Cytotoxic alkaloids from the root of *Zanthoxylum paracanthum* (mildbr) Kokwaro

Leonidah Kerubo Omosa, Vaderament-A Nchiozem-Ngnitedem, Justus Mukavi, Brenda Atieno Okoko, Helder Ombui Nyaboke, Ibrahim Hashim, Jackson Obegi Matundura, Thomas Efferth & Michael Spiteller

View supplementary material

Published online: 08 May 2021.

Submit your article to this journal

Article views: 245

View related articles

View Crossmark data

Citing articles: 4 View citing articles

Taylor & Francis
Taylor & Francis Group

Check for updates

Cytotoxic alkaloids from the root of *Zanthoxylum paracanthum* (mildbr) Kokwaro

Leonidah Kerubo Omosa[a] (iD), Vaderament-A Nchiozem-Ngnitedem[a,b] (iD), Justus Mukavi[c] (iD), Brenda Atieno Okoko[a], Helder Ombui Nyaboke[a], Ibrahim Hashim[a,d] (iD), Jackson Obegi Matundura[a], Thomas Efferth[e] and Michael Spiteller[b]

[a]Department of Chemistry, University of Nairobi, Nairobi, Kenya; [b]Institute of Environmental Research (INFU), Department of Chemistry and Chemical Biology, Chair of Environmental Chemistry and Analytical Chemistry, TU Dortmund, Dortmund, Germany; [c]School of Pure and Applied Sciences, University of Embu, Embu, Kenya; [d]Department of Chemistry, Federal University Lafia, Nasarawa State, Nigeria; [e]Department of Pharmaceutical Biology, Institute of Pharmaceutical and Biomedical Sciences, Johannes Gutenberg University, Mainz, Germany

ABSTRACT

Chemical investigation of the root of *Zanthoxylum paracanthum* afforded 1 new alkamide derivative, (2*E*,4*E*)-6-oxo-*N*-isobutyldeca-2,4-dienamide (**1**) together with 10 known congeners including one phenolic amide (**2**), four benzophenanthridines (**3 − 6**), three indolonaphthyridines (**7 − 9**) and two lignans (**10** and **11**). Their structures were elucidated by a combination of spectroscopic and spectrometric data. Using resazurin reduction assay, the crude extract (10 μg/mL) and isolates (10 μM) were screened for their cytotoxic activities against the drug-sensitive (CCRF-CEM) leukemia cell line and its multidrug-resistant counterpart (CEM/ADR5000). Compounds **3**, **4** and **6** showed cytotoxicity against CCRF-CEM with IC_{50} values of 2.00 ± 0.33, 2.31 ± 0.20 and 0.11 ± 0.04 μM, respectively. Only compound **6** exhibited strong cytotoxic activity against CEM/ADR5000 with an IC_{50} value of 2.34 ± 0.34 μM in comparison with the standard drug doxorubicin which showed IC_{50} values of 0.01 ± 0.14 (CCRF-CEM) and 26.78 ± 3.30 μM (CEM/ADR5000).

Zanthoxylum paracanthum
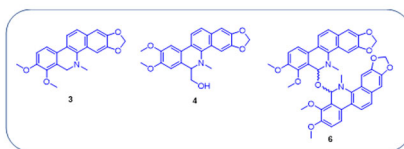
Table S3: IC_{50} (μM) values for cytotoxicity of benzophenanthridines alkaloids

Compounds	CCRF-CEM	CEM/ADR5000	[a]Degree of resistance
3	2.00 ± 0.33	55.56 ± 0.69	27.78
4	2.31 ± 0.20	35.00 ± 2.35	15.15
6	0.11 ± 0.04	2.34 ± 0.39	21.27
Doxorubicin	0.01 ± 0.14	26.78 ± 3.30	2.67

CONTACT Vaderament-A Nchiozem-Ngnitedem ✉ n.vaderamentalexe@gmail.com

Supplemental data for this article can be accessed online at https://doi.org/10.1080/14786419.2021.1913586.

