Natural Product Research

Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

Isolation of a new β-carboline alkaloid from aerial parts of *Triclisia sacleuxii* and its antibacterial and cytotoxicity effects

Fidelis Samita, Charles Otieno Ochieng, Philip Okinda Owuor, Lawrence Onyango Arot Manguro & Jacob Ogweno Midiwo

To cite this article: Fidelis Samita, Charles Otieno Ochieng, Philip Okinda Owuor, Lawrence Onyango Arot Manguro & Jacob Ogweno Midiwo (2017) Isolation of a new β-carboline alkaloid from aerial parts of *Triclisia sacleuxii* and its antibacterial and cytotoxicity effects, Natural Product Research, 31:5, 529-536, DOI: 10.1080/14786419.2016.1201666

To link to this article: https://doi.org/10.1080/14786419.2016.1201666

NATURAL PRODUCT RESEARCH, 2017
VOL. 31, NO. 5, 529–536
http://dx.doi.org/10.1080/14786419.2016.1201666

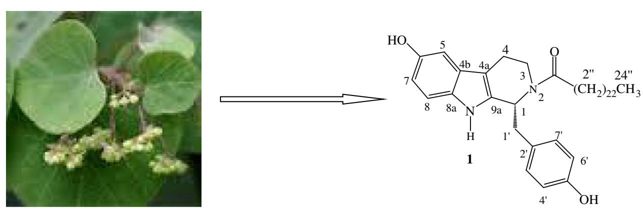
Isolation of a new β-carboline alkaloid from aerial parts of *Triclisia sacleuxii* and its antibacterial and cytotoxicity effects

Fidelis Samita[a], Charles Otieno Ochieng[a], Philip Okinda Owuor[a], Lawrence Onyango Arot Manguro[a] and Jacob Ogweno Midiwo[b]

[a]Department of Chemistry, School of Physical and Biological Sciences, Maseno University, Maseno, Kenya; [b]Department of Chemistry, School of Biological and Physical Sciences, University of Nairobi, Nairobi, Kenya

ABSTRACT

A new β-carboline alkaloid named sacleuximine A (**1**) together with known compounds palmatine (**2**), isotetrandrine (**3**), *trans*-N-feruloyltyramine (**4**), *trans*-N-caffeoyltyramine (**5**), yangambin (**6**), syringaresinol (**7**), sesamin (**8**), (+) epi-quercitol (**9**), 4-hydroxybenzaldehyde (**10**), β-sitosterol (**11**), quercetin 3-*O*-rutinoside (**12**) and myricetin 3-*O*-β-glucose (1→6) α-rhamnoside (**13**) have been isolated from methanol extract of *Triclisia sacleuxii* aerial parts. Compounds **1–10** were evaluated for their cytotoxicity against human adenocarcinoma (HeLa), human hepatocarcinoma (Hep3B) and human breast carcinoma (MCF-7) cells lines and also for antibacterial activities against both Gram-positive and Gram-negative bacteria. The cytotoxicity (IC_{50}) values ranged between 0.15 and 36.7 μM while the minimum inhibitory concentrations were found to be in the range of 3.9 and 125 μM, respectively. This is the first report of antibacterial compounds and the isolation of lignans together with a β-carboline alkaloid from *T. sacleuxii.*



Triclisia sacleuxii aerial part

ARTICLE HISTORY
Received 29 June 2015
Accepted 2 June 2016

KEYWORDS
Triclisia sacleuxii; Menispermaceae; secondary metabolites; cytotoxicity; antibacterial; aerial parts

1. Introduction

The species *Triclisia sacleuxii* (Pierre) Diels of the Menispermaceae family is a tendril plant that grows in the lowland and riverside forests of many African countries (Jacques & De Franceschi 2007). In traditional medicine, the plant root is chewed and the sap swallowed to manage kidney problems, sore throat, schistosomiasis, intestinal worms, venereal diseases

CONTACT Lawrence Onyango Arot Manguro chem@maseno.ac.ke
Supplemental data for this article can be accessed at http://dx.doi.org/10.1080/14786419.2016.1201666.

