf methanolic extract of *N. laevis*

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MATERIALS AND METHODS

General methods: All melting points were determined on a Kofler hot stage apparatus and are uncorrected. IR and UV spectra were recorded on a Pelkin Elmer model 983. Mass spectra were determined on an MS9025 Mass spectrometer. ^{1}H and ^{13}C-NMR including 2D-NMR, DEPT spectra were recorded on a Bruker WP300SY Spectrometer (300.133 MHz for ^{1}H and 75.468 MHz for ^{13}C).

Plant materials: The leaves of *N. laevis* collected from Jericho forest reserve, Ibadan, Nigeria was authenticated at source and confirmed by Mr. Felix Usang at the Forest Research Institute of Nigeria where a voucher specimen (No. KH11O6427) was deposited in the herbarium.

Extraction and isolation: The air dried leaves of *N. laevis* (800 g) were ground and soxhlet extracted first with hexane for 48 h to afford the hexane extract (34.3 g) and subsequently with methanol to yield the methanolic extract (76.6 g, ST-57, LD$_{50}$ 30 μg mL^{-1}). A portion of the methanol extract (30 g) was subjected to dry flash column (8×10 cm) chromatography on silica gel (230-400 mesh). Gradient elution of the column started with light petroleum (40-60°C) followed by increasing percentages of light petroleum (5, 10 and 15%). Fractions obtained were analysed by silica gel TLC and combined on the basis of similar TLC profiles to give 10 major fractions (NL01-NL10). Compounds were detected by UV light (254 nm), I_2 absorption or Vanillin-H_2SO_4 spray reagent. Subsequently, further investigations were only on the bioactive fractions (NL 6-8).

Fraction NL08 (250 mg, LD$_{50}$ 10 μg mL^{-1} *in vitro* against ST-57 brain tumor transformed fibroblasts) was obtained from 90% EtOAc in light petroleum and after repeated column and flash chromatographic separation yielded compound 1 which was recrystallised from methanol-acetone giving a white solid (mp 264-266°). On structural elucidation and comparison with reference data (Doddrell *et al*., 1974; Numata *et al*., 1989; Lontsi *et al*., 1990; Rahman and Ahmad, 1994), compound 1 was resolved as 2α, 3β, 19α-trihydroxy-12-ursen-28-oic acid or tormentic acid.

Fraction NL07 (250 mg, LD$_{50}$ 15 μg mL^{-1} *in vitro* against ST-57 brain tumor transformed fibroblasts) was eluted from 85% EtOAc in hexane yielding compound 2 as pure white crystalline substance on repeated column chromatographic separation. It was recrystallized from methanol-acetone (mp 280-82°). On structural elucidation and comparison with reference data (Kuang *et al*., 1989; Guang-Yi *et al*., 1989; Soares *et al*., 1998), the structure of compound 2 was deduced as 3β, 19α-dihydroxy-12-ursen-28-oic acid or pomolic acid.

Fraction NL06 (336 mg, LD$_{50}$ 20 μg mL^{-1} *in vitro* against ST-57 brain tumor transformed fibroblasts) was obtained from 85% EtOAc in hexane as a greenish solid which was purified in similar manner to NL08 giving compound 3 as a white crystalline solid (mp 277° {Decomp}). Compound 3 was identified as 3β-hydroxy-12-ursen-28-oic acid or bungeolic acid by spectral data and comparison with reference data (Connolly and Hill, 1991; Rahman and Ahmad, 1994).

Fraction NL06 (336 mg, LD$_{50}$ 20 μg mL^{-1} *in vitro* against ST-57 brain tumor transformed fibroblasts) was obtained as a greenish substance from 85% EtOAc in hexane. Repeated column chromatographic, LH 20 Sephadex (33% chloroform in methanol) separation and recrystallization from acetone-methanol mixture, yielded compound 4 as a white crystalline solid (mp 272° {Decomp.}) compound 4 was identified as 3β-hydroxy-12-oleanen-28-oic acid or Astrantiagenin-C by spectral data and comparison with reference data (Numata *et al*., 1989; Connolly and Hill, 1991; Rahman and Ahmad, 1994).

