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Biological activity of plant extracts and isolated compounds from *Alchornea laxiflora*: Anti-HIV, antibacterial and cytotoxicity evaluation

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ABSTRACT

This study was designed to assess the cytotoxicity, anti-HIV and antibacterial efficacy of various solvent extracts of roots, stem and leaves of *Alchornea laxiflora*, as well as five compounds isolated from its methanolic stem extract viz.; ellagic acid (**1**); 3-*O*-methyl-ellagic acid (**2**), 3-*O*-*β*-D-glucopyranosyl-*β*-sitosterol (**3**), 3-*O*-acetyl-oleanolic acid (**4**) and 3-*O*-acetyl-ursolic acid (**5**). The tested crude extracts were prepared from several solvent polarities including: hexane (Hex), chloroform ($CHCl_3$), ethyl acetate (EtOAc), ethanol (EtOH), methanol (MeOH) and water (H_2O). The anti-HIV properties were assessed on HIV-1 subtype C integrase while the cytotoxicity was tested against Hela cells. The antibacterial activity was studied on a panel of pathogens including gastrointestinal, skin, respiratory and urinary-tract infection causing Gram positive bacteria viz.; *Bacillus cereus* (ATCC 11778), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus saprophyticus* (ATCC 15305)] and Gram-negative bacteria, i.e., *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Moraxella catarrhalis* (ATCC 23246). All the tested samples were determined to be non-toxic due to the low inhibitions observed. The most potent anti-HIV activity was observed for the methanolic extract of *A. laxiflora* root (ALR4) with an IC_{50} value of 0.21 ng/ml, which was more active than chicoric acid used as reference drug (6.82 nM). Roots, stem and leaves of *A. laxiflora* extracts exhibited antibacterial activities against most of the Gram-positive bacteria with the minimum inhibitory concentrations (MIC) ranging between 50 and 63 μg/ml. Compounds **1**–**5** displayed antibacterial activities against *S. saprophyticus* with MIC values as low as 4 μg/ml. The results inferred from this study demonstrate the potential of *A. laxiflora* root as a source for new anti-HIV drugs and scientifically validate the traditional use of *A. laxiflora* in the treatment of gastrointestinal, skin, respiratory and urinary tract related infections. These results reaffirm the ethnopharmacological significance of African traditional medicines.

1. Introduction

The human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) was first reported in 1981 in the United States of America (USA) (Zhang et al., 2017). It has since become epidemic worldwide. More than 78 million people have suffered from HIV infection and about half of this number have died. HIV/AIDS reportedly remains the leading cause of death in Africa (Global HIV Statistics, 2017). The advances in treatment coupled to multiple campaigns of awareness to prevent HIV/AIDS have significantly slowed down the progression of the disease. Importantly, the antiretroviral therapy significantly impacted the progression of the virus, resulting in the decrease of HIV-related deaths worldwide (Zhang et al., 2017). Additionally, a

Abbreviations: ALL, *Alchornea laxiflora* leaf; ALR, *Alchornea laxiflora* root; ALS, *Alchornea laxiflora* stem; ATCC, American type culture collection; BSA, bovine serum albumin; CFU, colony forming units; CIN, HIV-1 subtype C integrase; DMEM, Dulbecco's Modified Eagle's Medium; DRC, Democratic Republic of Congo; FBS, fetal bovine serum; FDA, Food and Drug Administration; FITC, Fluorescein isothiocyanate; HIV/AIDS, human immunodeficiency virus/acquired immunodeficiency syndrome; HNC, Herbier National du Cameroun (National Herbarium of Cameroon); IC_{50}, 50% inhibitory concentration; INT, iodonitrotetrazolium chloride; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; PBS, Phosphate buffered saline..

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decrease by 19% of new infections was observed between 2005 and 2014 (Zhang et al., 2017). Due to the emergence of drug-resistance developed by many HIV/AIDS patients and the associated side effects such as a weakened immune system (this exposes the affected individuals to other microbial, viral and parasitic infections), there is a permanent need for the discovery of new treatments for HIV infection. Natural products have been an important source for new drug discovery leads and have led to the development of drugs approved by the USA Food and Drug Administration (FDA) for treatment of several diseases (Newman and Cragg, 2016).