1. Introduction

Zanthoxylum paracanthum (mildbr.) Kokwaro belongs to the family Rutaceae, which consists of over 1730 species distributed globally (Dalitz et al. 2011). The genus *Zanthoxylum* comprises of about 225 species (Appelhans et al. 2018), distributed worldwide in the tropics and temperate regions and Kenya is gifted with 7 species (Beentje 1994). Although, *Z. paracanthum* is rarely used in Kenyan folk medicine, a number of plants belonging to the genus *Zanthoxylum* are used to alleviate coughs, snake bites, chest pain, fever, toothache, malaria, edema, pneumonia, dizziness and asthma (Nguta et al. 2010; Nyaboke et al. 2018; Omosa et al. 2019). Previous phyto-chemical studies of *Zanthoxylum* plants have reported alkaloids as the main secondary metabolites (Rodríguez-Guzmán et al. 2011; Omosa and Okemwa 2017). For instance, phytochemical investigation of *Z. paracanthum* stem bark afforded aporphines type alkaloids (Samita et al. 2013). Recently, Kaigongi and collaborators investigated the root bark of *Z. paracanthum*, this led to the isolation of isoquinolines represented by benzophenanthridines skeleton (Kaigongi et al. 2020). Other minor phytoconstituents include coumarins, lignans, amides, terpenoids and quinolones (Chen et al. 1999; Omosa et al. 2019; Yang et al. 2009a). Diverse biological activities namely anti-viral, antioxidant, anti-fungal, larvicidal, analgesics, antibiotic, anti-inflammatory, cytotoxic and anti-plasmodial have been reported from the genus (Nyaboke et al. 2018; Omosa et al. 2019; Yang et al. 2009b; Ochieng et al. 2020).

As part of our ongoing search for bioactive compounds from Kenyan medicinal plants (Mukavi et al. 2020; Nchiozem-Ngnitedem et al. 2020b), we reported herein, iso-lation, structure elucidation and cytotoxicity of one new alkamide (**1**) along with ten known congeners (**2 − 11**) from the root extracts of *Z. paracanthum*.

2. Results and discussion

The MeOH/CH$_2$Cl$_2$ crude extract from the root of *Z. paracanthum* yielded 11 organic compounds, out of which 1 is reported here for the first time. The known compounds were identified as *N-p*-coumaroyltyramine (**2**) (Zhang et al. 2020), dihydrochelerythrine (**3**) (Omosa et al. 2019), 6-hydroxymethyldihydronitidine (**4**) (Khalid and Waterman 1985), arnottianamide (**5**) (Yang et al. 2009a), *bis*-[6-(5,6-dihydrochelerythrinyl)] ether (**6**) (Rodríguez-Guzmán et al. 2011), canthin-6-one (**7**) (Makong et al. 2019, Omosa et al. 2019), canthin-6-one-3-*N*-oxide (**8**) (Devkota et al. 2014), 9-methoxycanthin-6-one (**9**) (Ngoc et al. 2016), sesamin (**10**) (Lin et al. 2018) and piperitol (**11**) (Takaku et al. 2001) (Figure 1).

Compound **1** was purified as white amorphous solid. The HRESIMS (Figure S2, Supporting Information) observed at *m/z* 238.1803 [M + H]$^+$ (calcd, 238.1762) was con-sistent with a molecular formula C$_{14}$H$_{23}$NO$_2$, four unsaturation sites. The UV λmax (274 nm), IR (1650 and 1549 cm^{-1}) and NMR (Table S1, Supporting Information) spectra displayed characteristic bands of amidoamino and amidocarbonyl substituents, indicat-ing the occurrence of a *trans-2-trans*-4-dienamide moiety (Chen et al. 1999).