Spectral data

Compound 1:

- IR v_{max}cm^{-1} (KBr): 3577-2940 br (OH and COOH), 1688 (acid)
- HRMS m/z (rel.int.%): 488.077 (0.7, M^{+}, $C_{30}H_{48}O_5$)
- EIMS 70 eV: 264 (100), 249 (36.5, 264-CH_3), 219 (18, 264 -COOH), 223 (15.5)
- ^{1}HNMR (C_5D_5N, 300.133 MHz): δ 5.55 (1H, t, J = 3.5, H-12), 4.09 (1H, dt, J = 6, 9 Hz, H-2β), 3.38 (1H, d, J = 9Hz, H-3), 3.12 (1H, dt, J = 13,5 Hz, H-16α), 3.04 (1H, s; H-18), 2.3 (2H, d, J = 6 Hz; H-1), 2.28 (1H, dt, J = 13, 4 Hz, H-15β), 2.25 (1H, dt, J = 13, 5 Hz, H-16β),1.95 (1H, dt, J = 13. 5 Hz, H-15α), 1.70 (3H, s, 29-Me), 1.42 (3H, s, 27-Me), 1.26 (3H, s, 23-Me), 1.12 (3H, d, J = 6 Hz, 30-Me), 1.09 (3H, s, 26-Me), 1.07 (3H, s, 24-Me), 1.01 (3H,.s, 25-Me)
- ^{13}CNMR (C_5D_5N, 75.468 MHz (Table 1): δ 180.7 (C-28), 140.0 (C-13), 127.9 (C-12), 84.0 (C-3), 72.9 (C-19), 68.6 (C-2), 56.0 (C-5), 54.6 (C-18), 48.3 (C-17), 47.9 (C-1), 47.6 (C-9), 42.4 (C-14), 42.2 (C-20), 40.4 (C-8), 39.8 (C-4), 38.5 (C-22), 38.5 (C-10), 33.5 (C-7), 29.3 (C-23), 26.9 (C-21), 29.1 (C-29), 26.4 (C-16), 29.3 (C-15), 24.7 (C-27), 24.1 (C-11), 19.0 (C-6), 17.7 (C-26), 17.3 (C-30), 16.9 (C-25), 16.8 (C-24)

Compound 2:

- IR v_{max}cm^{-1} (pyridine): 3600-3300 br (OH and COOH), 1697 (acid)
- HRMS m/z (rel.int.%): 472.177 (0.87, M^{+}, $C_{30}H_{48}O_4$)
- EIMS 70 eV: 390 (M^{+}-COOH-$(H_2O)_2^{+}$), RDA fragment of ring C, 264 (100), 249 (25.5, 264-CH_3), 219 (11.264-COOH), 207 (25.5)

Table 1: ^{13}CNMR of compounds 1-4

	δ: ^{13}CNMR (C_5D_5N, 75.468 MHz) of compounds			
C	1	2	3	4
1	47.9	38.9	38.5	38.8
2	68.6	28.0	27.1	28.0
3	84.0	78.1	77.2	78.0
4	39.8	39.3	38.2	37.3
5	56.0	55.8	54.3	55.7
6	19.0	18.8	20.1	18.7
7	33.5	35.5	35.8	33.1
8	40.4	40.3	39.0	39.6
9	47.6	47.7	47.0	48.0
10	38.5	37.3	37.8	37.2
11	24.1	23.9	23.2	23.6
12	127.9	128.0	125.5	122.4
13	140.0	139.9	138.1	144.7
14	42.4	42.0	38.6	39.3
15	29.3	29.2	27.0	28.2
16	26.4	26.3	24.4	23.6
17	48.3	48.2	46.4	46.4
18	54.6	54.5	51.8	41.9
19	72.9	72.6	38.1	42.1
20	42.2	42.3	38.6	30.8
21	26.9	26.8	32.1	34.1
22	38.5	38.4	35.9	37.2
23	29.3	28.7	28.6	28.7
24	16.8	17.1	14.3	15.6
25	16.9	16.4	14.7	16.5
26	17.7	16.7	16.0	17.3
27	24.7	24.6	22.2	26.0
28	180.7	180.6	180.9	180.1
29	29.1	27.0	29.9	33.1
30	17.3	15.5	17.3	23.6

