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Biological evaluation of phytoconstituents from *Markhamia tomentosa* ethanolic leaf extract

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ABSTRACT

Phytochemicals are increasingly being used in cancer treatment due to affordability and potential anticancer effect with minimal adverse reactions compared to chemotherapy. *Markhamia tomentosa* is a medicinal plant used traditionally to treat cancer. In this study, the antiproliferative compounds from *M. tomentosa* were isolated using bioactivity-guided approach and characterized using various spectroscopic techniques. Through bioassay-guided fractionation of the crude ethanolic leaf extract, the dichloromethane (Mdf) and ethyl-acetate (Mef) fractions exhibited potent cytotoxicity activity against HeLa cells with IC_{50} values of 83.26 and 104.5 μg/ml respectively in the MTT assay. Trypan blue assay and cell cycle analysis showed that Mdf fraction demonstrated cytotoxic effect with more extensive cell death and induced G_0/G_1 phase cell cycle arrest with concomitant decrease in S phase. Mef fraction showed reduced percentage of stained dead cells as compared to Mdf fraction and induced G_2/M phase with increase in the size of sub-G_1 phase, corresponding to apoptosis. From the isolation and purification of Mdf and Mef fractions by repeated column chromatography, followed by identification by 1D and 2D NMR spectroscopy and by comparison of the NMR data with values reported in literature, sitosterol **1**, mollic acid **2**, phytol **3** and oleanolic acid **4** were isolated for the first time from *M. tomentosa*. Mollic acid **2** exhibited more potent cytotoxic activity compared to compounds **1**, **3** and **4**. The results from these findings suggest that mollic acid **2** isolated from Mef which exhibited apoptotic cell death may be responsible for the earlier reported apoptosis induction capability of *M. tomentosa* against cervical cancer cell line HeLa.

1. Introduction

Several bioactive compounds, particularly the secondary metabolites isolated from medicinal plants have potential therapeutic effects on a myriad of diseases (Ghani, 2003). Based on ethnopharmacological data which provides a significantly increased chance of finding active plants rather than a random approach, many pharmaceutical drugs have been discovered by screening natural products from plants (Reddy et al., 2012). The increasing cost and undesirable side effects of conventional treatments have encouraged people to depend more on folk medicine. Many herbal remedies have long been used as anticancer agents. Emerging evidence has demonstrated that the antineoplastic effects of phytochemicals from natural products may affect cells by various mechanisms, including prevention initiation and promotion of carcinogenesis, or apoptosis induction (Gupta et al., 2001). Identification of the activators of apoptosis may provide more effective strategies for cancer therapy (Wong and Abdul Kadir, 2012). In addition, medicinal plants used as cancer chemopreventive or therapeutic agents produce minimal adverse effects and anti-multidrug resistance (Wu et al., 2002).

Markhamia tomentosa (Benth.) K. Schum ex. Engl. is a tree in the Bignoniaceae family. Preliminary phytochemical investigations of the leaves of *M. tomentosa* showed the presence of major classes of bioactive compounds such as terpenes, steroids, saponins and flavonoids (Ugbabe et al., 2010). Pentacylic triterpenoids including pomolic acid, oleanolic acid, tormentic acid and β-sitosterol have been reportedly isolated from the stem bark of *Markhamia tomentosa* (Tantangmo et al., 2010). Ajugol, tormentic acid, carnasol and oxopomolic acid were identified by LC-ESI-MS analysis in the ethyl acetate fraction of the leaf extract of *M. tomentosa* (Sofidiya et al., 2014).

Previously, we have demonstrated the cytotoxic effect of the crude extract of the leaves of *Markhamia tomentosa* against brine shrimp larvae and HeLa cervical cancer cells, chromosomal aberrations induction on *Allium* root cells and non-toxic effect in the liver and kidney function parameters in rats (Ibrahim et al., 2013, 2014, 2016).

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In this study, the isolation and characterization of the bioactive compounds of *Markhamia tomentosa* as well as antiproliferative activity of the isolated compounds are discussed.

