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Research Paper

Anti-cancerous triterpenoid saponins from *Lecaniodiscus cupanioides*

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ABSTRACT

From the ethanol extract of the stem of *Lecaniodiscus cupanioides* Planch, two known compounds **1** and **2** were isolated and identified as triterpenoid saponins 3-O- [α-L-arabinofuranosyl- (1→3)-α-L-rhamnopyranosyl- (1→2)-α-L-arabinopyranosyl-]-hederagenin and 3-O- [α-L-arabinopyranosyl- (1→3)-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranosyl-]-hederagenin. The structures were established by physicochemical and spectroscopic investigations (MS and NMR) as well as comparison of literature data. The compound **1** exhibited anticancer activity against human colon carcinoma H-116, human lung carcinoma A-549 and human lung carcinoma HT-29 cell lines with IC_{50} 5.0, 2.5 and 2.5µg/ml respectively and compound **2** exhibited similar activities with IC_{50}5.0, 5.0 and 2.5µg/ml respectively. This suggests that the isolated triterpenoid saponins may be considered as potential anticancer leads for further studies.

Keywords: *Lecaniodiscus cupanioides;* Anticancer; Triperpenoid saponins; Cell lines.

INTRODUCTION

Cancer is a major public health burden and leading cause of death in both developed and developing countries. Despite advances in imaging and molecular diagnostic techniques, cancer still affects many people worldwide. It is the second leading cause of death after cardiovascular diseases. Improvement in surgery, chemotherapy and radiotherapy has helped, but not significantly for all forms of cancer. Also, many anticancer agents in circulation are cytotoxic and do not affect only tumor growth but worsen patient's recovery (Parkin, et al., 2005). This makes the search for new anticancer agents with low side effects valuable. Plants have been used as a source of medicinal agents for mankind for centuries. Plant-derived compounds have played a leading role in the provision of new structural leads for the discovery of many useful anti-cancer agents (Cragg and Newman, 2005; Siu, 2011). *Lecaniodiscus cupanioides* Planch (*Sapindaceae*) widely distributed in Southern Nigeria is a traditional medicinal plant that is commonly used as laxative, galactogen, febrifuge and for cough and infection (Adesegun, et al., 2008). The family Sapindaceae is a known potent source

of anticancer agents thus in our search for biologically active constituents of Nigerian medicinal plants, we report here on bioassay-guided fractionation, isolation and structure elucidation of known triterpenoid saponins with anticancer activities from the stem of the plant.

MATERIALS AND METHODS

Plant material: The stems of *L. cupanioides* were collected at Sango, Ogun State, Nigeria and identified by Mr. I. K. Odewo of Forest Research Institute of Nigeria (FRIN) then voucher specimen FHI 105,353 was deposited in the herbarium.

Extraction and isolation of compounds*:* The dried and powdered stem (1kg) of the plant was percolated with 96% ethanol (4L) for 48h using Soxhlet apparatus at the temperature of 80^{o}C. Ethanol was used because is known to dissolve large number of chemical agents. The extract was then concentrated to dryness (5.1% w/w) in vacuum using rotatory evaporator. The dried extract was chromatographed on Si gel column under vacuum using gradient of n-C_6H_{14}-$CHCl_3$-MeOH to give eleven fractions (V1-V11). The $CHCl_3$-MeOH (7:3) fraction was further chromatographed over Si gel isocratically using EtOAc-Acetone-AcOH-H_2O (6:2:1:1) to give eleven fractions. Fractions 8 and 9 as well as fractions 10 and 11 were purified by preparative TLC and HPLC to give compounds **1** (15 mg) and **2** (12 mg) (Figure 1).