- ^{1}HNMR (C_5D_5N, 300.133 MHz): δ 5.62 (1H, t, J = 3.5 Hz, H-12), 3.41 (1H, t, J = 9 Hz, H-3α), 3.14 (1H, dt, J = 13,5 Hz, H-16α), 3.05 (1H, s; H-18), 2.35 (1H, dt, J = 13,5, H-15β), 2.24 (1H, dt, J = 13.5 Hz, H-16β), 2.08 (1H, m; H-15α), 1.87 (2H, m; H-2), 1.72 (3H, s; Me-29). 1.44 (3H, s, Me-27), 1.21 (3H, s, Me-24), 1.10 (3H, d, J = 6 Hz, Me-30), 1.10 (3H, s, Me-26), 1.02 (3H, s, Me-23), 0.90 (3H, s, Me-25)
- ^{13}CNMR (C_5D_5N, 75.468 MHz (Table 1): δ: 180.6 (C-28), 139.9 (C-13), 128.0 (C-12), 78.1 (C-3), 72.6 (C-19), 55.8 (C-5), 54.5 (C-18), 48.2 (C-17), 47.7 (C-9), 42.0 (C-14), 40.3 (C-8), 39.3 (C4), 38.9 (C-1), 38.4 (C-22), 37.3 (C-10), 35.5 (C-7), 42.3 (C-20), 29.2 (C-15), 28.7 (C-23), 28.0 (C-2), 27.0 (C-29), 26.8 (C-21), 26.3 (C-16), 24.6 (C-27), 23.9 (C-11), 18.8 (C-6), 17.1 (C-24), 16.7 (C-26), 16.4 (C-25), 15.5 (C-30)

Compound 3:

- IR v_{max} cm^{-1} (KBr): 3430-2920 br (OH and COOH), 1695 (acid)
- HRMS m/z (rel.int.%): 456.606 (3.22, M^+, $C_{30}H_{48}O_3$),
- EIMS 70 eV: RDA fragment of ring C, 248 (100), 203 (48.8, 248-COOH), 207 (25.25)
- ^{1}HNMR (C_5D_5N, 300.133 MHz): δ 5.23 (1H, t, J = 3.5 Hz, H-12), 3.50 (1H, t, J = 9 Hz, H-3α), 2.18 (1H, d, J = 3.5, H-18), 1.82 (2H, m; H-2), 1.80 (2H, dt, J = 13.5 Hz, H-16), 1.27 (6 H, d, J = 6 Hz; Me-29, 30), 1.17 (2H, dt, J = 10.3, 4.3 Hz, H-15), 0.97 (3H, s, Me-27), 0.93 (3H, s, Me-23), 0.92 (3H, s, Me-25), 0.89 (3H, s, Me-24), 0.86 (3H, s; Me-26)
- ^{13}CNMR (C_5D_5N, 75.468 MHz (Table 1): δ 180.9 (C-28), 138.1 (C-13), 125.5 (C-12), 77.2 (C-3), 54.3 (C-5), 51.8 (C-18), 47.0 (C-9), 46.4 (C-17), 39.0 (C-8), 38.6 (C-20), 38.6 (C-14), 38.5 (C-1), 38.2 (C-4), 38.1 (C-19), 37.8 (C-10), 35.9 (C-22), 35.8 (C-7), 32.1 (C-21), 29.9 (C-29), 28.6 (C-23), 27.1 (C-2), 27.0 (C-15), 24.4 (C-16), 23.2 (C-11), 22.2 (C-27), 20.1 (C-6), 17.3 (C-30), 16.0 (C-26), 14.7 (C- 25), 14.3 (C-24)

Compound 4:

- IR v_{max} cm^{-1} (KBr): 3500-2941 br (OH and COOH), 1689 (acid)
- HRMS m/z (rel.int.%): 456.007 (1.55, M^+, $C_{30}H_{48}O_3$)
- EIMS 70 eV: RDA fragment of ring C, 248 (100), 233 (4.18, M^+-CH_3), 207 (25.25, M^+-COOH), 203 (16)
- ^{1}HNMR (C_5D_5N, 300.133 MHz): δ 5.48 (1H, t, J = 3.5 Hz, H-12), 3.45 (1H,t, J = 7 Hz,; H-3α), 3.29 (1H, t, J = 3.5 Hz, H-18), 1.82 (2H, m, H-2), 1.80 (1H, dd, J = 13.5 Hz, H-16α), 1.27 (3H, s; Me-27), 1.23 (3H, s; Me-23), 1.17 (1H, dd, J = 13, 4.5, H-15β), 1.0 (3H, s; Me-26), 1.0 (3H, s; Me-25), 0.98 (3H, s; Me-29), 0.92 (3H, s; Me-30), 0.85 (3H, s; Me-24)
- ^{13}CNMR (C_5D_5N, 75.468 MHz (Table 1): δ 180.1 (C-28), 144.7 (C-13), 122.4 (C-12), 78.0 (C-3), 55.7 (C-5), 48.0 (C-9), 46.4 (C-17), 42.1 (C-19), 41.9 (C-18), 39.6 (C-8), 39.3 (C-14), 38.8 (C-1), 37.3 (C-4), 37.2 (C-22), 37.2 (C-10), 34.1 (C-21), 33.1 (C-29), 33.1 (C-7), 30.8 (C-20), 28.7 (C-23), 28.2 (C-15), 28.0 (C-2), 26.0 (C-27), 23.6 (C-30), 23.6 (C-16), 23.6 (C-11), 18.7 (C-6), 17.3 (C-26), 16.5 (C-25), 15.6 (C-24)