2. Materials and methods

2.1. General experimental procedure

The 1D (^{1}H and ^{13}C) and 2D (COSY, HSQC and HMBC) NMR spectroscopy were recorded on a Bruker Avance^{111} 400 spectrometer in deuterated chloroform ($CDCl_3$) and methanol (CD_3OD) at room temperature with tetramethylsilane (TMS) as internal standard. The designations of ^{13}C spectra were done by analysis of DEPT (90 and 135) spectra to determine primary, secondary and tertiary carbons, by the use of 2D techniques and confirmed by comparison of chemical shifts with data in literature (Mahato and Kundu, 1994; Lacroix et al., 2009). A Perkin Elmer Spectrum 100 Fourier transform infrared (FT-IR) spectrometer with Universal attenuated total reflection (ATR) sampling accessory was used to obtain the Infrared (IR) spectra. GC–MS data of analysed samples were recorded on an Agilent gas chromatograph (GC) coupled to inert mass selective detector (MSD) operated in the electron impact (EI) mode with ionization energy of 70 eV. The GC-MSD apparatus was equipped with a DB-5SIL MS (30 m × 0.25 mm i,d., 0.25 μm film thickness) fused silica capillary column and helium - at a flow rate of 2 ml/min was used as the carrier gas.

2.2. Plant collection and extraction

Fresh leaves of *Markhamia tomentosa* (Benth) K. Schum ex Engl. collected from Oke-Igbo, Ondo state, Nigeria in February 2015 were used in the taxonomic identification and authentication in the Herbarium of Department of Botany and Microbiology, University of Lagos. A herbarium specimen was deposited and a voucher number LUH 5535 was obtained. The collected leaves were air dried at room temperature (23 °C ± 2 °C) for two days and pulverised in a mechanical grinder. The powdered leaf material (5 kg) was macerated in 50 L of absolute ethanol for 72 h at room temperature. The resulting ethanol extract was filtered through a Whatman filter paper and evaporated to dryness on a rotatory evaporator (Buchi, Switzerland) at 40 °C to yield 13.31% (*w*/w). The dried, deep brown crude extract (128.98 g) was dissolved in 100 ml of water and partitioned with solvents of increasing polarity: hexane (HEX; 3 × 500 ml), dichloromethane (DCM; 4 × 500 ml), ethylacetate (EtOAc; 3 × 500 ml) and *n*-butanol (BuOH; 2 × 500 ml). The resulting fractions were evaporated to dryness using a rotary evaporator (40 °C) to afford the Hex (Mhf; 19.56 g), DCM (Mdf; 53.07 g), EtOAc (Mef; 9.80 g) BuOH (Mbf; 6.27 g) and aqueous (Maf; 14.97 g) residues.

2.3. Cytotoxicity assay

The cytotoxic effect of the crude extract (Mcr), solvent fractions (Mhf, Mdf, Mef, Mbf and Maf) and isolated compounds from *M. tomentosa* in HeLa cells was investigated using the MTT assay as earlier described by Ibrahim et al. (2013). The cells were treated with the compounds in the final concentrations ranging from 25 to 100 μM for 48 h. IC_{50} values for the fractions and compounds were calculated from dose–response curves using GraphPad Prism 4 software package.

2.4. Evaluation of cell viability

Viability of cells was determined by the trypan blue dye exclusion (TBE) method and analysed by flow cytometry 48 h after initiation. Cells were treated with 100 μg/ml Mef and 80 μg/ml Mdf. Spent tissue culture media containing detached cells, and all wash solutions, were combined and added to the cells obtained from trypsinisation of the remaining adherent monolayer. After centrifugation, the cells were stained with trypan blue (0.4%) and immediately analysed by flow cytometry on a Beckman-Coulter FC500.A minimum of 10,000 events were recorded per sample in FL3 (red fluorescence), using a 488 nm Argon ion laser for excitation.

2.5. Cell cycle analysis

As earlier described by Ibrahim et al. (2013), cell cycle analysis of Mef (100 μg/ml) and Mdf (80 μg/ml) fractions was performed using the Coulter® DNA Prep™ Reagents Kit (Beckman Coulter CA, USA).