and root scrapings applied in topical scarifications to treat snakebites (Kokwaro 2009). Previous phytochemical studies reported the isolation of a series of tertiary bisbenzylisoquinoline alkaloids (Murebwayire et al. 2006), which displayed good antiplasmodial and antityrpanosomal activities *in vitro* against *Plasmodium falciparum* (Pollard 2008), *Leshmania donovani* promastigotes, *Trypanosoma cruzei* and *Trypanosoma brucei* (Murebwayire et al. 2008). Further biological activity studies using the same alkaloid fractions and isolates showed strong inhibition against acetylcholinesterase with lindoldhamine being reported as the most active compound, thus suggesting its potential in managing Alzheimer's disease (Murebwayire et al. 2009).

In the current study, preliminary antimicrobial and cytotoxicity assay results of MeOH extract of *T. sacluexii* aerial parts prompted us to phytochemically investigate it, resulting in the isolation of a β-carboline alkaloid, sacleuximine A (**1**) together with known compounds palmatine (**2**) and isotetrandrine (**3**) (Brazdovlcova et al. 1980), *trans*-N-feruloyltyramine (**4**) and *trans*-N-caffeoyltyramine (**5**) (Al-Taweel et al. 2012), yangambin (**6**) (Ahmed et al. 2002; Rubens et al. 2007), syringaresinol (**7**) (Gohari et al. 2011) and sesamin (**8**) (Houghton 1985; Liang et al. 2012), (+) epi-quercitol (**9**) (Rodríguez-Sánchez et al. 2010), 4-hydroxybenzaldehyde (**10**) (Tian et al. 2012) and β-sitosterol (**11**) (Wright et al. 1978), quercetin 3-*O*-rutinoside (**12**) (Manguro et al. 2004), myricetin 3-*O*-β-glucose (1→6) α-rhamnoside (**13**) (Manguro et al. 2005) (Figure 1). The compounds were identified using spectroscopic methods as well as comparison with literature data.

2. Results and discussion

Compound **1** was isolated as white amorphous powder with a molecular formula of $C_{42}H_{64}N_2O_3$ as determined by HR-ESI-MS $[M + Na]^+$ at $m/z = 667.4917$ (calcd. 667.4920). The UV spectrum absorption peaks at 242 (4.12), 260 (3.32) and 280 (3.60) nm suggested the presence of hydroxy indole and hydroxy benzyl chromophores (Wu et al. 1989). Its IR spectrum showed significant absorption bands at 3414 (NH), 3316 (OH) and 1653 (C=O) cm^{-1}. The ^1H NMR spectrum data (Table S1) revealed two sets of aromatic signals, a 3H AMX system {δ 7.00 (d, $J = 8.6$ Hz), 6.67 (dd, $J = 8.6, 2.4$ Hz) and 6.86 (d, $J = 2.4$ Hz)} and a 4H AA'XX' system {δ 7.15 (d, $J = 8.4$ Hz) and 6.82 ($J = 8.4$ Hz)} suggesting the presence of two aromatic rings (Manguro et al. 2003). In addition, the presence of a relatively upfield methylene at δ 2.35 (t, $J = 7.5$ Hz), a methylene multiplet at δ 1.25 and a terminal methyl group 0.88 (t, $J = 6.7$ Hz) together with a NH signal at δ 6.90 (s) suggested the presence of a long aliphatic chain having a CH_2-C = O connected to the carboline nucleus (Koskinen et al. 2014; Zhang et al. 2015). Furthermore, the presence of mutually coupled methylene protons δ 4.07 (d, $J = 13.7$, 4.1 Hz) and 3.38 (dd, $J = 13.0$, 4.6 Hz) signified a tetrahydro-β-carboline skeleton (Koskinen et al. 2014; Ngoc et al. 2016; Yang et al. 2016). The ^{13}C NMR spectrum (Table S1) displayed 14 aromatic carbon signals and an amide carbonyl carbon (δ 177.1) as evidenced by 135 DEPT NMR spectrum. The methylene peaks at δ 3.14 (H-1'a) and 2.93 (H-1'b) exhibited ^1H-^1H COSY correlation with a pair of the aromatic protons at δ 7.15 (H-3' and H-7') and in turn with H-1 indicating the presence of a benzyl moiety connected to a methine carbon. Analyses of the ^1H-^1H COSY, HSQC and HMBC spectra (Figure S9) established the presence of molecular features >CH–CH=CH–CH_2–N< and >N–CO–$(CH_2)_{22}CH_3$, which on comparison with spectral data of shepherdine and oldhamiaines (Salmoun et al. 2002; Zhang et al. 2015) and further supported by HMBC correlations between H-1/C-2', and H-3'/C-4', deduced the presence of