Cytotoxicity assay: The *in vitro* cytotoxicity assay were carried out to the standard protocols established by the National Cancer Institute (Geran *et al*., 1972). Different concentration (4 and 0.4 μg mL^{-1}) of the compounds were tested *in vitro* against ST-57 brain tumor transformed fibroblasts.

Primary culture of fibroblasts was prepared by method of explantate culture (Meier *et al*., 1990a, b). Fibroblasts were cultured as mono layers on all to a confluency (5×10^4 cells cm^{-2}) on plastic stips in a 4 well multiwell. The cells were innoculated in 2 mL medium containing the compounds (dissolved in methanol and diluted with 0.1 phosphate buffer saline) in sterile atmosphere in multiwells. Control cells contained

Table 2: Cytotoxicity of compounds 1-4 against brain tumor transformed fibroblasts

	LD 50 µg mL^{-1}						
Compounds	0.04	0.1	0.2	0.4	1	2	4
1	-	-	-	+++	+++	+++	+++
2	-	-	-	-	++	+++	+++
3	-	-	-	-	-	++	+++
4	-	-	-	-	-	-	++
Control	-	-	-	-	-	-	-
Adriamycin	+++	+++	+++	+++	+++	+++	+++

+++: Good activity; +: Low activity; ++: Moderate activity; -: No activity

methanol and phosphate buffer saline. Cytotoxicity was appreciated by evaluation of LD_{50} values up to the 3rd day of treatment. LD_{50} value ≤4 µg mL^{-1} for pure compounds are considered significant. Results are shown in Table 2.

RESULTS AND DISCUSSION

In an initial study, the methanol extract of *N. Laevis* was found to be cytotoxic when tested *in vitro* against ST-57 brain tumor transformed fibroblasts (LD_{50} 30 µg mL^{-1}). As a result of subjecting this methanol extract to flash column and Sephadex LH 20 chromatography on silica gel and cytotoxic assay, three (NL 06, NL 07, NL O8) of the ten combined fractions were found to demonstrate significant cytotoxic activity (ST 57, LD_{50}<20 µg mL^{-1}). Fractions NL 08 and NL 07 yielded compounds 1 and 2, respectively while fraction NL 06 yielded compounds 3 and 4.

Compound 1 exhibited a molecular formula of $C_{30}H_{48}O_4$ based on its high resolution EIMS data. It gave rise to IR absorption bands for a carboxylic acid and a vinyl bond at v_{max} 1688 and 1640 cm^{-1}, respectively in addition to the observed bands at 3300-2900 (OH, COOH). The ^{13}CNMR displayed signals for 30 carbon atoms: one carbonyl (δ 180.6), two ethylenic carbon atoms (-C-C = , δ 127.9 and 140.0), two oxymethine, an oxygen bearing quaternary carbon, (δ 72.9), seven methyls, eight methylene, four methines and five quaternary carbon atoms. Six methyls were tertiary and the olefinic proton gave a signal at δ 5.55. The signal for H 18 was a broad singlet indicating its β-diposition relative to the D-ring (Doddrell *et al*., 1974). The presence of the double bond at C-12 was confirmed by the chemical shifts at δ 127.9 (C-12) and δ 139.9 (C-13), characteristic of Δ^{12} urs 12 enes (Doddrell *et al*., 1974). Six tertiary methyl at δ 1.01, 1.07, 1.09, 1.26, 1.42, 1.70 (each, 3 H, s) and a secondary methyl at δ 1.12 (3H, d, 30-Me) are indicative of a pentacyclic ursene triterpene (Doddrell *et al*., 1974). Since, in ursene skeleton, the proton at C-18 appeared as a singlet, the tertiary hydroxy group could only be located at C-19. The two secondary carbinol protons resonated at δ 3.38 (1 H, d, J = 9 Hz) and 4.09 (1 H, dt, J = 6.9 Hz) indicating that both were neighbours coupling with each other (J = 9 Hz) while the doublet of triplet was in addition coupled to two other neighbouring protons (J = 6 Hz). Based on these deductions, the one proton doublet was assigned to C-3 with the neighbouring carbinol proton at C-2. This was in accordance with the ^{13}C-nmr resonances observed at δ 84.0 and 68.6. The value of the coupling constants and carbon resonances confirm that the hydroxy group is attached β to C-3 (J = 9 Hz) and strongly deshielded hence, the high resonance of δ 84.0 observed while the other hydroxyl group is attached α to C-2 and shielded thereby resonating at δ 68.6. These value are consistent with reference data (Connolly and Hill, 1991). From comparison of the ^{1}HNMR and ^{13}CNMR of compound 1 with reference data (Kuang *et al*., 1989; Numata *et al*., 1989), the structure of compound 1 was identified as 2α, 3β, 19α-trihydroxy-12-ursen-28-oic acid or tormentic acid.

