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ORIGINAL ARTICLE

Rhizomatoflavonoid D and Other Flavonoids from the Twigs of Ochna Rhizomatosa as a Potential Inhibitor of HIV-1

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Abstract

Currently, HIV morbidity and mortality in sub-Saharan Africa remain a huge concern and awaiting interventions. Even though the combination antiretroviral therapy (cART) has recorded significant success, drug resistance and limited access to available therapeutics are major factors responsible for the low impact of cART in several African communities. Herein, as part of our continuous effort on the investigation of bioactive metabolites of *Ochna rhizomatosa*, we report the isolation of a new flavonoid; Rhizomatoflavonoid D (**1**), alongside with four known ones (**2**–**5**). The structures of these compounds were elucidated by using spectroscopic techniques (^{1}H NMR, ^{13}C NMR, HSQC, HMBC, ^{1}H-^{1}H COSY, and ROESY) and mass spectrometry. The antiviral activity of the resulting compounds was assessed using deCIPhR assay run in parallel with the Alamar Blue based cytotoxicity assay. This assay revealed a moderate activity for compound **4** (72% inhibition at 2.5 µg/mL) while compound **1** had minimal activity (36% inhibition at 2.5 µg/mL). The prominent inhibitory effect on HIV-1 was showed by compound **4** (IC_{50} = 3.1 µM). Unfortunately, compound **4** proved to be non-selective as it demonstrated also a CC_{50} = 5.2 µg/mL (Selectivity index of 1.7). The prominent inhibitory effect on HIV-1 showed by compound **4** (IC_{50} = 3.1 µM) could be due the presence of a methoxy group at C-7, since this group enhances the lipophilicity of biflavonoids, thereby improving its incorporation into cells.

Keywords HIV-1 Inhibitors · Rotamers · Rhizomatoflavonoid D · *Ochna Rhizomatosa* · Biflavonoids

1 Introduction

HIV/AIDS has been a devastating global epidemic since the 1980s. More than 39.0 million people are currently living with HIV including 37.5 million [31.8 million – 43.6 million] adults (15 years or older) and 1.5 million [1.2 million–2.1 million] children (0–14 years) [1]. More than 29 million (about 78%) of those people being in Sub-Saharan Africa which is the center of this epidemic. In Cameroon, 450 000 adults between the age of 15 and 49 are living with HIV. AIDS is reported to be the leading cause of death among the 15–49 age groups with about 3300 AIDS-related deaths in 2022 [2]. The combination antiretroviral therapy (cART) which consists largely of integrase strand transfer inhibitors (INSTIs) and nucleoside reverse transcriptase inhibitors (NRTIs) are widely used [3]. Despite the beneficial effects of current treatment options in improving the quality of life of HIV and AIDS patients, prolonged use of these agents is

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limited due to the development of resistance, toxicity, high costs and unavailability [4–6]. Due to these constraints, some communities use medicinal plants which are claimed to have therapeutic properties against HIV including the management of HIV/AIDS related opportunistic infections [7] and many other health problems in Africa such as microbial infections, liver problems [8, 9]. Currently, natural products appear to be a major source of anti-HIV-1 drug discovery and development [10, 11]. Many studies showed that alkaloids, flavonoids and polyphenols are effective in suppressing HIV-1 replication [12–14]. Biflavonoids also reported to display anti-HIV activity are natural dimers of monomeric flavonoids often connected together with a C-C or C-O-C molecular linkage [15], which are often substituted by either a methoxy or a glycoside [16, 17]. They are known to have a wide range of pharmacological potentials as anticancer, cytoprotective, and in most cases found superior to their monomeric counterparts [18].