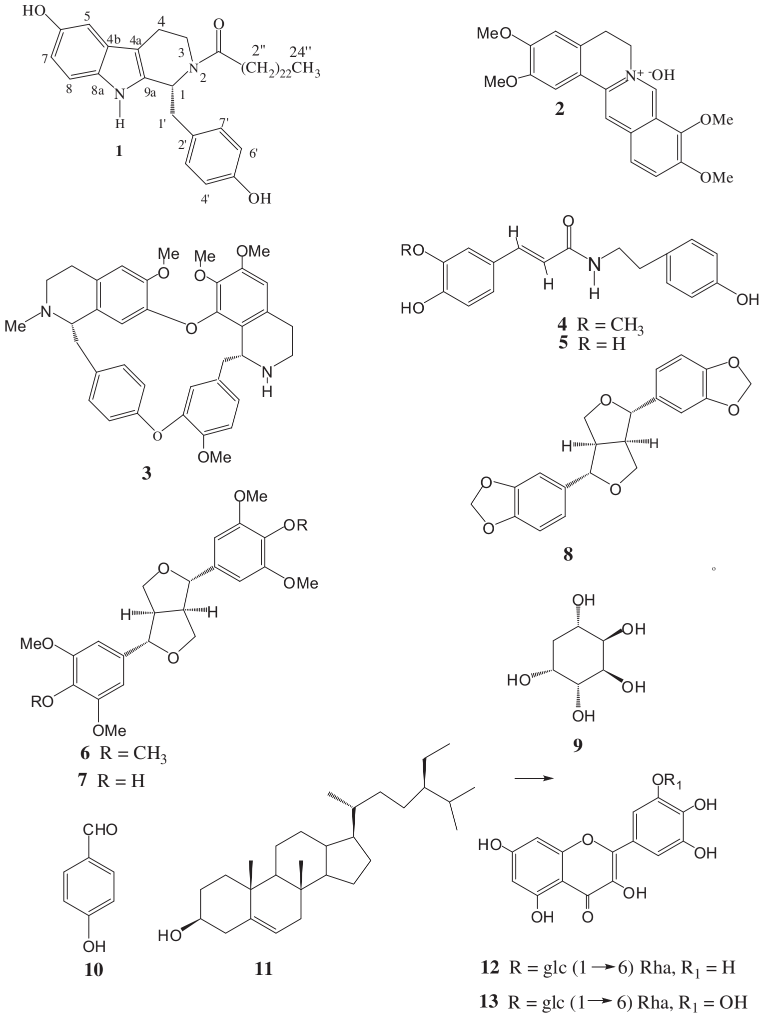


Figure 1. Compounds isolated from aerial parts of *T. sacluexii.*

4-hydroxybenzyl group bonded to a methine carbon attached to an SP^3 hybridised nitrogen atom and an SP^2 hybridised carbon (Camacho et al. 2002; Jain 2016). The NOSY correlation between the aromatic proton at δ 7.00 (H-8) and the indolic NH proton at δ 6.90 coupled with the HMBC cross peaks observed between H-1 and C-2′and in turn with C-4a allowed the allocation of the benzyl moiety to C-1. Similarly, the HMBC correlation between H-1 and the carbonyl carbon (C-1‴) confirmed the attachment of the aliphatic chain on the second nitrogen. The aliphatic chain was deduced to be a tetracosan-1-one based on the ESIMS fragmentation (Fig. S10) caused by α-cleavage of the amide bond *m/z* 354 $[M-C_{18}H_{18}N_2O_2]^+$ and a less-intense McLafferty rearranged fragment ion *m/z* 337 $[M-C_{22}H_{46}]^+$. Comparison of spectral data of **1** with those of shepherdine and oldhamiaines (Salmoun et al. 2002; Zhang et al. 2015) and the NOESY correlation between the benzylic methylene protons and the indolic proton NH suggested the configuration at C-1 as a β for H-1. This was further supported by the spin decoupling experiments which showed that the 4-hydroxylbenzyl moiety was linked in a $=C–CH_{eq}(C_6H_4OH_{ax})–N<$ requiring it to be at C-1 as α. Thus, on the basis of spectroscopic data and comparison with literature data, compound **1** was deduced to be 1-(6-hydroxy-1-(4-hydroxybenzyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)tetracosan-1-one, a new alkaloid named sacleuximine A.