Compound 2 exhibited a molecular formula of $C_{30}H_{48}O_4$, based on its high resolution EIMS data. It gave rise to IR absorption bands for a carboxylic acid and a vinyl bond at v_{max} 1697 and 1640 cm^{-1}, respectively. The ^{13}CNMR with DEPT spectrum revealed 30 carbon signals including characteristic signals due to a trisubstituted double bond-δ 128.0 (d) and 140.0 (s), one carbonyl (δ 180.6), one oxymethine, an oxygen bearing quaternary carbon (δ 72.6), seven methyls, nine methylene, four methines and five quaternary carbon atoms. The difference between compound 1 and 2 was mainly the absence of one oxymethine which was evident in the low δ value of C-2 (28.0). Based on these and comparison with compound 1 and reference data (Kuang *et al*., 1989; Guang-Yi *et al*., 1989), compound 2 was identified as 3β, 19α-dihydroxy-12-ursen-28-oic acid or pomolic acid.

Compound 3 exhibited a molecular formula of $C_{30}H_{48}O_3$, based on its high resolution EIMS data. It gave rise to IR absorption band at v_{max} 3430-2920 br (OH and COOH), 1695 (acid) 1695 cm^{-1}. The ^{13}CNMR gave signals for 30 carbon atoms, one carbonyl (δ 180.6), two ethylenic carbon atoms (-C-C = , δ 125.5 and 138.1), one oxymethine, seven methyls, nine methylene, four methines and five quaternary carbon atoms. The structure of compound 4 was deduced based on its spectral properties and comparison with reference data (Numata *et al*., 1989; Rahman and Ahmad, 1994). Comparison of the spectral data (IR, MS, NMR) with those of 3β-hydroxy-12-ursen-28-oic acid or Bungeolic acid confirmed that they were the same compound.

Compound 4 exhibited a molecular formula of $C_{30}H_{48}O_3$, based on its high resolution EIMS data. It gave rise to IR absorption bands at v_{max} cm^{-1} (KBr): 3500-2941 br (OH and COOH), 1689 (acid). ^{13}CNMR gave signals for 30 carbon atoms: one carbonyl (δ 180.6), two ethyleniic carbon atoms (-C-C = , δ 125.5 and 138.1), one oxymethine, seven methyls, nine methylene, four methines and six quartarary carbon atoms. The structure of compound 4 was deduced based on its spectral properties and comparison with reference data (Rahman and Ahmad, 1994; Spengel, 1996). Comparison of the spectral data (IR, MS, NMR) with those of 3β-hydroxy-12-oleanen-28-oic acid or Astrantiagenin-confirmed that they were the same compound.

These compounds were tested for their cytotoxicity and found to posses significant cytotoxicity against ST 57 brain tumor transformed fibroblasts. Compound 1 was the most active (LD_{50} 0.4 μg mL^{-1}) while compound 4 (LD_{50} 4 μg mL^{-1}) was the least active (Table 2). The presence of hydroxy group appears to have some effect on the activity as the activity was observed to increase as the number of hydroxy groups in the molecule increased from compound 4 to compound 1.

CONCLUSION

Although, extensive research had been done on the root, stem and flowers of *N. laevis*, limited research has been carried out on the leaves of the plant (Usman and Osuji, 2007). This report therefore, establishes the cytotoxicity of the leaf methanolic extract. In addition, four of the compound responsible for cytotoxic antitumor activity of the leaf methanolic extract has been identified, isolated and characterised. To the best of the knowledge, there is no prior report of the presence of terpenoids in the plant.

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