Alchornea laxiflora (Benth.) Pax & K. Hoffm. (Euphorbiaceae) is a shrub widely spread over large parts of Africa. It occurs from eastern Nigeria to Ethiopia and South to the Democratic Republic of Congo (DRC), throughout East Africa to Zimbabwe, Mozambique, north-eastern parts of South Africa as well as Swaziland. It can grow as high as 6 m (Dalziel, 1937; Schmelzer, 2007). *Alchornea laxiflora* is widely used as a folk medicine in many parts of Africa, as a remedy for numerous diseases ranging from inflammation to heart diseases (Burkill, 1998; Dan et al., 2004; Kayode and Omotoyinbo, 2008). In Cameroon, uses of *Alchornea* species have been described in the traditional pharmacopoeia to treat a range of microbial infections and parasitic diseases (Adjanohoun et al., 1996; Jiofack et al., 2009). However, *A. laxiflora* leaves in particular, have been reported to be effective in the treatment of kidney, urinary bladder inflammation and related infectious diseases (Olatunde Farombi et al., 2003; Sofowora, 2008). Furthermore, in the upper Nyong valley forest in Cameroon, *A. laxiflora* leaves are used to treat dysentery, haemorrhoids and urinary-tract infections (Jiofack et al., 2009) and in Ndop Central sub-division in Cameroon, a decoction (locally known as *Mechango* or *Bambalang*) is taken orally for post-partum pain and inflammation (Focho et al., 2009). In Nigeria, *A. laxiflora* is reportedly used as a topical application to alleviate teething problems in children (Olatunde Farombi et al., 2003). Phytopharmacological studies gleaned from various investigations include the anti-inflammatory (Ogundipe, 1999), hyposensitive and antihistaminic (Kayode and Omotoyinbo, 2008; Sofowora, 2008) properties of leaf extracts.

2. Materials and methods

2.1. Chemicals

The tested pure compounds include ellagic acid (**1**); 3-*O*-methyl-ellagic acid (**2**), 3-*O*-β-D-glucopyranosyl-β-sitosterol (**3**), 3-*O*-acetyl-oleanolic acid (**4**) and 3-*O*-acetyl-ursolic acid (**5**) obtained from the Phytochemical's Bank of the Medicinal Organic Chemistry and Nanomaterials Laboratory, Department of Chemistry, Rhodes University, Grahamstown, South Africa. These compounds were recently isolated and identified from the methanolic extract of *Alchornea laxiflora* (Euphorbiaceae) stem bark by our research team (Sandjo et al., 2011). Chicoric acid > 98%, Emetine > 98% and Ciprofloxacin hydrochloride > 98% were purchased from Sigma–Aldrich.

2.2. Plant materials

The plant material was collected in bulk from uncultivated farmland on the Elounden Mount in Yaoundé, Cameroon, in January 2010. The species was authenticated by Mr. Victor Nana, a botanist from the National Herbarium of Cameroon in Yaoundé, where a voucher specimen (N° 45363/HNC) was deposited.

2.3. Extraction of plant materials for biological assessment

All the collected plant materials (leaf, root and stem) were dried individually at ambient temperature and ground into fine powder. Each of the powdered plant materials was soaked in the solvent (10 g plant material/ 50 ml solvent) in an air-tight container at ambient temperature for 72 h. Selected solvents - hexane, chloroform, ethyl acetate, methanol, ethanol and water (in that order) were used for successive extraction of each sample, respectively.