Ochna rhizomatosa, is a small tree that can grow up to one meter (1 m) tall (Fig. 1). In Cameroon, leaves of *Ochna rhizomatosa* are used for the treatment of headaches, malaria and toothache. Leaves and twigs of *Ochna rhizomatosa* were reported to display antimicrobial activity. The phytochemical profile of this plant revealed that it contains flavonoids, triterpenoids alkaloids and fatty acids [19]. In our previous studies, the chemical constituents of the root bark of *Ochna rhizomatosa* were investigated for the first time in this genus to find a potential inhibitor of HIV-1 integrase and antiplasmodial lead compounds. Three uncommon C-C type isobiflavonoids were described along with four known compounds [6]. In the present study, the chemical constituents of the ethyl acetate fraction of the twigs of *Ochna rhizomatosa* were investigated. The resulted isolated compounds were assessed for their potential inhibitory activity on HIV-1 in the interest of identifying potential lead antiviral compounds. One previously undescribed flavonoid rotamer (**1**) is reported herein for the first time from *Ochna rhizomatosa* along with known biflavonoids (**3–5**) and an additional flavonoid glycoside **2**. Their structures were elucidated using 1D and 2D NMR and HRESIMS spectral data.

Fig. 1 Picture of *Ochna rhizomatosa* [20]

2 Materials and Methods

2.1 General Methods

The UV spectra were recorded on UV-570/ VIS/ NIP and Shimadzu UV-24012 A double-beam spectrophotometer (Shimadzu, Japan). IR measurements were obtained on a PerkinElmer (model 1600) FTIR spectrometer. The NMR spectral data were recorded using a Bruker Avance DPX-400 spectrometer (Bruker, Germany) operating at the frequencies of 400 MHz (^{1}H) and 100 MHz (^{13}C). The samples were dissolved in MeOH-d_4 (Sigma Aldrich, Germany, 99.8%) prior to recording. Chemical shift values were given in parts per million (ppm) from the internal standard (Tetramethylsilane, Sigma Aldrich, Germany, 99.9%). HRESIMS was performed using a MSQ Thermo Finnigan (ThermoFischer Scientific, USA). The purifications were carried out on a Medium Pressure Liquid Chromatography (MPLC), Chromatography system - sepacore® flash systems x10 / x50 (Büchi, Switzerland) system on RP-18, 0.040–0.063 mm, Merck MGaA (Darmstadt, Germany), Open column chromatography on Sephadex LH-20 (0.025–0.100 mm; GE Healthcare, Danderyd, Sweden) and on silica gel 60 (Merck, 0.040–0.063 mm Qingdao, China). Thin layer chromatography (TLC) experiments were performed on pre-coated Merck Kieselgel 60 F_{254} plates (20×20 Cm^2, 0.25 mm, (Qingdao, China). Methanol, ethyl acetate, methylene chloride, hexane were obtained from Reflecta Laboratory Supplier, Johannesbourg, South Africa (Purity > 98% for these solvents).

2.2 Plant Collection

The twigs of *Ochna rhizomatosa* were collected from "Mbe" district, Adamawa region, Cameroon (GPS data 7°34′30″ N 13°30′35″ E 1.84 km) in April 2022. The plant

was identified at the National Herbarium of Cameroon, where a voucher specimen (N° 39120HNC) was deposited.

2.3 Extraction of Plant Materials and Isolation of Compounds

Dried and powdered twigs of *Ochna rhizomatosa* (200 g) were extracted by maceration in MeOH (3 L) for 72 h at room temperature. The mixture was then filtered, and the filtrate was evaporated to dryness to yield a crude extract. The process was repeated twice to yield 80 g of crude methanol extract. A portion of the extract (70 g) was dissolved in H_2O and partitioned with hexane (3 × 100 mL) and ethyl acetate (3 × 100 mL). The ethyl acetate fraction (40 g) was subjected to column chromatography over silica gel eluting with gradients of CH_2Cl_2-MeOH to afford 109 sub-fractions of 250 mL each. These sub-fractions were combined based on their TLC profiles into 4 major sub-fractions: A (0.07 g, 1–50); B (0.17 g, 51–60); C (13.20 g, 61–95) and D (21.70 g, 96–109). Sub-ractions A (CH_2Cl_2 100%) and B (CH_2Cl_2/MeOH 50:1; 40:1 and 30:1) contained mainly fatty acids. Sub-fraction C (CH_2Cl_2/MeOH 20:1) was purified over silica gel column chromatography to afford compounds **1** (17.0 mg) and **3** (7.0 mg); Sub-fraction D (CH_2Cl_2/MeOH 10:1) was purified by column chromatography over Sephadex LH-20 with MeOH to afford compounds **4** (5.0 mg); **5** (9.0 mg) and **2** (26.0 mg).