2.6. Isolation by column chromatography (CC)

The Mef and Mdf fractions were subjected to column chromatography (silica gel 60; 0.040–0.063 mm; Merck, Germany) on suitably sized columns and the collected fractions were monitored by TLC (Merck 20 cm × 20 cm silica gel 60 F254 aluminium plates) which was analyzed under UV (254 and 366 nm) and visualized as the plates were sprayed with a 10% sulphuric acid in methanol solution and heated for 30 s.

For the Mef fraction (7.46 g), a gradient elution of hexane: ethylacetate was used on a 4 cm diameter column starting with 10% EtOAc in Hex as eluent. This was followed by a gradient of increasing EtOAc percentage, and finally with 100% MeOH. The collected fractions (100 ml each) were combined based on TLC analysis to afford four main fractions E- 1 to E- 4. Fraction E- 2 (280 mg) was re-chromatographed over silica gel in a 2 cm column with Hex:EtOAc (9:1) as solvent system to yield sitosterol **1** (10 mg). Fraction E- 4 (1.23 g) was purified by silica gel CC with EtOAc:MeOH gradient (9.5:0.5) to yield a colourless amorphous solid: mollic acid **2** (230 mg).

Similarly, 30.5 g of Mdf fraction was separated by CC using Hex: DCM and DCM: EtOAc gradient starting with 100% Hex stepped to 50 and 100% DCM. This was followed by 20 and 40% increase of EtOAc every 250 ml to afford seven main fractions D- 1 to D- 7. With Hex:DCM (1:9), yellow thick viscous oil, phytol **3** (850 mg) was obtained as fraction D- 4. Fraction D- 6 (5.13 g) was re-chromatographed over a silica gel column with a Hex:EtOAc step gradient as eluent to give six subfractions d- 1 to d- 6. Sub fraction d- 4 (770 mg) was purified further by silica gel CC eluted with Hex:EtOAC (9:1) gradient to afford oleanolic acid **4** (90 mg).

3. Results and discussion

This present study is a continuation of our previous study that demonstrated the cytotoxic activity of *Markhamia tomentosa* leaf extract against HeLa (cervical cancer) cells through the induction of intrinsic pathway of apoptosis (Ibrahim et al., 2013).

Table 1
Cytotoxic activity of extract, fractions and isolated compounds isolated from the leaves of *M. tomentosa*.

Fractions/compounds	HeLa cells IC_{50} μg/m (μM)
Crude extract	182.0 ± 8.9 (ND)
Hexane	>250
Dichloromethane	83.26 ± 13.8 (ND)
Ethyl-acetate	104.50 ± 5.1 (ND)
n-Butanol	>250
Aqueous	>500
Sitosterol **1**	>100
Mollic acid **2**	34.74 ± 5.4 (73.93 ± 11.5)
Phytol **3**	>100
Oleanolic acid **4**	>100
Melphalan	40 μM ± 2.3

ND: Not determined.

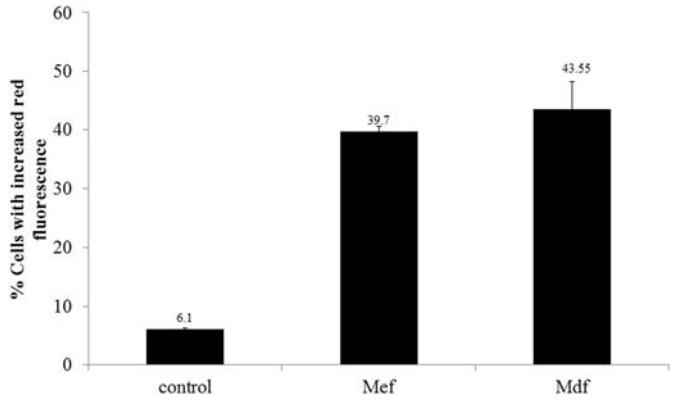

Fig. 1. Cell viability determined by trypan blue staining.