2.4. HIV-1 integrase strand transfer reaction assay

The HIV-1 subtype C integrase (CIN) strand transfer inhibition assay was adapted from previously described method (Grobler et al., 2002). Briefly, 20 nM double-stranded biotinylated donor DNA (5′-5 Biotin TEG/ACCCTTTTAGTCAGTGTGGAAAATCTCTAGCA-3′ annealed to 5′ ACTGCTAGAGATTTTCCACACTGACTAAAAG-3′) was immobilised in wells of streptavidin coated 96-well microtiter plates (R&D Systems, USA). Following incubation at room temperature for 40 min and a stringent wash step, 5 μg/ml purified recombinant HIV- 1 CIN in buffer 1 (50 mm NaCl, 25 mM Hepes, 25 mM $MnCl_2$, 5 mM β-mercaptoethanol, 50 μg/ml BSA, pH 7.5) was added to individual wells. Test samples and chicoric acid were added to individual wells to a final concentration of 20 μM (pure compounds and chicoric acid) and 50 mg/ml (crude extracts). Recombinant HIV-1 subtype C IN was assembled onto the pre-processed donor DNA through incubation for 45 min at room temperature. Strand transfer reaction was initiated through the addition of 10 nM (final concentration) double-stranded FITC-labelled target DNA (5′-TGACCAAGGGCTAATTCACT/36-FAM/—3′ annealed to 5′-AGTGAATTAGCCCTTGGTCA—/36-FAM/—3′) in integrase buffer 2 (same as buffer 1, except 25 mm $MnCl_2$ replaced with 2.5 mm $MgCl_2$). After an incubation period of 60 min at 37 °C, the plates were washed using PBS containing 0.05% Tween 20 and 0.01% BSA, followed by the addition of peroxidase-conjugated sheep anti-FITC antibody (Thermo Scientific, USA) diluted 1:1000 in the same PBS buffer. Finally, the plates were washed and peroxidase substrate (Sure Blue ReserveTM, KPL, USA) was added to allow for detection at 620 nm using a Synergy MX (BioTek®) plate reader. Absorbance values were converted to percentage enzyme activity relative to the readings obtained from control wells (enzyme without inhibitor).

2.5. Cytotoxic activity

This was adopted from our previously described method (Mbosso Teinkela et al., 2018). Briefly, to assess the overt cytotoxicity, samples were incubated at 25 μg/ml for extracts and 20 μM for pure compounds in 96-well plates containing HeLa cells (human cervix adenocarcinoma), maintained in a culture medium made of Dulbecco's Modified Eagle's Medium (DMEM) with 5 mM L-glutamine (Lonza) and supplemented with 10% fetal bovine serum (FBS) and antibiotics (penicillin/streptomycin/fungizone - PSF) for 24 h. The number of cells surviving drug exposure were counted using the resazurin based reagent and resorufin fluorescence was quantified (Excitation560/Emission590) in a multiwell plate reader.

2.6. Single concentration screening

The percentage of cell viability was calculated at a fixed concentration of 20 μM for pure isolated compounds or 25 μg/ml for natural plant extracts. Experiments were performed in triplicate wells, and the standard deviation (SD) was derived. For comparative purposes, Emetine (which induced cell apoptosis) was used as a positive control drug standard at a concentration of 10 μM. Samples were tested for HIV-1 integrase at a concentration of 20 μM in the case of pure compounds and 25 μg/ml for crude extracts and Chicoric acid was used as positive control for HIV-1 integrase at a concentration of 20 μM.

2.7. Dose response

The IC_{50} (50% inhibitory concentration) values of tested extract/compound exhibiting a low percentage viability or low integrase activity were determined from the resulting dose–response curve by non-linear regression using Prism 5 program (Version 5.02, Graph Pad

Software, Inc). IC50 values for cytotoxicity were not determined due to the low inhibition observed by the preliminary single concentration screening.

2.8. Antibacterial activity

The antibacterial activity of the crude extracts and pure compounds was evaluated by the micro-dilution assay (Siwe Noundou et al., 2016) against four Gram-positive bacteria i.e. *B. cereus* ATCC 11778, *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923 and *S. saprophyticus* ATCC 15305, as well as four Gram-negative bacterial strains i.e. *E. coli* ATCC 25922, *K. pneumoniae* ATCC 13883, *M. catarrhalis* ATCC 23246 and *P. mirabilis* ATCC 43071. All bacterial strains were confirmed pure from stock cultures and maintained in the Pharmaceutical Microbiology Laboratories at the University of Witwatersrand, Johannesburg, South Africa. The stock solutions of samples (32 mg/ml for crude extracts and 1 mg/ml for isolated compounds) were prepared as follows: Acetone was used for the extracts i.e. Hex, $CHCl_3$ and EtOAc and compounds **4** and **5** while a solution of DMSO/H_2O (5:95, v/v) was used for EtOH and MeOH extracts and compounds **1**, **2** and **3**. The aqueous extracts were used as are. All the above stock solutions were placed in duplicate (using adjacent wells) and further serially diluted (2-fold dilution) with sterile water in a 96-well microtitre plate to obtain a 1 mg/ml and 0.031 mg/ml solution in the final row for the extracts and isolated compounds respectively. To the above dilutions in the well plates, equal volumes (100 μl) of bacterial suspension yielding approximately an inoculum size of 1×10^6 colony forming units (CFU/ml) were added. The plates were sealed with sterile seals and incubated at 37 °C for 24 h. Thereafter, 0.04% (w/v) *p*-iodonitrotetrazolium (INT) was added to each well and the plates were kept at ambient temperature for 6 h. The results were recorded visually under a light source. All well plate experiments were carried out in triplicate (2×3). Sterile broth containing bacterial suspension was used to monitor the viability of the test organism, while ciprofloxacin hydrochloride (0.01 μg/ml) was used as the positive antibacterial control. Acetone or DMSO were used as negative control. The final concentration of acetone or DMSO in the well had no effect on the bacterial growth.