2.4 In vitro anti-HIV-1 and Cytotoxicity Screening of Compounds Isolated from *Ochna Rhizomatosa*

To assess the HIV-1 inhibitory activities, all compounds were dissolved in dimethyl sulfoxide (99.7%, Molecular Biology grade, Sigma-Aldrich, USA) to a stock concentration of 10 mg/mL and stored at -20 °C prior to biological evaluation. The anti-HIV replication of the four compounds was assessed against the prototypic subtype B clone of HIV-1, NL4-3, in vitro using the previously described dual enhancement of cell infection to phenotypic resistance (deCIPhR) assay [21, 22]. All compounds were screened across four concentration points (0.025, 0.25, 2.5 and 10 mg/mL), in technical duplicates and with one independent biological repeat. Efavirenz (Sigma-Aldrich, USA, 98%) served as a positive inhibition control of viral replication while dilution buffer containing the same concentration of DMSO as the extracts was used as negative control. Cytotoxicity of the compounds was assessed parallel to the anti-HIV replication studies under similar cell culture conditions but without addition of the virus. Plates were purchased from Thermo-Fischer Scientific, USA. They were read out following 96 h of incubation, using Alamar Blue reagent (Sigma-Aldrich, USA, 98%).

3 Results and Discussion

3.1 Structure Elucidation of Isolated Compound 1

Compound **1** (Fig. 2) was obtained as a yellow powder and gave a positive reaction with Neu's reagent. The HRESIMS (-) of compound **1** revealed a *pseudo* molecular ion $[2\text{ M-H}]^-$ at *m/z* 947.2238 m.a.u, corresponding to $C_{46}H_{43}O_{22}^-$ (calc. 947.2251), indicative of the molecular formulae $C_{23}H_{22}O_{11}$ (calc. 474.1162), with 13 degrees of unsaturation.

The ^{1}H NMR spectrum. revealed two units of apigenin-type flavones supported by the signal at δ_H 6.63 (s, 1H, H-3 M) and 6.60 (s, 1H, H-3 m) as well as by the two sets of AA′BB′ type signals at δ_H 8.22 (d, J = 8.3 Hz, 2 H, H-2′/6′M) and 7.88 (d, J = 7.8 Hz, 2 H, H-2′/6′m) (Table 1). Their respective analogues at δ_H 6.90 (o, 2 H, H-3′/5′M) and 6.92 (o, 2 H, H-3′/5′m) identified through (^{1}H-^{1}H) COSY correlations. The letters M and m after each position refer to major and minor rotamers, respectively. The resonances at δ_H 2.04 (s, 3 H, OAcM) and 2.02 (s, 3 H, OAcm) were assigned to acetyl groups. The generated information from the HSQC spectrum contributed to identify two correlations from the protons at δ_H 4.94 (H-1″M) and 5.06 (H-1″m) with the carbons at δ_H 74.3 (C-1″M) and 75.4 (C-1″m), respectively, attributed to the anomeric protons and carbons of two C-glycosides. However, the splitting patterns observed for most signals in the NMR spectrum suggested the presence of an atropoisomer [23] with each molecule consisting of one apigenin unit, one C-glycoside and one acetyl group.

The careful examination of the ^{13}C NMR spectrum permitted the identification of several signals at δ_C 182.7 (C-4 M/m), 161.4 (C-4′M/m), 129.3 (C-2′/6′M), 128.3 (C-2′/6′ m), 115.5 (C-3′/5′M), and 115.5 (C-3′/5′M); which confirmed the apigenin moieties. In addition, the signals at δ_C 19.4 (OAcM), 19.3 (OAcm), 171.5 (C = OM) and 171.3 (C = Om) supported the acetyl groups in each rotamer. Meanwhile, the signals exhibited in the interval 64–78 ppm combined with ^{1}H NMR data supported two units of C-β-D-glucopyranosides [24, 25].