3.1. Cytotoxicity assay

In an attempt to determine the compounds responsible for the cytotoxic effect, the different solvent fractions: Hex (Mhf), DCM (Mdf), EtOAc (Mef) BuOH (Mbf) and aqueous (Maf) fractions obtained from the ethanolic leaf extract were investigated for cytotoxic effect on HeLa cells. The results showed that the Mhf, Mbf and Maf fractions did not exhibit cytotoxic effect as IC_{50} values greater than 250 μg/ml were obtained. On the other hand, Mdf and Mef fractions were cytotoxic against HeLa cells with IC_{50} values of 83.26 μg/ml and 104.5 μg/ml respectively (Table 1).

3.2. Cell viability and cell cycle analysis

The cytotoxic effect of the Mdf and Mef was further substantiated by the cell viability assay and cell cycle analysis. The TBE assay measures the cell membrane integrity as the dye enters dead cells through their compromised cell membranes and colours them blue (Wong et al., 2011). The dye also fluoresces when bound to protein and can therefore be detected using flow cytometry (Avelar-Freitas et al., 2014).As shown in Fig. 1, the untreated control cells were not permeable to the trypan blue dye compared to the treated cells which demonstrated an increased percentage of stained dead cells.

According to Ibrahim et al. (2013), incubation of HeLa cells with *M. tomentosa* crude extract showed cell cycle arrest in the G_0/G_1 phase after 12 h with a large increase in the size of the sub G_1 peak and a significant increase in the Annexin V stained positive cells, confirming apoptosis induction. The cell cycle phase-specific arrest of the cell growth inhibitory activity of the fractions from *M. tomentosa* showed that Mef fraction induced G_2/M phase arrest with increase in the size of sub-G_1 phase, corresponding to apoptosis (Fig. 2) while the Mdf fraction induced a small increase in percentage cells in G_0/G_1 phase with concomitant decrease in S phase (Fig. 2), but without nuclear fragmentation. The increase in size of sub-G_1 phase of the cell cycle shown by the Mef fraction correlate with the results obtained from *M. tomentosa* crude extract. Our results suggest that the cytotoxic effect of Mef fraction may be due to apoptosis induction while Mdf fraction exhibited a necrotic cell death.

3.3. Structural elucidation

Following the successful evaluation of the cytotoxicity potential of the fractions, isolation of the phyto-constituents responsible for the cytotoxic activity of Mdf and Mef fractions was carried out by column chromatographic technique. The isolated compounds were identified and structurally determined by 1D (^{1}H and ^{13}C) and 2D (COSY, HSQC and HMBC) NMR spectroscopy. The dichloromethane (Mdf) and ethylacetate (Mef) fractions from the leaves of *Markhamia tomentosa* yielded four terpenoids: three triterpenoids and one diterpenoid (Fig. 3). As shown in Tables 2 and 3, sitosterol **1** (Lee et al., 2013; Awolola et al., 2014) and mollic acid **2** (Facundo et al., 1993 and 2008) were isolated and identified from the Mef fraction whilst phytol **3** (Arigoni et al., 1999) and oleanolic acid **4** (Mahato and Kundu, 1994) were obtained from the Mdf fraction.

Compounds **1** to **4** (Tables 2 and 3) are known secondary metabolites isolated from natural sources but this is the first report on the isolation and identification of these compounds from the leaf extract of *Markhamia tomentosa*. Compound 1 was identified as sitosterol by comparison of chemical shifts from the 1H and 13C NMR (Table 2; Suppl. 1) with data in literature. Sitosterol **1** is ubiquitous to many plant species, and it has been reportedly isolated from the stem bark of *Markhamia stipulata* and *M. tomentosa* and from the leaf extract of *Markhamia lutea* (Joshi et al., 1978; Kernan et al., 1998; Tantangmo et al., 2010).