Anticancer Assay: The fractions and compounds isolated were studied *in vitro* on human lung carcinoma A-549, human colon carcinoma HT-29 and human colon carcinoma H116 tumor cells. These were cultured in RPMI medium containing glutamine (2 mM), penicillin (50 IU/ml), streptomycin (50µg/ml), supplemented with 5% FBS (A-549 , HT-29 and H-116). The dye reduction assay involving 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT; Sigma Chemical Co., St. Louis, MO) is based on the reduction of MTT by mitochondrial dehydrogenases of viable cells to a blue formazan product, which could be measured spectro photometrically. For each experiment, the cells were harvested from subconfluent cultures using trypsin and resuspended in fresh medium before planting. The tumor cells were incubated in each well with serial dilutions of the tested compounds in 200 µl of complete medium. A separate set of wells was seeded as a growth control to ensure that cells remained in the exponential growth phase. After 2 days of incubation at 37 °C and 5% CO_2 in an atmosphere with 98% humidity, MTT (5mg/ml in PBS) were added to each well and the plate incubated for a further 2 h (37 ºC). The resulting formazan was dissolved in DMSO and read at 490 nm. All determinations were carried out in triplicate. The ED50 values (Con.n of drug yielding 50% cell survival by comparing the OD in wells with drug to the OD in the control wells) were obtained by probit analysis a VAX computer program (Instituto Biomar, S. A., Leon, Spain).

RESULTS

Chemical Analysis:

Compound* 1:* colour less crystals; $[\alpha]_D^{25}$: +11.2^{o} (c 0.5, EtOH); ESI-HRMS m/z 882.3 ($[M-H]^-$); 750 [M-arabinose-H]$^-$; 604 [M-(arabinose+rhamnose)-H]$^-$; 471.3[M-2(arabinose+ rhamnose)-H]$^-$ (calc. for $[C_{46}H_{74}O_{16}-1]$, 882.49767400).

^{1}H NMR (400.13 MHz, C_6D_5N) δ 0.79 (3H, s, H-24), 0.80 (3H, s, H-26), 0.86 (3H, s, H-29), 0.88 (3H, s, H-30), 0.99 (3H, s, H-25), 1.11 (3H, s, H-27), 5.33 (1H, br s, H-12), 4.16 (1H, m, H-3α), 4.94 (1H, d, *J*=7.6Hz, H-1'), 6.25 (1H, d, *J*=7.8 Hz, H-1"), 6.05 (1H, d, *J*=7.9 Hz, H-1'").

^{13}C NMR data (100 MHz, C_6D_5N) δ 38.4 (C-1, t), 25.7 (C-2, t), 80.7 (C-3, d), 43.0 (C-4, s), 47.3 (C-5,d), 17.6 (C-6, t), 32.3 (C-7, t), 39.1 (C-8, s), 47.6 (C-9, d), 36.3 (C-10, s), 23.0 (C-11, t), 122.0 (C-12, d), 144.2 (C-13, s), 41.5 (C-14, s), 27.7 (C-15, t), 23.2 (C-16, t), 46.0 (C-17 s), 41.1 (C-18, d), 45.8 (C-19, t), 30.3 (C-20, s), 33.6 (C-21, t), 32.6 (C-22, t), 63.5 (C-23, s), 13.5 (C-24,q), 15.5 (C-25, q), 16.8 (C-26, q), 25.5 (C-27, q), 179.0 (C-28, s), 32.7 (C-29, q), 23.1 (C-30, q), 104.1 (C-1′, d), 74.7 (C-2′, d), 74.6 (C-3′, d), 69.1 (C-4′, d), 65.6 (C-5′, t), 100.5 (C-1′′, d), 71.2 (C-2′′, d), 78.7 (C-3′′, d), 71.8 (C-4′′, d), 68.8 (C-5′′, d), 18.0 (C-6′′, q), 110.3 (C-1′′′, d), 81.8 (C-2′′′, d), 78.2 (C-3′′′, d), 87.5 (C-4′′, d), 62.1 (C-5′′, t).

***Compound* 2**: colour less crystals; $[\alpha]_D^{25}$: +15.4^o (c 0.5, EtOH); ESI-HRMS m/z 882.2 ([M-H]$^-$), $^-$ (calc. for [$C_{46}H_{74}O_{16}$ –1], 882.49767400).

^{1}H NMR (400.13 MHz, C_6D_5N): δ 1.16 (3H, br s, H-24), 0.95 (3H, s, H-25), 1.05 (3H, s, H-26), 1.57 (3H, s, H-6′′), 1.25 (3H, s, H-27), 1.00 (3H, s, H-30), 0.95 (1H, s, H-29). 4.90 (1H, d, *J*=7.8Hz, H-1'), 6.30 (1H, d, *J*=7.8 Hz, H-1"), 6.15 (1H, d, *J*=7.6 Hz, H-1'").