The interpretation of HMBC spectrum allowed to establish ^{3}J correlations from protons to carbons. In fact, the correlations from the protons at δ_H 4.94 (H-1″M) and 5.06 (H-1′′m) to the respective carbons at δ_C 103.4 (C-8 M) and 101.6 (C-8 m) justified the attachment of C-β-D-glucopyranoside to apigenin moieties thus corresponding to vitexin. But the higher value of carbon 6″M/m suggested an esterification [24]. The correlations from the protons at δ_H 4.34 (H-6″M/m) to carbons at δ_C 171.5 (OAcM) and 171.3 OAcm) justified the linkage between the acetyl groups and the sugar moieties (Fig. 3). However, these data were very close to those of the reported (2 S)5,7,4 -trihydroxyflavanone-8-C-β-D-(6″-O-acetyl)glucopyranoside [25]. But the main

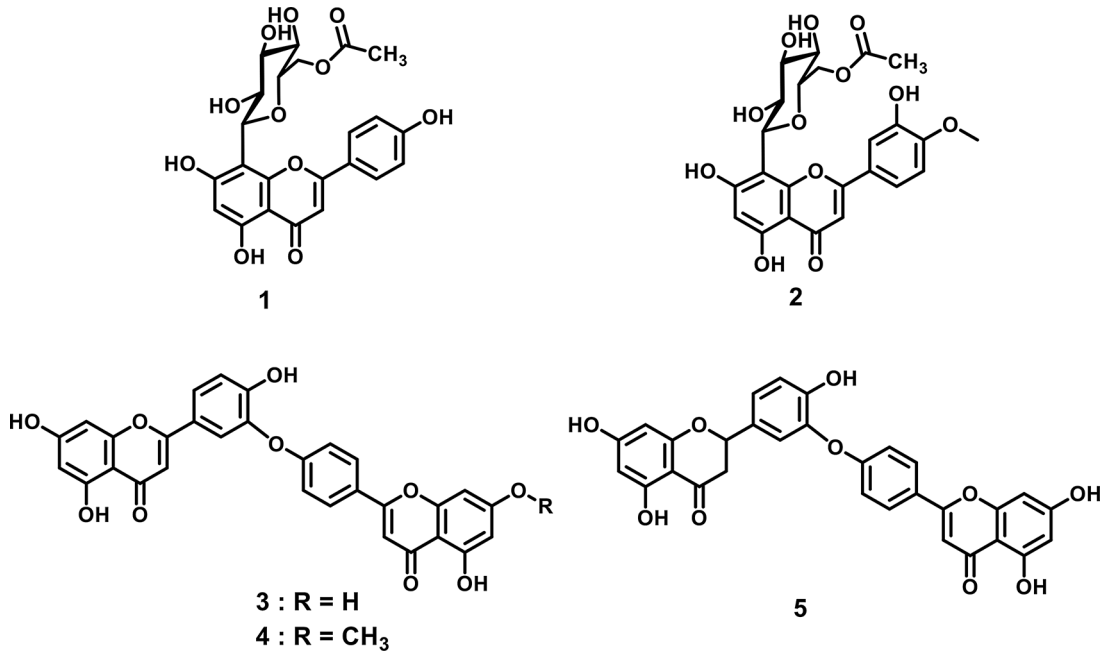

Fig. 2 Structures of compounds (**1**–**5**) isolated from *Ochna rhizomatosa*

Table 1 ^{1}H (400 MHz, CD_3OD) and ^{13}C NMR (400 MHz, CD_3OD) spectroscopic data for compounds **1** and **2** (δ in ppm, J in hz)