3. Results and discussion

3.1. HIV-1 Integrase inhibitory activity

Most of the root extracts of *A. laxiflora* (ALR series) inhibited the activities of HIV-1 Integrase (Fig. 1a and Table 1). The methanolic root extract (ALR4) exhibited noteworthy HIV-1 Integrase inhibitory activity with an IC_{50} value of 0.21 ng/ml, chicoric acid was taken as a reference ($IC_{50} = 6.82$ μM). The ethanolic root extract (ALR5) also displayed marked HIV-1 Integrase inhibitory activity with an IC_{50} value of 67.07 μg/ml, while the ethyl acetate root fraction (ALR3) IC_{50} value was found to be 6.034 μg/ml. The cytotoxicity studies results (Table 1) showed that none of these extracts were cytotoxic. The results of our findings are in agreement with previous reports on the anti-HIV efficacy on *A. laxiflora*. In fact, Buhner, (2012), reported that *A. laxiflora* was found to be strongly active against HIV-1 and HIV-2 in vitro, more so than Azidothymidine (AZT) (no IC_{50} value shown nor the part of the plant used). AZT is the first drug approved by the US FDA in the fight against AIDS (Zhang et al., 2017). A closely related species of the same genus namely *Alchornea cordifolia* was also found to be active against HIV-1 and HIV-2 (Ayisi and Nyadedzor, 2003).

The methanolic extract of the stem of *A. laxiflora* (ALS4) inhibited the activities of HIV-1 Integrase by 91.75% (Table 1), but the IC_{50} was not determined. The activity of isolated compounds from ALS4 (Fig. 2) on HIV-1 Integrase was also investigated (Fig. 1b and Table1). While all the isolated compounds were found to be non-cytotoxic (Table 1), some of these compounds inhibited the activities of HIV-1 Integrase (Table 1). Ellagic acid (1) displayed the best anti-HIV-1 Integrase activity with IC_{50} value of 90.23 μM. The IC_{50} values of 3-*O*-methylellagic acid (**2**) and 3-*O*-acethyl of oleanolic acid (**4**) were > 100 μM. This result follows the same trend as observed in the literature. To this instance, ellagic acid isolated from *Lagerstroemia speciosa* L. was found to be non-toxic and to inhibit HIV-1 activity with an IC_{50} value of 73 μg/ml (Nutan et al., 2013). This result is the first report on the identification of compounds that can be responsible for the anti-HIV activity of *A. laxiflora*. It is therefore suggested that ellagic acid can be a potential agent for the development of novel drugs against HIV-1.

3.2. Antibacterial activity of solvent extracts

The extracts of three plant parts (leaves, stems and roots) of *A. laxiflora* prepared in the order of solvent polarity (i.e. hexane (Hex), chloroform ($CHCl_3$), ethyl acetate (EtOAc), Ethanol (EtOH), methanol (MeOH) and water (H_2O) are depicted in Table 2.

The antibacterial observations include inhibitory effects on many of the selected pathogens representing gastrointestinal, skin, respiratory and urinary tract infections. Methanol extracts of all three plant parts were found to be the most active when compared to other extracts. The best results for the leaf extracts were observed for the EtOAc, MeOH and EtOH extracts against *Klebsiella pneumoniae* (MIC 63 μg/ml, for each of the three extracts), a Gram-negative strain associated with respiratory ailments (Peleg and Hooper, 2010). *Staphylococcus saprophyticus*, a Gram-positive urinary tract pathogen (Hovelius and Mardh, 1984), was particularly susceptible to most extracts (MIC ranging between 63 and 250 μg/ml) and this is in agreement with one of the most commonly listed traditional uses for *Alchornea laxiflora*, in the treatment of diarrhoea. However, the activity of all extracts on *Proteus mirabilis* appeared to be weak compared to *S. saphrophyticus*. It would be prudent to consider a study on other urinary tract pathogens which could provide further information on the wider use of *A. laxiflora* extracts. The root extracts showed higher activity against *B. cereus* and *E. faecalis* at MICs of 63 μg/ml and on *S. aureus* at 50 μg/ml. The stem extracts of ethanol and methanol showed significant MIC values on *S. saprophyticus* at 63 μg/ml. However, the EtOAc, CHCl3 and Hex extracts