To the best of our knowledge, this is the first report on the isolation and identification of mollic acid **2**, a cycloartane triterpenoid from the genus *Markhamia*. Mollic acid has however been reported from *Combretum leprosum* (Facundo et al., 2008).A total of 30 carbons were resolved in the ^{13}C–NMR spectrum (Fig. 3). As shown in Suppl. 2, the ^{1}H–NMR displayed a pair of doublets δH 0.48 and 0.68 ($J = 4.16$ Hz), a characteristic of the cyclopropane ring of a cycloartane triterpene. Table 2 shows a full NMR structural elucidation of compound **2.** Compound **3** was obtained as a thick yellow viscous oil and its HR-ESI-MS indicated by the molecular ion peak, M^+ at m/z 296 corresponding to the formula, $C_{20}H_{40}O$ matched with phytol MS data from the MS library. A

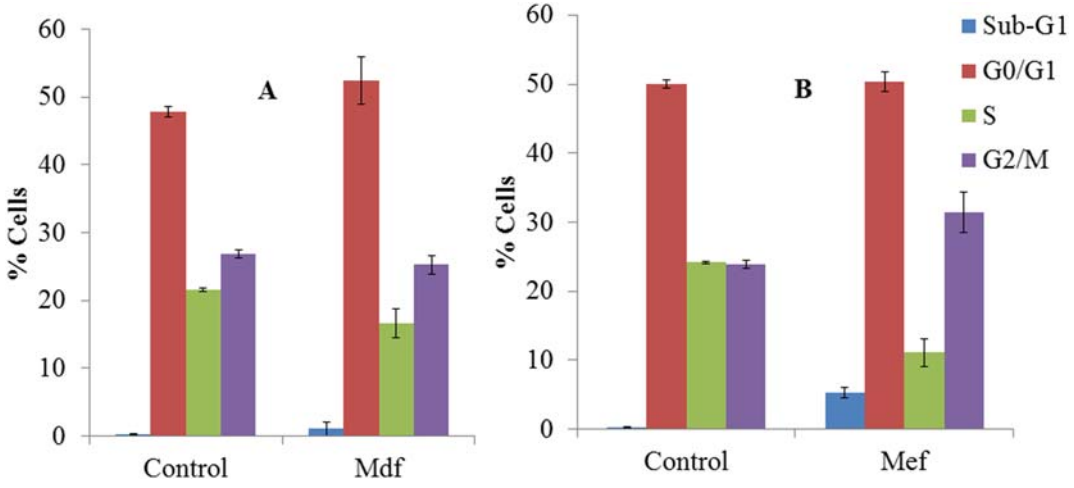

Fig. 2. Cell cycle analysis for A: Mdf (80 μg/ml) and B: Mef (100 μg/ml) fractions.