Position	1				2	
	Rotamer M		Rotamer m			
	δ_H	δ_C	δ_H	δ_C	δ_H	Δc
2		165.3		164.9		164.5
3	6.63 (1 H, s)	101.6	6.60 (1 H, s)	102.5	6.98 (1 H, s)	104.7
4		182.7		182.7		182.7
5		161.2		161.2		156.6
6	6.29 (1 H, s)	98.0	6.28 (1 H, s)	99.8	6.28 (1 H, s)	98.5
7		163.3		163.2		163.2
8		103.4		103.3		104.5
9		156.7		156.7		161.0
10		103.3		103.9		102.9
1’		121.6		122.0		121.7
2’	8.22 (1 H, d, J=8.3 Hz)	129.3	7.88 (1 H, d, J=7.8 Hz)	128.3	7.56 (1 H, s)	116.4
3’	6.90 (1 H, m)	115.5	6.92 (1 H, m)	115.6		148.3
4’		161.4		161.4		151.2
5’	6.90 (1 H, m)	115.5	6.92 (1 H, m)	115.6	6.90 (1 H, d J=7.8)	110.4
6’	8.22 (1 H, d, J=8.3 Hz)	129.3	7.88 (1 H, d, J=7.8 Hz)	128.3	8.26 (1 H, d, J=7.8 Hz)	123.3
Glc-1”	4.94 (1 H, d, J=9.0 Hz)	74.3	5.06 (1 H, d, J=9.0 Hz)	75.4	4.69 (1 H, d, J=9.7 Hz)	74.3
2”	4.23 (1 H, t, J=9.5 Hz)	69.7	4.44 (1 H, t, J=9.5 Hz)	68.6	3.86 (1 H, m)	69.9
3”	3.67 (1 H, m)	74.9	3.65 (1 H, m)	75.4	3.78 (1 H, m)	77.3
4”	4.06 (1 H, m)	75.6	4.02 (1 H, m)	76.7	4.20 (1 H, m)	68.3
5”	3.98 (1 H, m)	77.4	3.94 (1 H, m)	77.4	3.46 (1 H, m)	75.5
6”	4.33–4.38 (2 H, m)	64.5	4.31–4.33 (2 H, m)	63.9	4.21 (1 H, d, J=9.7 Hz) 4.08 (1 H, m)	65.4
C=O		171.5		171.3		170.9
CH_3	2.04	19.4	2.02	19.3	1.98 (3 H, s)	21.2
OCH_3			-		3.89 (3 H, s)	56.5

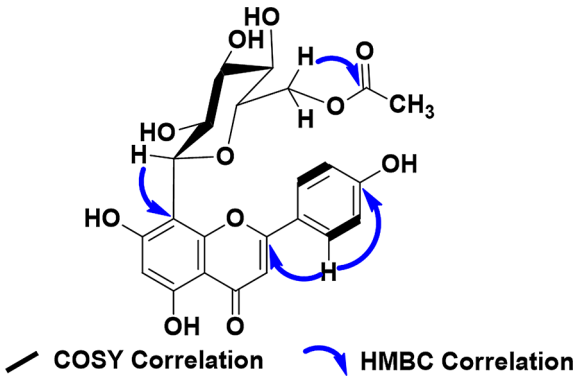

Fig. 3 Major correlations (COSY and HMBC) observed for compound **1**

difference lies on the double bond 2–3, therefore, these molecules were elucidated as being apigenin-8-C-β-D-(6″-O-acetyl)glucopyranoside. The combination of ^{1}H NMR, ^{13}C NMR, COSY, HSQC and HMBC allowed to assign all the values of protons and carbons (Table 1). It was reported that the main characteristic of rotamers is the ROESY correlations between protons at identical positions in both conformers [17, 24]. From the ROESY spectrum (supplementary data) of compound **1**, there is a correlation between the proton at δ_H 8.22 (H-2′/6′M) and 7.88 (H-2′/6′m) as well as between the proton at δ_H 4.94 (H-1″M) and 5.06 (H-1″m). This clearly supports that apigenin-8-C-β-D-(6″-O-acetyl) glucopyranoside coexists in solution with its rotamer, this phenomenon has been evidenced by Franck and co-workers that it is due to the free rotation of the bond C1″-C8 in such molecules [26]. This compound was trivially named Rhizomatoflavonoid D (**1**)/5,7-Dihydroxy-2-(4-hydroxyphenyl)-4 H-1-benzopyran-4-one-8-C-(2″R,3″S,4″S,5″R,6″S)-2″-(hydroxyl)-6″-O-acetyloxane-3″,4″,5″-triol (Fig. 2).

3.2 Structure Elucidation of Isolated Compound 2

Compound **2** was obtained as a yellow amorphous powder, which had some similarities with **1** based on NMR comparison. The spectral data (Table 1) was characteristic of tetrahydroxyflavone system of luteolin with a C-glycoside substitution at position 8. The HRESIMS of **2** gave an $[M+H]^+$ ion at *m/z* 505.1332, corresponding to $C_{24}H_{25}O_{12}$ (calc. 505.1346). Upon comparison with literature data [27], **2** was named 4'-methoxyluteolin-8-C-6"acetylglucopyranoside /4'-methoxy-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-chromen-4-one-8-C-(2″R,3″S,4″S,5″R,6″S)-2″-(hydroxyl)-6″-O-acetyloxane-3″,4″,5″-triol.