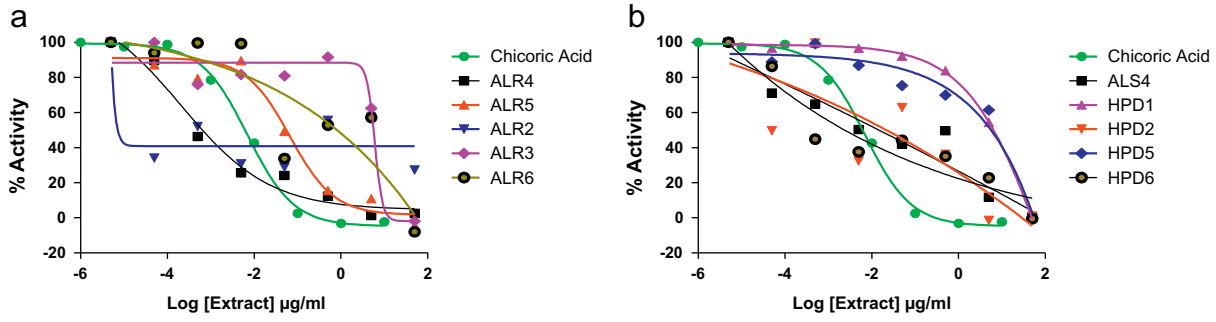

Fig. 1. Dose–response curve of anti-HIV integrase assay for (a): *A. laxiflora* root extracts and (b): *A. laxiflora* stem bark MeOH extract and isolated compounds.

Table 1
Bioassay data showing percentage activity, IC_{50} values for inhibition of HIV-1 C integrase activity and PV values for toxicity against HeLa cells.

Sample		HIV-1 integrase activity[a]		Cytotoxicity activity[b,c]	
Name	Code	PA (%)	IC_{50}	PV (%)	IC_{50}
Crude extracts					
A. laxiflora root (hexane)	ALR1	7.06	nd	121.87 ± 3.60	nd
A. laxiflora root (chloroform)	ALR2	28.47	nd	107.27 ± 10.29	nd
A. laxiflora root (ethylacetate)	ALR3	−2.03	6.034	101.69 ± 7.30	nd
A. laxiflora root (methanol)	ALR4	−1.91	0.0002083	113.45 ± 13.93	nd
A. laxiflora root (ethanol)	ALR5	−2.39	0.06707	99.57 ± 10.87	nd
A. laxiflora root (water)	ALR6	−2.99	>500	115.32 ± 9.29	nd
A. laxiflora stem (methanol)	ALS4	91.39	nd	138.58 ± 2.44	nd
Pure isolated compounds from ALS4					
Ellagic acid (**1**)	HPD1	12.80	90.23	109.64 ± 3.49	nd
3-*O*-methylellagic acid (**2**)	HPD2	1.08	>100	110.64 ± 6.53	nd
3-*O*-*β*-D-glucopyranoside of *β*-sitosterol (**3**)	HPD4	34,41	nd	112.84 ± 8.39	nd
3-*O*-acethyl of oleanolic acid (**4**)	HPD5	16.39	>100	124.00 ± 1.14	nd
3-*O*-acethyl of ursolic acid (**5**)	HPD6	91.75	nd	100.85 ± 3.45	nd
References					
	Chicoric acid	−0.48	0.00682		
	Emetine				0.044

PA: percentage activity; PV: percentage viability; nd: not determined;
PV values are from triplicate experiments 20 μM (compounds) or 25 μg/ml (extracts);
IC50: 50% inhibitory concentration, i.e. the concentration of sample that inhibits/reduces by 50% the activity/growth or proliferation of enzyme integrase/HeLa cells in μg/ml for extracts and in μM for pure compounds and reference drugs.
[a] HIV-1 integrase inhibitory activity;
[b] HeLa (human cervix adenocarcinoma) cells;
[c] IC50 values of samples were not determined due to the low inhibition observed by the preliminary single concentration screening for cytotoxicity.