The cytotoxicity of the isolated compounds was evaluated by MTT method (Alley et al. 1988). The alkaloids (**1–3**) and the lignans (**6–8**) showed strong cytotoxicity effects (Fig. S11) against the three cancer cell lines while *trans*-N-feruloyltyramine (**4**) and *trans*-N-caffeoyltyramine (**5**) showed better activities. The latter compound was more active than compound **4** which may be attributed to the presence of *ortho* dihydroxyl groups. The activities of the alkaloids were in agreement with the previously reported cytotoxicity of β-carboline alkaloids against several human cancer cell lines with IC_{50} values ranging from 0.3 to 1.2 µg/mL (Cao et al. 2007). Compounds (**1–10**) were also evaluated for their antibacterial activities against Gram-positive and Gram-negative bacteria strains (Table S2). Compound **1** exhibited moderate to low antibacterial activities while compound **3** showed significant (MIC 7.8 µM) inhibition against Gram-positive strains compared to compound **2** and the two ferrulamides (**4** and **5**). Lignans showed better antibacterial activity compared to the other compounds against the tested strains with **7** displaying higher activity (minimum inhibitory concentration [MIC] 3.9 µM) against the Gram-positive bacteria (*Staphylococcus epidermis* and *Staphylococcus aureus*).

3. Experimental

3.1. General experimental procedure

Column chromatography and thin-layer chromatography were performed using silica gel 0.035–0.070 mm, 60A and $60F_{254}$, respectively. Optical rotation was measured on a Jasco P-1020 Polarimeter (Jasco Corporation, Tokyo, Japan). Melting points were determined on a Gallenkamp apparatus (Manchester, UK). UV spectra were done on a Shimadzu UV-2401 A spectrophotometer (Shimadzu Corporation, Kyoto, Japan). IR data were recorded on a Bruker Tensor 27 FTIR spectrophotometer (Bruker Corporation, Bremen, Germany) as KBr pellet. The ^{1}H, ^{13}C and 2D-NMR spectra were recorded on Bruker AVANCE III-600 MHz spectrometer (Bruker Corporation, Zurich, Switzerland) equipped with a 5-mm TCI cryogenic probe head (*z*-gradient) using standard pulse sequences. Semi-preparative RP-HPLC was performed on a Hitachi Chromaster system (Hitachi Corporation, Japan) equipped with an YMC-Triart C18 column (250 × 10 mm i.d., 5 µm, YMC Corporation, Tokyo, Japan) using a flow rate of 0.5 mL/min, and eluent of 1:4-mixture of H_2O + 0.1% HCO_2H and H_3CCN + 0.1% HCO_2H. LR-MS was performed using an Agilent Technology 1200 series (Agilent Corporation, Bobingen, Germany) apparatus. LR-MS and HR-ESI-MS data were recorded on a Q-TOF ULTIMA-III (Waters Corporation, Manchester, UK) equipped with a LockSpray Interface.

3.2. Plant material

The aerial parts of *T. sacleuxii* were collected in August 2012 from Cha-Simba Rocks, Kilifi County, Kenya. The plant material was identified by Mr. Mutiso of Botany Department, Nairobi University, and a voucher specimen (EAH/2012/608) was deposited at the East African herbarium, Nairobi, Kenya.