3.3 Structure Elucidation of Isolated Compound 3

Compound **3** was obtained as a pale-yellow amorphous powder. The HRESIMS of **3** gave an $[M+H]^+$ ion at *m/z* 539.0884, corresponding to $C_{30}H_{19}O_{10}$ (calc. 539.0978). The ^{1}H and ^{13}C NMR spectra data of compound **3** is presented in Table 2. The data confirms the presence of two flavone units with a C-O-C linkage between positions 3' and 4'. The NMR data was consisted with those reported in literature [28] for the biflavonoid, ochnaflavone /2-[4-[5-(5,7-dihydroxy-4-oxochromen-2-yl)-2-hydroxyphenoxy]phenyl]-5,7-dihydroxychromen-4-one.

3.4 Structure Elucidation of Isolated Compound 4

Compound **4** was obtained as a yellow amorphous powder. The HR-ESI-MS of **4** showed an $[M+H]^+$ ion at *m/z* 553.1071, corresponding to $C_{31}H_{21}O_{10}$ (calc. 553.1134). The NMR spectral data showed that **4** differs from **3** with the presence of methoxy group in position 7" as indicated in Table 2. Compound **4** was therefore named 7″-*O*-methylochnaflavone /7″-*O*-methyl-2-[4-[5-(5,7-dihydroxy-4-oxochromen-2-yl)-2-hydroxyphenoxy]phenyl]-5,7-dihydroxychromen-4-one in agreement with previous report [28].

3.5 Structure Elucidation of Isolated Compound 5

Compound **5** was obtained as a yellow amorphous powder. The HRESIMS of **5** gave a $[M-H]^-$ ion at *m/z* 553.1147, corresponding to $C_{31}H_{21}O_{10}$ (calc. 553.1135). The skeleton of **5** showed C-O-C linkage between a flavanone and a flavone rather than two flavone units as observed in **3** and **4**. This resulted in a more deshielded C-4 carbonyl (δc 196.4) compared to C-4" (δc 182.3) and the dissimilar methylene protons (δ_H 3.11 and 2.80) of H-3 (Table 2), which are characteristics of a flavanone moiety. Compound **5** was named 2,3-dihydroochnaflavone 7-*O*-methyl ether /7″-*O*-methyl-2-[4-[5-(5,7-dihydroxy-4-oxochroman-4-one-2-yl)-2-hydroxyphenoxy]phenyl]-5,7-dihydroxychromen-4-one in agreement with a previous report [29].

4 Anti-HIV Activities of Compounds Isolated from *Ochna Rhizomatosa*

Preliminary in vitro anti-HIV replication potency of compounds (Table 3) was assessed using the deCIPhR assay run in parallel with the Alamar Blue based cytotoxicity assay. Efavirenz, used as the positive control, inhibited viral replication (78, 72, 82, and 100% inhibition at 215, 100, 46.5, and 20.48 nM) as expected. Of the four compounds, compound **4** emerged as the most active (55% and 72% inhibition at 10 and 2.5 µg/mL, respectively) closely followed by compound **1** (36% inhibition at both 10 and 2.5 µg/mL) which demonstrated moderate activity. The prominent inhibitory effect

Table 2 ^{1}H (400 MHz, CD_3OD) and ^{13}C NMR (400 MHz, CD_3OD) spectroscopic data for compounds **3–5** (δ in ppm, J in hz)