showed MICs of about 250 μg/ml. The leaf extracts exhibited highest MICs of above 100 μg/ml for activity on *B. cereus* and *E. faecalis* and significant in the case of the respiratory pathogen *K. pneumoniae* and the urinary pathogen *S. saprophyticus.*

According to Bueno, (2012), plant extracts displaying MIC values below or equal to 100 μg/ml are considered to show noteworthy antimicrobial activity (Bueno, 2012). Based on the above statement, we could consider the *A. laxiflora* plant extract concentrations showing significant MICs below 100 μg/ml as having significant antimicrobial activity. This is the first report on the comparative antibacterial study of *A. laxiflora* different parts.

3.3. Antibacterial activity of isolated compounds

The five isolated compounds (Fig. 2) were screened for antibacterial activity against the same eight pathogens as the extracts (Table 2).

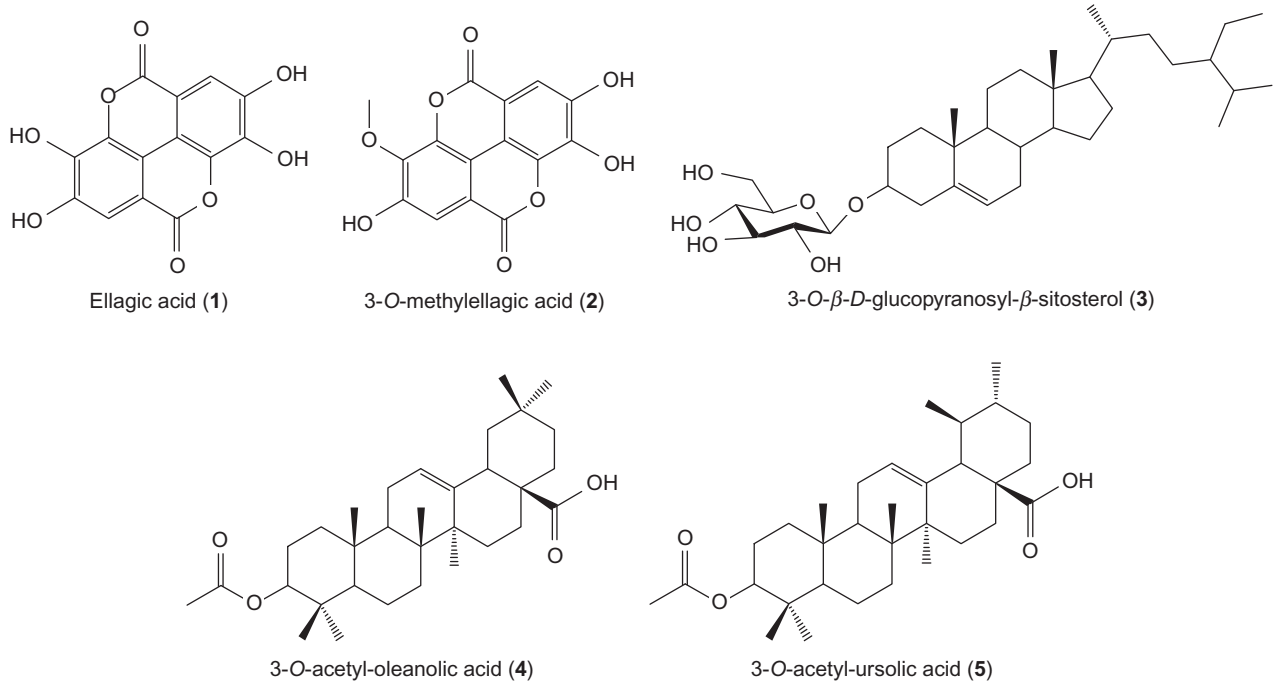

Fig. 2. Chemical structure of compounds **1–5.**

Table 2
The MIC values (in μg/ml) exhibited against selected pathogens by the crude extracts of *A. laxiflora*, as well as the pure compounds isolated from the stem bark. Highest activities are indicated in bold.