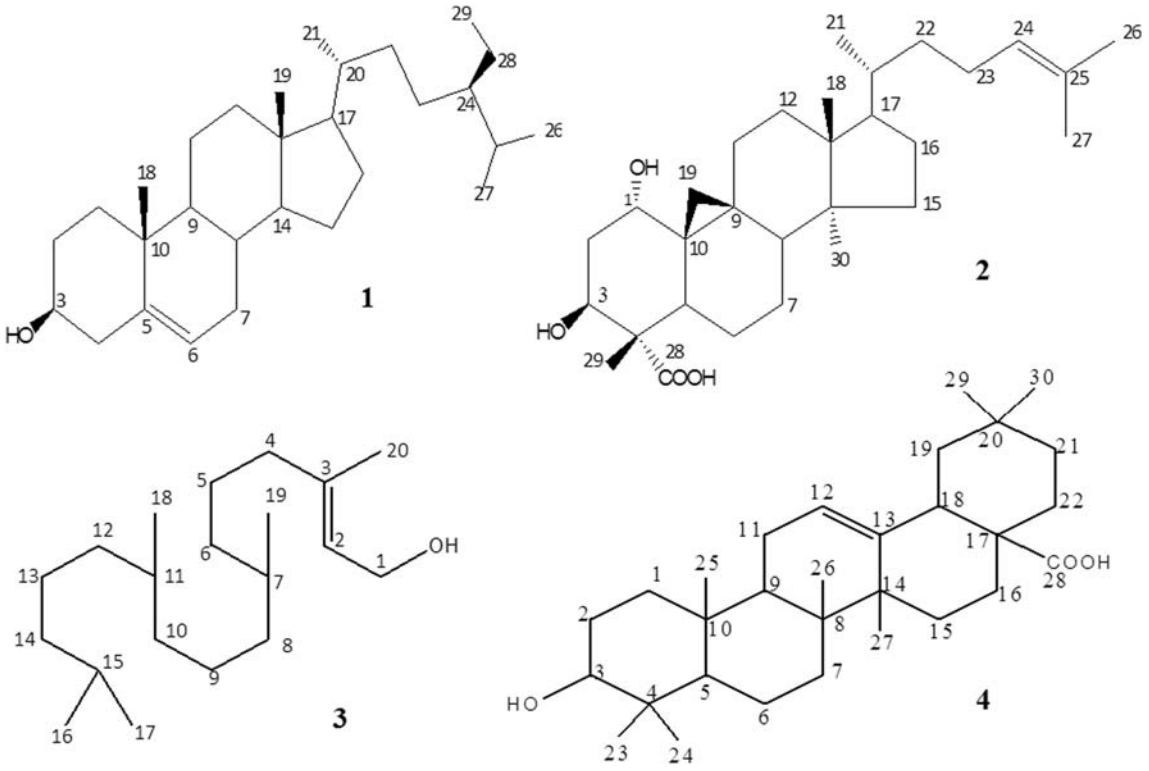

Fig. 3. Chemical structures of compounds isolated from *Markhamia tomentosa*. Sitosterol (1), Mollic acid (2), Phytol (3) and Oleanolic acid (4).

total of 20 carbons were resolved in the ^{13}C–NMR spectrum (Fig. 3). As shown in Suppl. 3, the doublet at δ_H 4.11 (d, 1H, J = 6.88 Hz, H-1) indicating the presence of oxygenated methylene group (-OCH2-) and a doublet of doublet of doublets atδ_H5.37 (ddd, 1H, J = 6.76; 0.92 Hz, H-2) indicating the presence of double bond compared well with the data in the literature for phytol (Arigoni et al., 1999).

The ^{13}C–NMR (Suppl. 4) and DEPT spectra for compound **4**, had the required methyl, methylene, methine and quaternary carbon resonances for oleanolic acid with the molecular ion peak observed at m/z 439.45. The C-12 and C-13 resonances occurred at δ_C 122.6 and δ_C 143.6 respectively, a characteristic of oleanene triterpenes (Moodley et al., 2011). The H-NMR spectrum (Suppl. 4) showed characteristic resonances with a doublet at δ_H1.88 (d, J = 3.12 Hz, H-11) and a doublet of doublets at δ_H3.22 (dd, J = 10.72, 4.24 Hz, H-3) and δ_H2.82 (dd, J = 13.52, 3.72 Hz, H-18). The NMR data (Table 3) corresponded well with the data in literature for oleanolic acid (Mahato and Kundu, 1994; Barreiros et al., 2002).

3.4. Biological activity of isolated compounds

Secondary metabolites present in medicinal plants could be responsible for the observed pharmacological effects. According to Sofidiya et al. (2014), characterization of the chemical constituents of ethyl-acetate fraction of *M. tomentosa* by electrospray ionization mass spectrometry (LC–MS) led to the identification of phenolic, iridoid and terpenoid compounds. This present study however, reports the isolation and identification of four terpenoid compounds: three triterpenoids (sitosterol **1**, mollic acid **2**, oleanolic acid **4**) and one diterpenoid (phytol **3**) from the bioactive ethyl-acetate and dichloromethane fractions of the leaves of *M. tomentosa*. The compounds **1** to **4** (Fig. 3) were evaluated for their cytotoxic effect on HeLa cervical cancer cells using MTT assay. Table 1 shows the cytotoxic effect of the compounds with their IC_{50} values. Mollic acid **2** exhibited effective cytotoxic effect with an IC_{50} value of 34.74 μg/ml (73.93 μM) while sitosterol **1**, phytol **3** and oleanolic acid **4** showed minimal cytotoxic effect against HeLa cells. Phytosterols are among the subclass of terpenoids, which are derived from tetracyclic triterpenes and exist in several forms in plants including stigmasterol, β-sitosterol and cycloartenol (Abumweis et al., 2008). Several *in-vitro* cytotoxic effects of these terpenic compounds isolated from different