3.3. Extraction and isolation of compounds

The air-dried ground aerial parts of *T. sacleuxii* (2 kg) were extracted three times with 95% aqueous methanol (each 5 L), each extraction lasting three days with occasional swirling. The extracts were combined and solvent removed under *vacuo* leaving behind a green residue, 60 g. The extract (56 g) was suspended in water (0.3 L), then partitioned successively into dichloromethane (1 L × 4) and *n*-butanol (0.5 L × 4), each yielding 26.0 and 19.0 g of extracts, respectively. The remaining H_2O portion was freeze-dried affording ≈ 6 g of dark green extract.

3.3.1. Fractionation of dichloromethane extract

A portion of the CH_2Cl_2 extract (approx. 24.0 g) was subjected to column chromatography on silica gel (SiO_2 240 g; 3 × 60 cm), starting with 100% cyclohexane as eluent followed by cyclohexane–ethyl acetate mixture with increasing polarity of the more polar solvent (increment 10%) and elution concluded with 100% ethyl acetate. A total of 270 fractions (each 20 mL) were collected and their homogeneity monitored by TLC using cyclohexane–EtOAc (4:1, 3:2, 2:1 and 1:1) and CH_2Cl_2–MeOH (98:2). Depending on TLC profiles, the eluants were combined to give six pools (I–VI). The first 20 fractions showed no spots and the solvent was recovered. Pool II (Fraction 21–33, eluent: cyclohexane–EtOAc, 4:1) showed a single spot with same solvent system, and upon crystallisation, afforded **11** (100 mg). Pool III constituted fractions 34–47 (3 g) and was further purified using cyclohexane–EtOAc (3:2) to give **10** (23 mg). Pool IV (fractions 50–100, 5 g), upon further purification on silica gel column using 0.5% methanol in CH_2Cl_2 as eluent, afforded **7** (32 mg), **4** (15 mg) and **5** (17 mg). Fractions 101–200 (Pool V, 6 g) showed one major spot on TLC using 1% methanol in CH_2Cl_2 and purification on silica gel column with 2–4% MeOH–CH_2Cl_2 mixture, followed by further purification on Sephadex LH-20 (100% MeOH, 500 mL), yielded a colourless substance that was Dragendorff reagent positive. Final purification using preparative RP-HPLC C-18 column yielded **1** (16 mg). The remaining fractions constituted Pool VI (4 g) which upon further purification (150 g SiO_2; 2.5 x 50 cm; 2–6% MeOH-CH_2Cl_2) yielded **8 (**29 mg) and **6** (42 mg).

3.3.2. Fractionation of n-butanol extract

The *n*-butanol extract (15 g) was fractionated over silica gel column using a mixture of CH_2Cl_2–methanol with increasing polarity of the more polar solvent. A total of 53 fractions, each 100 mL were collected and those exhibiting similar TLC profiles were pooled together (Pools VII andVIII). Pool VII (fractions 1–20, 8 g) upon further purification afforded more of **8 (**18 mg) and **6 (**20 mg). Fractions 21–52 (pool VIII, 7 g) on further fractionation (SiO_2 150 g; 2.5 × 50 cm; 5–10% MeOH–CH_2Cl_2) gave three fractions (A–C). Fraction A (1 g) was purified on Sephadex LH-20 (100% MeOH, 500 mL) to yield **2** (27 mg). On the other hand, fraction B (1.9 g) yielded **3** (21 mg) under similar purification procedure. Fraction C (1.2 g) was triturated with methanol to yield (+) epi-quercitol **9** (19 mg).

3.3.3. Fractionation of H_2O extract

Approximately 5.5 g of the extract was fractionated over 2% oxalic acid-deactivated silica gel starting with CH_2Cl_2/MeOH gradient and elution concluded with MeOH, fractions of 20 mL each being collected and their homogeneity determined by TLC [eluent: CH_2Cl_2–MeOH (9:1, 3:2 and 2:1); *n*-BuOH–HOAC–H_2O (4:5:1)]. Fractions 30–80 showed a major spot and was crystallised in MeOH–H_2O (95:5) to give **12** (50 mg). Fractions 93–150 upon repeated fractionation using CH_2Cl_2–MeOH (4:1) gave **13** (60 mg).