Samples		Gastro-intestinal pathogens			Skin pathogen	Respiratory pathogens		Urinary pathogens	
Solvent	Code	*B. cereus*[a] ATCC 11778	*E. faecalis*[a] ATCC 29212	*E. coli*[b] ATCC 25922	*S. aureus*[a] ATCC 25923	*K. pneumoniae*[b] ATCC 13883	*M. catarrhalis*[b] ATCC 23246	*P. mirabilis*[b] ATCC 43071	*S. saprophyticus*[a] ATCC 15305
A. laxiflora roots									
Hex	ALR1	500	**63**	1000	**63**	2000	2000	1000	125
$CHCl_3$	ALR2	**63**	**50**	500	**50**	125	500	500	**63**
EtOAc	ALR3	250	2000	500	**50**	125	1000	250	**63**
MeOH	ALR4	**63**	**63**	500	**50**	125	1000	250	**63**
EtOH	ALR5	**63**	**63**	500	**50**	125	500	250	**63**
H_2O	ALR6	8000	8000	8000	8000	8000	>8000	>8000	>8000
A. laxiflora stem									
Hex	ALS1	8000	8000	8000	1000	2000	>8000	>8000	250
$CHCl_3$	ALS2	250	2000	500	500	500	2000	2000	250
EtOAc	ALS3	250	500	250	500	500	2000	8000	250
MeOH	ALS4	1000	1000	500	500	500	500	4000	**63**
EtOH	ALS5	500	1000	250	500	500	500	4000	**63**
H_2O	ALS6	8000	8000	8000	8000	4000	>8000	>8000	>8000
A. laxiflora leaves									
Hex	ALL1	500	500	500	250	1000	1000	>8000	250
$CHCl_3$	ALL2	125	125	125	250	500	1000	8000	**63**
EtOAc	ALL3	125	125	125	250	**63**	125	8000	**63**
MeOH	ALL4	125	250	125	250	**63**	2000	8000	**63**
EtOH	ALL5	125	250	125	400	**63**	1000	2000	250
H_2O	ALL6	4000	1000	4000	1000	8000	>8000	>8000	>8000
Compounds isolated from *A. laxiflora* stem									
Compound 1		125	**63**	**63**	125	**16**	125	125	**31**
Compound 2		125	**63**	**63**	125	**31**	250	250	**16**
Compound 3		125	**63**	**63**	125	**31**	125	250	**4**
Compound 4		125	125	**63**	125	**16**	**16**	**63**	**4**
Compound 5		125	125	**63**	125	**31**	**16**	**63**	**4**
Ciprofloxacin HCl (+ control)		0.313	0.625	0.156	0.312	0.625	0.625	0.078	0.313
Culture*		>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000
Acetone (− control)*		>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000
5% DMSO/Water (− control)*		>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000

[a] Gram positive;
[b] Gram negative; + positive; − negative.

3.3.1. Gastrointestinal (GI) pathogens

All the compounds showed an MIC of 125 μg/ml against *B. cereus*; compounds **1**, **2** and **3** exhibited an MIC of 63 μg/ml against *E. faecalis*, whereas, MICs of 125 μg/ml were observed for compounds **4** and **5** against *E. faecalis*. The minimum inhibitory concentration against *E. coli* was observed at 63 μg/ml for all compounds.

3.3.2. Skin pathogen

All the five compounds displayed an MIC of 125 μg/ml against *S. aureus*.

3.3.3. Respiratory pathogens

All the five compounds exhibited an MIC of <31 μg/ml against Gram-negative *K. pneumoniae*, compounds **4** and **5** displayed antibacterial activity with MIC value of 16 μg/ml against *M. catarrhalis*; compounds **1** and **3** exhibited an MIC of 125 μg/ml against *M. catarrhalis* while compound **2** showed an MIC of 250 μg/ml against the same pathogen.

3.3.4. Urinary pathogens

Compounds **3**–**5** were the most active with an MIC value of 4 μg/ml against Gram-positive *S. saprophyticus*. Compounds **4** and **5** were the most active against *P. mirabilis* whith MIC value of 63 μg/ml each.