Table 2
^{1}H and ^{13}C NMR data of sitosterol and mollic acid with compound **1** and **2.**

	Sitosterol (300 MHz, $CDCl_3$)		Compound **1** (600 MHz, $CDCl_3$)		Compound **2** (600 MHz, CD_3OD)	
C	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	37.4	–	73.1	3.95 (s)	73.7	3.53 (br. s)
2	31.8	–	39.3	2.53 (t, 12.8)	37.8	–
3	71.9	3.52 (m)		2.33 (dd, 12.8, 2.6)	71.5	4.54 (dd, 11.92; 4.7)
4	42.4	2.27 (m)	71.3	5.60 (dd, 12.1, 4.4)	55.9	–
5	141.0	–	56.3	–	38.4	2.57 (dd, 12.2; 3.84)
6	121.9	5.35 (m)	38.3	3.46 (dd, 12.1, 4.4)	24.1	–
7	32.0	–	23.9	1.75; 1.25	26.6	–
8	32.0	–	26.4	2.15; 1.52	50.2	1.50
9	50.3	–	48.7	1.60	22.2	–
10	36.7	–	21.4	–	30.2	–
11	21.2	1.99 (m)	30.9	–	26.6	–
12	39.8	–	26.8	2.78; 1.25	34.0	–
13	42.4	–	33.8	1.67; 1.45	46.4	–
14	57.0	–	46.1	–	49.9	–
15	24.5	–	49.7	–	37.0	–
16	28.4	–	36.4	–	29.2	–
17	56.1	–	28.9	–	53.7	1.61
18	12.0	–	53.1	1.60	18.9	0.95 (s)
19	19.1	–	19.0	1.07 (s)	31.1	0.68 (d, 4.16)
						0.48 (d, 4.16)
20	36.3	–	30.5	0.85 (d, 4.0)	37.1	–
21	18.9	–		0.57 (d, 4.0)	18.9	–
22	34.1	–	36.7	1.28	37.5	–
23	26.2	–	18.9	0.97 (d, 6.2)	25.9	–
24	46.0	–	37.2	1.75; 1.48	126.2	5.10 (t, 7.0)
25	29.1	–	25.8	2.10; 1.92	131.8	–
26	19.1	0.81 (d, 6.3)	126.4	5.24 (t, 7.0)	17.8	1.61 (s)
27	19.6	0.86 (d, 4.2)	131.3	–	26.0	1.68 (s)
28	23.2	–	18.2	1.65 (s)	180.8	–
29	12.1	0.8 (t, 5.8)	26.3	1.71 (s)	9.2	1.08 (s)
30	–	–	–	–	19.9	0.96

Table 3
^{1}H and ^{13}C NMR data of phytol and oleanolic acid with compound **3** and **4.**