Sacleuximine A (**1**), white amorphous powder, mp 212–214 °C; UV ($CHCl_3$ c, 0.02) λ_{max}: 242 (4.12), 260 (3.32) and 280 (3.60) nm; IR ν_{max} (KBr): 3414 (NH), 3316 (OH), 2911, 1653, 1524, 1453, 1238, 1167, 1124 cm^{-1}; ^1H NMR ($CDCl_3$) ppm: 7.15 (2H, d, *J* = 8.4 Hz, H-3′ and H-7′), 7.00 (1H, d, *J* = 8.6 Hz, H-8), 6.90 (1H, s, NH), 6.86 (1H, d, *J* = 2.4 Hz, H-5), 6.82 (2H, d, *J* = 8.4 Hz, H-4′ and H-6′), 6.67 (1H, dd, *J* = 8.6, 2.4 Hz, H-7), 5.82 (1H, dd, *J* = 9.6, 4.3 Hz, H-1), 4.07 (1H, dd, *J* = 13.7, 4.1 Hz, H-3a), 3.38 (1H, dd, *J* = 13.0, 4.6 Hz, H-3b), 3.14 (1H, dd, *J* = 13.0, 4.6 Hz, H-1′a), 2.93 (1H, dd, *J* = 12.9, 9.8 Hz, H-1′b), 2.76 (2H, m, H-4), 2.35 (1H, t, *J* = 7.5 Hz, H-2″), 1.67 (2H, m, H-3″), 1.25 (40H, m, H''_4-H''_{23}), 0.88 (3H, t, *J* = 6.7 Hz, end CH_3); ^{13}C NMR ($CDCl_3$) ppm: 177.1 (C-1″), 154.9 (C-5′), 149.5 (C-6), 135.2 (C-9a), 131.1 (C-8a), 131.0 (C-3′ and C-7′), 129.8 (C-2′). 127.1 (C-4b), 115.7 (C-4′ and C-6′), 111.6 (C-8), 111.3 (C-7), 107.7 (C-4a), 102.9 (C-5), 51.1 (C-1), 41.0 (C-3), 39.3 (C-1′), 34.1 (C-2″), 29.9–32.1 (C″4-C″23), 24.9 (C-3″), 22.9 (C-4), 14.1 (C-24″); ESI-MS (positive): *m/z* 646 $[M + H]^+$ (100), 354 $[M-C_{18}H_{18}N_2O_2]^+$ (20), 337 $[M-C_{22}H_{46}]^+$ (5); HR-ESIMS: *m/z* 667.4917 $[M + Na]^+$ (calcd. $C_{42}H_{64}N_2O_3Na$: 667.4920).

3.4. Cell culture and cytotoxicity tests

Human cervical adenocarcinoma (HeLa), human hepatocarcinoma (Hep3B) and human breast carcinoma (MCF-7) cells were obtained from American Type Culture Collection (Manassas, VA). HeLa and Hep3B cells were cultured in Dulbecco's modified essential medium, and MCF-7 cells were maintained in a RPMI-1640 medium in a humidity of 5% CO_2 atmosphere at 37 °C. All media were supplemented with penicillin (100 ug/mL), streptomycin (100 μg/mL) and 10% heat-inactivated foetal bovine serum (FBS). Cytotoxicity assays were carried out according to Alley et al. (1988) method. The cancer cells seeded in 96-well plates at concentration of 1×10^4 cells/well were treated with various concentrations of test compounds and incubated in a humidity of 5% CO_2 atmosphere at 37 °C. After 72-h incubation, 10 μL of 5 mg/mL MTT was added to each well and incubated for another 4 h. After removal of the supernatant, formazan crystals were dissolved in 100 μL DMSO and the absorbance measured at 570 nm using a microplate reader. Doxorubicin was used as a positive control and the IC_{50} value cancer cells against the 10 compounds (**1–10**) were obtained using the Log probit analysis.