	3		4		5	
Position	δ_H	δc	δ_H	δc	δ_H	δc
2		163.4		163.4	5.41 (1 H, dd, *J* = 12.7 & 2.6 Hz)	78.5
3	6.69 (1 H, s)	103.7	6.85 (1 H, s)	104.4	3.11 (1 H, dd, *J* = 17.1 & 12.6 Hz) 2.80 (1 H, dd, *J* = 17.1 & 3.0 Hz)	42.5
4		182.1		182.3		196.4
5		163.4		161.9		164.9
6	6.23 (1 H, d, *J* = 2.1 Hz)	99.0	6.20 (1 H, d *J* = 2.1 Hz)	99.4	6.03 (1 H, d, *J* = 2.2 Hz)	94.4
7		164.2		164.8		168.1
8	6.48 (1 H, d, *J* = 2.1 Hz)	93.9	6.48 (1 H, d *J* = 2.1 Hz)	94.4	6.01 (1 H, d, *J* = 2.2 Hz)	96.3
9		157.8		157.8		163.0
10		104.3		105.2		102.6
1’		122.8		122.2		130.9
2’	7.74 (1 H, d, *J* = 2.1 Hz)	120.7	7.93 (1 H, dd,*J* = 9.2 and 2.1 Hz)	121.8	7.21 (1 H, d, *J* = 1.96 Hz)	120.3
3’		142.5		142.0		142.0
4’		153.4		154.4		149.6
5’	7.17 (1 H, d, *J* = 8.5 Hz)	118.3	7.17 (1 H, d, *J* = 9.2 Hz)	118.5	7.04 (1 H, d, *J* = 8.3 Hz)	117.1
6’	7.83 (1 H, dd, *J* = 8.5 & 2.1 Hz)	125.2	7.93 (1 H, d, *J* = 9.2 Hz)	125.9	7.25 (1 H, dd, *J* = 8.3 & 2.1 Hz)	124.1
2”		162.4		163.4		163.8
3”	6.64 (1 H, s)	104.1	6.93 (1 H, s)	104.0	6.61 (1 H, s)	103.4
4”		182.1		182.2		182.3
5”		163.2		161.5		161.8
6”	6.23 (1 H, d, *J* = 2.1 Hz)	98.9	6.36 (1 H, d, *J* = 2.1 Hz)	98.5	6.20 (1 H, d, *J* = 2.2 Hz)	98.8
7”		164.4		165.6		163.9
8”	6.44 (1 H, d, *J* = 2.1 Hz)	93.9	6.81 (1 H, d, *J* = 2.1 Hz)	93.1	6.43 (1 H, d, *J* = 2.2 Hz)	93.7
9”		157.8		157.7		158.0
10”		104.3		104.4		103.9
1"’		125.0		124.7		124.7
2"’	8.00 (1 H, d, *J* = 9.0 Hz)	128.2	8.05 (1 H, d, *J* = 8.9 Hz)	128.8	7.91 (1 H, d, *J* = 8.9 Hz)	127.8
3"’	7.11 (1 H, d, *J* = 9.0 Hz)	116.5	7.04 (1 H, d, *J* = 8.9 Hz)	116.4	7.02 (1 H, d, *J* = 8.9 Hz)	116.3
4"’		161.1		161.3		161.4
5"’	7.11 (1 H, d, *J* = 9.0 Hz)	116.5	7.04 (1 H, d, *J* = 8.9 Hz)	116.4	7.02 (1 H, d, *J* = 8.9 Hz)	116.3
6"’	8.00 (1 H, d, *J* = 9.0 Hz)	128.2	8.05 (1 H, d, *J* = 8.9 Hz)	128.8	7.91 (1 H, d, *J* = 8.9 Hz)	127.8
OCH_3			3.84 (3 H, s)	56.5		

Table 3 Inhibition of HIV-1 replication and cytotoxicity of compounds isolated from *Ochna Rhizomatosa*

Compound	% Inhibition of HIV-1 replication (µg/mL)				% Cytotoxicity (µg/mL)			
	10	2.5	0.25	0.025	10	2.5	0.25	0.025
1	36.7 ± 2.1	36.8 ± 0.9	NA	NA	6.3 ± 8.1	8.5 ± 6.6	14.9 ± 1.2	10.3 ± 4.0
4	55.7 ± 17.1	72.6 ± 9.4	NA	NA	12.4 ± 15.9	0.2 ± 17.2	10.2 ± 7.4	9.9 ± 0.7
5	NA	NA	NA	NA	46.1 ± 76.5	22.6 ± 51.5	17.2 ± 1.6	11.6 ± 4.5
2	NA	NA	NA	NA	49.5 ± 73.9	28.8 ± 54.0	14.9 ± 0.8	9.7 ± 1.3