	Phytol (400 MHz, $CDCl_3$)		Compound 3 (600 MHz, $CDCl_3$)		Oleanolic acid (400 MHz, $CDCl_3$)		Compound **4** (600 MHz, $CDCl_3$)	
C	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	59.39	4.14 (d)	59.3	4.11 (d, 6.88)	38.5	–	38.4	–
2	123.09	5.39 (t)	123.2	5.37 (ddd, 6.76, 0.92)	27.4	–	27.2	–
3	140.0	–	140.0		78.7	–	79.0	3.22 (dd, 10.72, 4.24)
4	39.85	1.97 (m)	39.9	1.96 (t, 15.16, 7.48)	38.7	–	38.7	–
5	25.12	1.40 (m)	25.1		55.2	–	55.2	–
6	36.65	1.24(m),1.05(m)	36.7		18.3	–	18.3	–
7	32.67	1.36 (m)	32.7		32.6	–	32.4	–
8	37.35	1.23(m), 1.03(m)	37.4		39.3	–	39.3	–
9	24.45	1.29, 1.15 (m)	24.5		47.6	–	47.6	–
10	37.41	1.23(m), 1.03(m)	37.4		37.0	–	37.1	–
11	32.77	1.35 (m)	32.8		23.1	–	23.4	1.88 (d, 3.12)
12	37.28	1.23(m), 1.03(m)	37.3		122.1	–	122.6	–
13	24.79	1.25 (m)	24.8		143.4	–	143.6	–
14	39.35	1.11, 1.03(m)	39.4		41.6	–	46.5	–
15	27.95	150 (m)	28.0		27.7	–	27.7	–
16	22.60	0.84 (d)	22.6		23.4	–	22.9	–
17	22.69	0.84 (d)	22.7		46.6	–	41.6	–
18	19.69	0.83 (d)	19.7		41.3	–	41.0	2.82 (dd, 13.52, 3.72)
19	19.72	0.82 (d)	19.7		45.8	–	45.9	–
20	16.14	1.65 (s)	16.14		30.6	–	30.7	–
21					33.8	–	33.8	–
22					32.3	–	32.6	–
23					28.1	–	28.1	–
24					15.6	–	15.3	–
25					15.3	–	15.5	–
26					16.8	–	17.1	–
27					26.0	–	25.9	–
28					181.0	–	183.4	–
29					33.1	–	33.1	–
30					23.6	–	23.6	–

plants against various cancer cell lines including cervical cancer cells are well documented in literature. The cytotoxic effect of sitosterol in human leukaemic U937 cells, mouse fibrosarcoma, prostate (PC-3) and breast (MDA-MB-231) cancer cells have been reported in literature (Awad et al., 2001, 2005; Park et al., 2007; Moon et al., 2008). Phytol, a diterpene alcohol produced from chlorophyll, has demonstrated apoptosis inducing activity against human gastric adenocarcinoma (AGS) cells (Song and Cho, 2015). George et al. (2012) reported the apoptosis induction capability of oleanolic acid in HACaT (keratinocyte) cell line at relatively low cytotoxicity that points to the potential anticancer properties of this compound. Although the cytotoxic effect of these phytosterol and terpenoids in different cell lines have been reported, the results obtained from this study showed a non-cytotoxic effect of sitosterol, phytol and oleanolic acid against HeLa (cervical cancer) cells. Amidst the isolated compounds from *M. tomentosa* leaf extract, mollic acid exhibited effective antiproliferative activity. The antiproliferative effect of this cycloartane triterpenoid against HeLa (cervical cancer) cells is consistent with the report of the apoptotic inducing effects of two cycloartane triterpene glycosides, mollic acid arabinoside and mollic acid xyloside isolated from ethylacetate fraction of *Leea indica* against Ca Ski (cervical) cancer cell line (Wong et al., 2011; Wong and Abdul Kadir, 2012). Our results suggest that mollic acid **2** isolated from the ethyl-acetate fraction may be responsible for the cytotoxic and apoptosis induction activities of *Markhamia tomentosa* leaf extract against HeLa cells.

4. Conclusion

Sitosterol **1**, mollic acid **2**, phytol **3** and oleanolic acid **4** were isolated from the leaf extract of *Markhamia tomentosa*. To the best of our understanding, this study reports for the first time the isolation, structural identification and pharmacological study of compounds **1** to **4** from the plant. The cytotoxic cycloartane triterpene: mollic acid **2**, isolated from the ethyl-acetate fraction which exhibited apoptotic cell death associated with G_2/M phase arrest and increased size of sub-G_1 phase may be responsible for the apoptosis inducting capability of *M. tomentosa* against HeLa cells. The results obtained from this study contribute to the pharmacological validation and support the use of *Markhamia tomentosa* in traditional medicine.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sajb.2017.12.014.

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