3.5. In vitro *antibacterial activity*

The compounds were evaluated for their *in vitro* antibacterial activities against *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *S. epidermis* ATCC 25924) and *S. aureus* (ATCC 25923) according to the procedure of National Committee for Clinical Laboratory Standards (NCCLS) microdilution assay format (Murray et al. 1999). The test compounds were dissolved in 5% DMSO at an initial concentration of 1 mg/mL and serially diluted in plate to provide decreasing concentrations. Maximum tested concentration was 500 μg/mL while the minimum was 3.9 μg/mL. Each well was then inoculated with 2–5 × 10^5 bacterial cells and incubated at 37 °C for 24 h. One well containing the micro-organisms and 5% DMSO without test compounds was for control while the other containing only growth medium was used for sterility control. Tetracycline was used as a positive control. The MIC was evaluated as the lowest concentration of the test substances that inhibited the growth of the bacteria strains.

4. Conclusion

The results of the study showed that compound **1** exhibited both cytotoxicity and antibacterial activities.

Acknowledgments

This work was supported by the National Commission for Science, Technology and Innovation (NACOSTI) and Germany Academic Exchange Services (DAAD). The authors are grateful to Mr. Mutiso (Taxonomist) of Botany department, University of Nairobi for identification and collection of the plant materials and Mr. James Odhiambo of the School of Public Health, Maseno University for conducting the bioassay experiments. The Institute of Organic Chemistry, Johannes Gutenberg University at Mainz, Germany is acknowledged for the spectroscopic data.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the National Commission for Science, Technology and Innovation (NACOSTI) and Germany Academic Exchange Services (DAAD).

References

Ahmed AA, Mahmoud AA, Ali ET, Tzakou O, Couladis M, Mabry TJ, Gáti T, Tóth G. 2002. Two highly oxygenated eudesmans and 10 lignans from *Achillea holosericea*. Phytochemistry. 59:851–856.

Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, Abott BJ, Mayo JG, Shoemaker RH, Boyd MR. 1988. Feasibility of drug screening with panels of human tumor cells using a microculture tetrazolium assay. Cancer Res. 48:589–601.

Al-Taweel AM, Perveen S, El-Shafae AM, Fawzy GA, Malik A, Afza N, Iqbal L, Latif M. 2012. Bioactive phenolic amides from *Celtis africana*. Molecules. 17:2675–2682.

Brazdovlcova B, Kostalova D, Tomko J, Jin HY. 1980. Isolation and identification of alkaloids from fruits of *Berberis thunbergii DC*. Chem Zvesti. 34:259–262.

Camacho RM, Phillipson JD, Croft SL, Rock P, Schiff PL Jr. 2002. *In vitro* activity of *Triclisia patens* and some bibenzylisoquinoline alkaloids against *Leishmania donovani* and *Trypanosoma brucei*. Phytother Res. 16:602–611.

Cao R, Peng W, Wang Z, Xu A. 2007. Carboline alkaloids: biochemical and pharmacological functions. Curr Med Chem. 14:479–500.

Gohari AR, Saeidnia S, Bayati-Moghadam M, Amin Gh. 2011. Lignans and neolignans from stelleropsis antoninae. DARU. 19:74–79.

Houghton PJ. 1985. Lignans and neolignans from *Buddleja davidii*. Phytochemistry. 24:819–826.

Jacques FMB, De Franceschi D. 2007. Menispermaceae wood anatomy and cambial variants. Iawa J. 28:139–172.

Jain P. 2016. Secondary metabolites for antiulcer activity. Nat Prod Res. 30:640–656.

Kokwaro JO. 2009. Medicinal plants of East Africa. Nairobi: University of Nairobi Press; p. 276.

Koskinen AMP, Lood C, Laine AE. 2014. Pharmacological importance of optically active tetrahydro-β-carbolines and synthetic approaches to create the C1 stereocenter. Molecules. 19:1544–1567.