^{13}C NMR data (100 MHz, C_6D_5N) δ 39.2 (C-1, t), 26.5 (C-2, t), 81.4 (C-3, d), 43.8 (C-4, s), 47.9 (C-5,d), 18.4 (C-6, t), 33.1 (C-7, t), 39.9 (C-8, s), 48.3 (C-9, d), 37.0 (C-10, s), 23.8 (C-11, t), 122.8 (C-12, d), 144.9 (C-13, s), 42.3 (C-14, s), 28.5 (C-15, t), 24.4 (C-16, t), 46.8 (C-17 s), 39.9 (C-18, d), 46.5 (C-19, t), 31.1 (C-20, s), 34.4 (C-21, t), 33.3 (C-22, t), 64.2 (C-23, s), 14.3 (C-24, q), 16.2 (C-25, q), 17.6 (C-26, q), 26.3 (C-27, q), 180.4 (C-28, s), 33.4 (C-29, q), 23.9 (C-30, q), 104.8 (C-1′, d), 75.2 (C-2′, d), 74.7 (C-3′, d), 69.7 (C-4′, d), 66.4 (C-5′, t), 101.5 (C-1′′, d), 72.2 (C-2′′, d), 83.1 (C-3′′, d), 73.2 (C-4′′, d), 69.9 (C-5′′, d), 18.6 (C-6′′, q), 107.7 (C-1′′′, d), 73.3 (C-2′′′, d), 79.9 (C-3′′′, d), 69.9 (C-4′′′, d), 67.3 (C-5′′′, t).

Anticancer assay: The results of anticancer assay for extract, fractions and pure compounds are as shown in Table 1**.**

DISCUSSION

Compound **1** was colorless crystal obtained from column fractions 8 and 9. The negative ion ESI-HRMS showed the [M-H] at m/z 882.3 calculated as 882.49767400 which deduced the formula $C_{46}H_{74}O_{16}$ with fragment peaks appearing at m/z 750.9, 604.0 and 471.3 indicating loss of arabinose, arabinose + rhamnose and 2 x arabinose + rhamnose. The ^{1}H and ^{13}C NMR and HMBC spectra showed signal of trisubstituted double bond δ_H 5.33 ppm (br s, H-12) and δ_C 144.2 and 122.0 ppm, specific for Δ12 double bond in an oleanane skeleton (Silverstein and Webster, 1997; Taskini, et al., 2005). The ^{13}C NMR displayed 46 carbon resonances and anomeric signals at δC 110.0, 104.9 and 101.3 indicating trisaccharide with two pentoses, one hexose and aglycone with 30 signals. The DEPT spectra showed 7 methyl, 13 methylene, 18 methine and 8 quaternary carbon atoms. Comparism of ^{13}C NMR spectra with those reported literature revealed that is a monodesmosides of 3-O-glycoside (Podolak, et al., 2010) with α-L-arabinofuranose, α-L-arabinopyranose and α-L-rhamnopyranose and aglycone as hederagenin (Adesegun, et al., 2008). On the basis of the spectra data, compound **1** was identified as 3-O- [α-L-arabinofuranosyl- (1→3)-α-L-rhamnopyranosyl- (1→2)-α-L-arabinopyranosyl-]-hederagenin.

Compound **2** was also a colour less crystal obtained from column fractions 10 and 11. The anomeric signals at δC 107.0, 104.8 and 101.5 suggested a trisaccharide and δ_C 144.9 and 122.8 ppm, showed the presence of unsaturation at C-12 as found in an oleanane skeleton. Comparison of the ^{13}C NMR spectra with corresponding methylglycosides indicated different sugar composition from compound **1** composed

of -2 units of α-L-arabinopyranose and 1 unit of α-L-rhamnopyranose as sugar while hederagenin was the aglycone. The negative ion ESI-HRMS showed the [M-H] at m/z 882.2 corresponding to the empirical molecular $C_{46}H_{74}O_{16.}$ The DEPT spectra showed 7 methyl, 13 methylene, 18 methine and 8 quaternary carbon atoms. Mass spectra data indicated that compounds **1** and **2** structures were identical but differed in ^{13}C NMR spectra which showed the terminal arabinose C-5′′′ was attached directly to oxygen to form pyranose in **2** unlike furanose in **1.** Consequently, **2** was identified as 3-O- [α-L-arabinopyranosyl- (1→3)-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranosyl-]-hederagenin (Adesegun, et al., 2008).