Data presented as Mean ± SD, NA: non active

on HIV-1 showed by compound **4** (IC_{50} = 3.1 µM) may be explained by the presence of a methoxy group at C-7, since this group enhances the lipophilicity of the biflavonoid, thus improving cellular absorption [30]. However, compounds **1** and **4** showed cytotoxicity in the same concentration range. The presence of one glucoside attached at C-8 flavone aglycone and a higher number of free hydroxyl groups in compound **1**, resulted in an affinity for nucleophilic interactions. These functional groups may increase the inhibitory activity of HIV-1 of compound **1.** This result has been demonstrated by Messi and coworkers [6]. It is also likely that an additional free hydroxyl group on compound **4** (due to the replacement of one apigenin with luteolin) contributed to better activity [6]. More so, there is a difference in the linkage pattern of **1** (4’-7 C-O-C) and **4** (3’-4"’ C-O-C). While compound **4** showed a good IC_{50} value of 3.1 µg/mL, it unfortunately proved to be non-selective as it also demonstrated a CC_{50} value of 5.2 µg/mL (Selectivity index of 1.7)

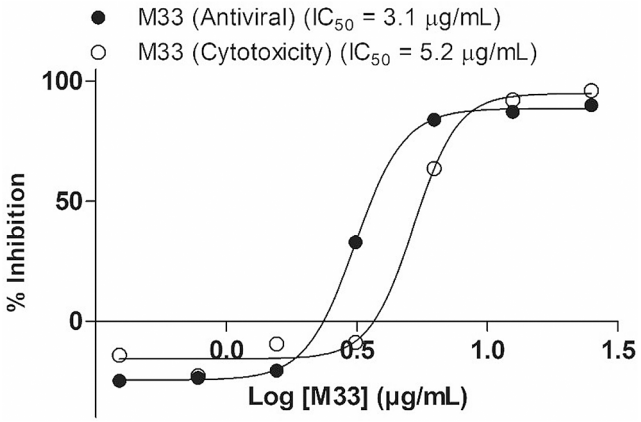

Fig. 4 Full dose-response curve for antiviral and cytotoxicity activity of M33 (**4**). The sigmoidal IC_{50} curve was generated on GraphPad (v5), using the "log(inhibitor) vs. response - Variable slope" non-linear fit option. Raw data was processed in Microsoft Excel

(n = 1, in technical triplicates) (Fig. 4). Compound **4** (M33) was subsequently prioritized for further full-dose investigations. Compounds **5** and **2** were inactive at all concentrations evaluated. The major difference between compounds **4** and **5** is the flavanone skeleton of unit 1 in compound **5**. The absence of a C-C double bond between positions 2 and 3, which introduces a bridge in the extended conjugation of compound **5** may be responsible for its inactivity.

5 Conclusion

Five compounds, including the unreported rotamers rhizomatobiflavonoid D (**1**), 4'-methoxyluteolin-8-C-6"acetylglucopyranoside (**2**), ochnaflavone (**3**), 7″-*O*-methylochnaflavone (**4**), 2,3-dihydroochnaflavone 7-*O*-methyl ether and an 8-C glycosyl flavone (**5**), were reported from the chemical study of the twigs of *Ochna rhizomatosa*. The prominent inhibitory effect on HIV-1 showed by compound **4** (IC_{50} = 3.1 µM) may be explained by the presence of a methoxy group at C-7, since this group enhances the lipophilicity of the biflavonoid, thereby improving its incorporation into cells. Unfortunately, compound **4** proved to be non-selective as it also demonstrated a CC_{50} = 5.2 µg/mL. Further studies are suggested to be performed on HIV integrase enzyme and if possible on the virus to determine the direct effects of our reported isolated compounds. Moreover, the antimicrobial potency of compound **4** is recommended in order to investigate its multi-effect as antiviral and antimicrobial lead molecule.

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Data Availability Data are contained within the article or supplementary material.

Declarations

Ethical Approval Not applicable.

Informed Consent Not applicable.

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Conflict of Interest The authors declare no conflict of interest.

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