Ellagic Acid (**1**) was evaluated against three strains of *Bacillus;* i.e. *B. subtilis*, *B. cereus* and *B. polymyxa* and the results showed antibacterial activity of ellagic acid (**1**), with MICs <10 μg/ml for the three species (Cetin-Karaca, 2011). In the same study, ellagic acid (**1**) was also evaluated against *E. coli* and displayed an antibacterial activity with MIC value of 20 μg/ml. Previous antibacterial investigation on 3-*O*-*β*-*D*-glucopyranosyl-*β*-sitosterol (**3**) was reported by Seukep et al. (2016) against *P. aeruginosa*, *K. pneumoniae* and *E. coli* strains and all the MICs ranged from 32 to 256 μg/ml. 3-*O*-acetyl-oleanolic acid (**4**) was studied against several bacterial strains including *S. aureus*, *B. cereus*, two strains of *E. coli*, *P. aeruginosa* and *K. pneumoniae* by Do Nascimento et al. (2014). 3-*O*-acetyl-oleanolic (**4**) acid exhibited significant activity (MIC ≤1024 μg/ml) against all tested strains except for *S. aureus*. The best results were found against *E. coli* with an MIC value of 32 μg/ml, and against *K. pneumoniae* and *S. aureus* with MIC values of 64 μg/ml and 128 μg/ml, respectively. In another antibacterial study by Cunha et al. (2010), the antibacterial activity of 3-*O*-acetyl-oleanolic acid (**4**) against *B. cereus*, *K. pneumoniae*, and *Streptococcus pneumoniae* was evaluated. The reported MICs were greater than 1000 μg/ml, except for *S. pneumoniae* (50 μg/ml). 3-*O*-acetyl-ursolic acid (**5**) was tested against *B. cereus, S. aureus, S. epidermidis,* methicillin-resistant *S. aureus* (MRSA), *E. coli* and *Pseudomonas aeruginosa* by Sanpa et al. (2015) and 3-*O*-acetyl-ursolic acid (**5**) exhibited an MIC value of 25 μg/ml against all the tested bacteria.

4. Conclusions

In continuation of the quest for discovery of effective anti-HIV and antimicrobial compounds, screening of herbal extracts and compounds is indeed an expedient procedure to discover new entities that may be used in developing future drugs. In this study, the isolated compounds did not display significant anti-HIV integrase activity. The most potent anti-HIV activity was observed for the methanolic extract of *Alchornea laxiflora* root (ALR4) with an IC_{50} of 0.21 ng/ml. This is an indication that further investigations are required to isolate and identify putative new anti-HIV integrase from the roots of *A. laxiflora*. Our next objective is therefore to isolate and identify the anti-HIV integrase compounds from the roots of *A. laxiflora*. The five isolated compounds exhibited significant activity with MIC values ranging between 4 and 63 μg/ml against all the bacteria except *B. cereus*. 3-*O*-*β*-D-glucopyranosyl-*β*-sitosterol, 3-*O*-acetyl-oleanolic acid and 3-*O*-acetyl-ursolic acid displayed the highest antibacterial activity (MIC value of 4 μg/ml) against *S. saprophyticus*. The roots seem to be the most active plant component of *A. laxiflora* as the root extracts (MIC as low as 50 μg/ml) were more active as compared to the stem and leaves extracts. The highest antibacterial activities were observed for the medium polarity extracts (EtOH, MeOH, EtOAc and $CHCl_3$). The antibacterial activity of *A. laxiflora* against gastro-intestinal (*B. cereus, E. faecalis* and *E. coli*), skin (*S. aureus*), respiratory (*K. pneumoniae* and *M. catarrhalis*) and urinary (*P. mirabilis* and *S. saprophyticus*) pathogens is indeed scientifically demonstrated. The isolated phytosteroid (3-*O*-*β*-D-glucopyranosyl-*β*-sitosterol) and triterpenoids (3-*O*-acetyl-oleanolic acid and 3-*O*-acetyl-ursolic acid) showed more potent antibacterial activity than the phenolic compounds (ellagic acid and its methyl derivative). These isolated compounds are reported here for the first time to be responsible for antibacterial activities of *A. laxiflora*. It is important to note that the roots seem to be the most anti-HIV and antibacterial active component of *A. laxiflora*. The overall results provide evidence that *A. laxiflora* as well as some of its isolated components (3-*O*-*β*-D-glucopyranosyl-*β*-sitosterol, 3-*O*-acetyl-oleanolic acid and 3-*O*-acetyl-ursolic acid) might be potential sources of new anti-HIV and antimicrobial drugs.

Competing interest

The authors declare no conflict of interest.

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