Anti-cancer activity: Presently, chemotherapy is known as one of the intervention measures for cancer treatment, with remarkable effect on the symptoms and quality of life of patients however, the survival rate is poor. Many cancer patients are known to embrace traditional medicine including herbal therapies (Parkin, et al., 2005). Anticancer compounds are known to inhibit, delay or reverse cancer progression mainly through cytotoxic or apoptosis effect (Naveen, et al., 2012). This led to the development of anticancer therapeutics for several decades. In this experiment, the effect of ethanolic extract of stem of *L. cupanioides*, fractions and isolated compound **1** and **2** on the growth of HT-29, A-431 and A549 cell lines were examined. Some of the fractions were found to have good cytotoxic activity on the cell lines but compounds **1** and **2** isolated from *L. cupanioides* had significant antiproliferative effects (IC50 2.5-5.0 µg/ml) on the human colon carcinoma H-116, human lung carcinoma A-549 and human lung carcinoma HT-29 cancer cell lines.

Triterpenoids are structurally diverse organic compounds characterized by a basic backbone modified in multiple ways, allowing the formation of several naturally occurring triterpenoid varieties (Man, et al., 2010). They have been increased interest in saponins due to their numerous attributes as cardiac, anti-inflammatory immunostimulating and antitumor activities and many are undergoing trials at different phases (Sparg, et al., 2004). Over the years, many biologically active triterpenoids are found to have cytotoxicity against a variety of tumor cells**.** Many oleanane triterpenoid saponins with hederagenin as aglycone have also been reported to show promising antineoplastic activities (Setzer & Setzer, 2003; Tian, et al., 2006). The isolated compounds **1** and **2** have at C-3 position of the aglycone, α -L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl moiety and free carboxylic acid at C-28 of the oleanane skeleton that have been reported to be structural requirements for potent cytotoxic activity of hederagenin glycosides (Chwalek, 2006; Gauthier, et al., 2009). The number and the variety of glycosides of saponins also play key roles in anticancer activity (Yan, et al., 2009).

Saponins have high cholesterol binding properties. Cancer cells have higher amounts of cholesterol analogs in their membranes versus normal cells and saponins act by binding with membrane cholesterol and interfering with cell growth and division. Saponins also bind to bile acids and reduce formation of secondary bile acids and consequently the risk of colon cancer. It was reported that they reduced the number of preneoplastic colon lesions in mice and also inhibited the growth of human carcinoma cells in cultures. Saponins from soybean were also known to suppress the growth of HT-29 colon cancer cells (Gurlinkel & Rao, 2003). The antitumor activity of the isolated triterpenoid saponins could be linked to their ability to block nuclear factor-κB activation, induce apoptosis, inhibit signal transduction, and activate transcription or angiogenesis (Petronelli, et al., 2009).

CONCLUSION

Our study demonstrated that known oleanane triterpenoid saponins isolated from stem extract of *L. cupanioides* have significant *in vitro* anticancer activities against human lung carcinoma A-549, human colon carcinoma HT-29 and human colon carcinoma H116 tumor cells.