Liang M, Liang R, Huang L-R, Hsu P-H, Wu Y-H. 2012. Separation of Sesamin and Sesamolin by a Supercritical Fluid-Simulated Moving Bed. American Journal of Analytical Chemistry. 3:931–938.
Manguro LOA, Ugi I, Lemmen P, Hermann R. 2003. Flavonol glycosides of *Warburgia ugandensis* leaves. Phytochemistry. 64:891–896.
Manguro LOA, Ugi I, Lemmen P. 2004. Further flovonol glycosides of *Embelia schimperi* leaves. Bull Chem Soc Ethiop. 18:51–57.
Manguro LOA, Ugi I, Lemmen P. 2005. Flavonol glycosides from the leaves of *Embelia keniensis*. J Chinese Chem Soc. 52:201–208.
Murebwayire S, Diallo B, Luhmer M, Vanhaelen-Fastré R, Vanhaelen M, Duez P. 2006. Alkaloids and amides from *Triclisia sacleuxii*. Fitoterapia. 77:615–617.
Murebwayire S, Frédérich M, Hannaert V, Jonville MC, Duez P. 2008. Antiplasmodial and antitrypanosomal activity of *Triclisia sacleuxii* (Pierre) Diels. Phytomedicine. 15:728–733.
Murebwayire S, Ingkaninan K, Changwijit K, Frédérich M, Duez P. 2009. *Triclisia sacleuxii* (Pierre) Diels (Menispermaceae), a potential source of acetylcholinesterase inhibitors. J Pharm Pharmacol. 61:103–107.
Murray PR, Baron EJ, Pfaller MA, Tenover FC, Myolken RH. 1999. *National committee for clinical laboratory standards antibacterial susceptibility tests: dilution and disk diffusion methods manual of clinical microbiology*. Washington, DC. American Society for Microbiology, p. 1526–1543.
Ngoc PB, Pham TB, Nguyen HD, Tran TT, Chu HH, Chau VM, Lee J, Nguyen TD. 2016. A new anti-inflammatory β-carboline alkaloid from the hairy-root cultures of *Eurycoma longifolia*. Nat Prod Res 30:1360–1365.
Pollard BJ. 2008. *Triclisia dictyophylla* Diels. In: Schmelzer GH, Gurib-Fakim A, editors. Prota 11(1): medicinal plants/plantes medicinales 1[CD-Rom]. Wageningen: PROTA; p. 45.
Rodríguez-Sánchez S, Ruiz-Matute A, Alañón M, Pérez-Coello MS, de Julio-Torres LF, de Julio-Torres R, Martinez-Castro I. 2010. Analysis of cyclitols in different Quercus species by gas chromatography-mass spectrometry. J Sci Food Agric. 90:1735–1738.
Rubens L, Netoa M, Jose M, Filhoa B, Sousab LMA, Filhoa PFA, Diasa CS, Oliveiraa MR. 2007. Crude ethanolic extract, lignoid and yangambin from *Ocotea duckei* (Lauraceae) show antileishmanial activity. Z Naturforsch 62c: 1173–1176.
Salmoun M, Devijver C, Daloze D, Braekman JC. 2002. 5-Hydroxytryptamine-derived alkaloids from two marine sponges of the genus *Hyrtios*. J Nat Prod. 65:1173–1176.
Tian M, Zhu T, Park H, Row K. 2012. Purification of 4-hydroxybenzoic acid and 4-hydroxybenzaldehyde from *Laminara japonica* Aresch using commercial and monolithic sorbent in spe cartridge. Analyt Lett. 45:2359–2366.
Wright JLC, McInnes AG, Shimizu S, Smith DG, Walter JA. 1978. Identification of C-24 alkyl epimers of marine sterols by ^{13}C nuclear magnetic resonance spectroscopy. Can J Chem. 56:1898–1903.
Wu FE, Koike K, Nikaido T, Sakamoto Y, Ohmoto T, Ikeda K. 1989. New beta-carboline alkaloids from a Chinese medicinal plant, *Arenaria kansuensis*. Structures of arenarines A, B, C and D. Chem Pharm Bull. 37:1808–1809.
Yang H, Luo Y, Zhao H, Wu J, Chen Y. 2016. Isolation of a new carboline alkaloid from *Trigonostemon lii*. Nat Prod Res. 30:42–45.
Zhang Y, Wang G, Lv, H, Luo J. 2015. Two new β-carboline alkaloids from the roots of *Gypsophila oldhamiana*. Nat Prod Res. 29:1207–1211.