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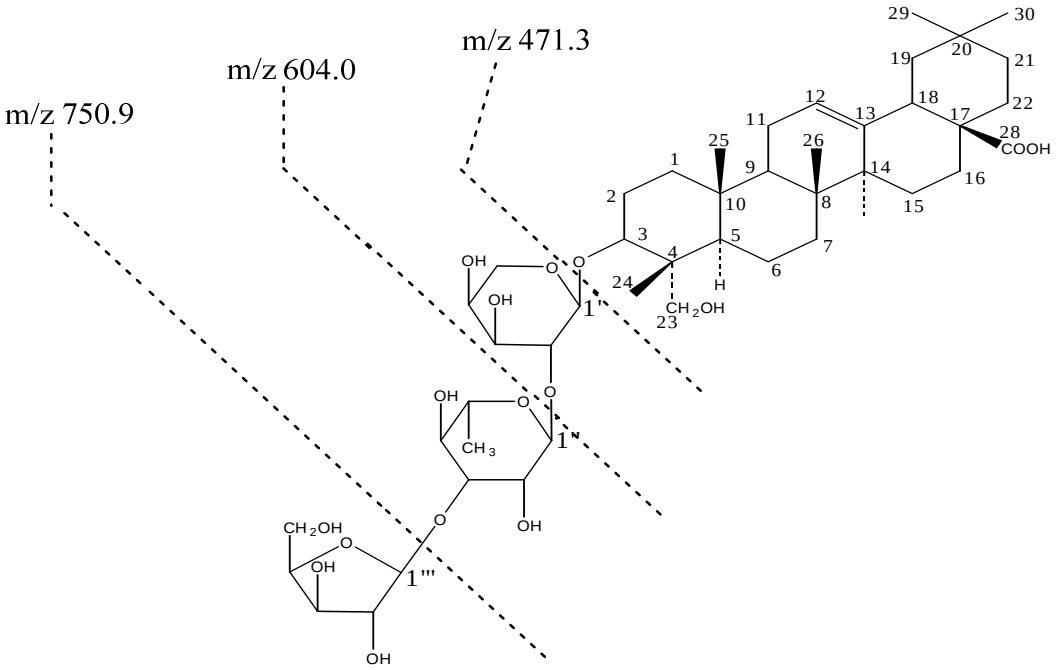
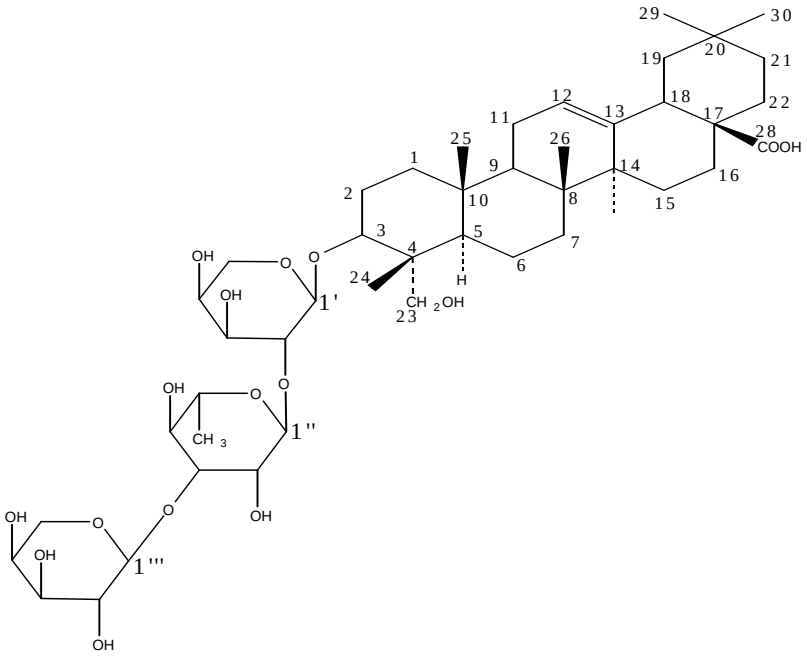
Table-1 : A**ntitumor activity of the extract, column fractions and isolated compound from *L. cupanioides.***

Samples	*In Vitro* Anticancer Activity (IC50 µg/ml)		
	A549	**HT-29**	**H-116**
Extract	<25	<25	<25
V-7	<25	25	5.0
V-8	5	12.5	2.5
V-9	<25	5.0	<25
V-10	<25	<25	5.0
V-11	<25	<25	<25
Compound 1	2.5	2.5	5.0
Compound 2	5.0	5.0	2.5



Compound 1



Compound 2

Figure -1: Fractionation of *L. cupanioides* Stem Extract.