The large coupling constants observed in the ^{1}H NMR (Table S1 and Figures S5–S7, Supporting Information) spectrum of **1** at δ$_H$ 6.46 (1H, *d*, *J* = 14.1 Hz, H-2), 7.24 (1H, *dd*, *J* = 14.1, 11.3 Hz, H-3), 7.30 (1H, *dd*, *J* = 15.0, 11.3 Hz, H-4) and 6.53 (1H, *d*, *J* = 15.0 Hz,
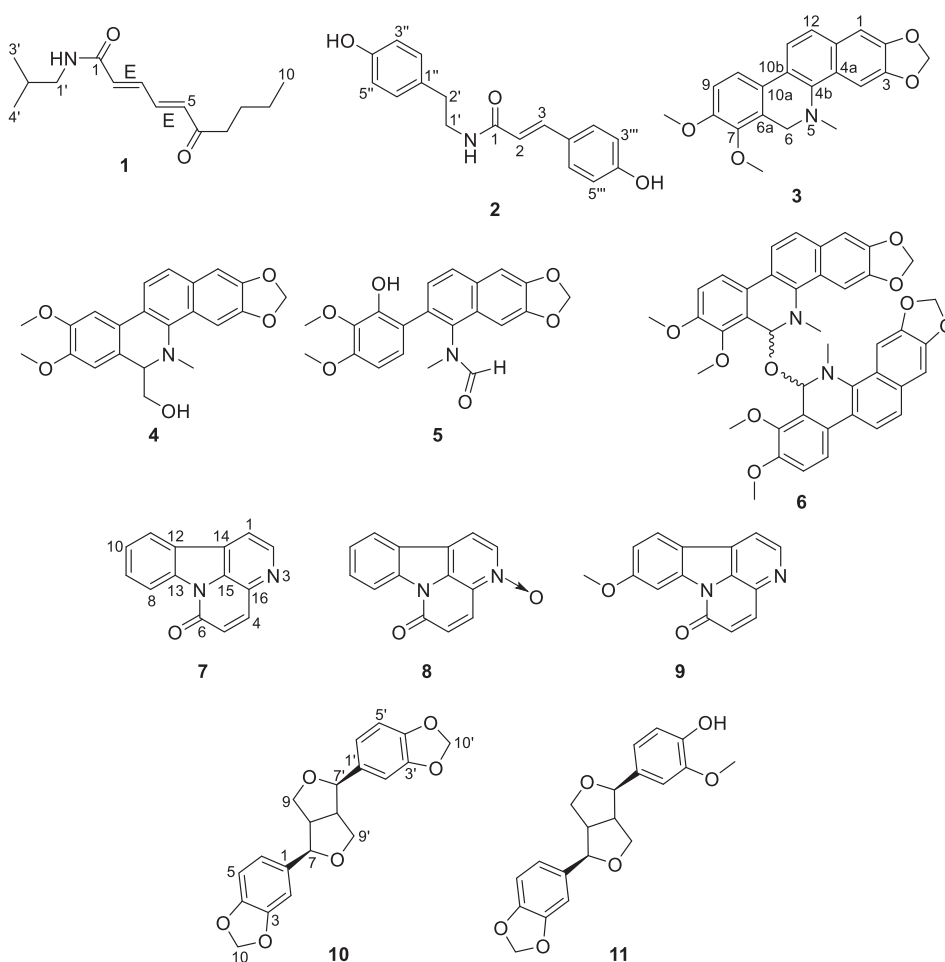
Figure 1. Structure of compounds isolated from the root of *Z. paracanthum*.

H-5) were also in good agreement with the *trans-2-trans-4*-dienamide moiety. Further, signals depicted at δ_H 3.12 (2H, *d*, *J* = 6.9 Hz, H-1′), 1.84 (1H, *m*, H-2′) and 0.95 (6H, *d*, *J* = 6.7 Hz, H-3′/4′) were characteristic of *N*-isobutyl substituent. Besides the ^{1}H NMR data from the *n*-butyl chain, the fragment was perfectly visualised through successive correlations shown in the COSY spectrum. The *n*-butyl chain was evidenced by the signals observed at δ_H 2.68 (2H, *t*, *J* = 7.4 Hz, H-7), 1.61 (2H, *p*, *J* = 7.4 Hz, H- 8), 1.38 (2H, *m*, H-9) and 0.96 (3H, *t*, *J* = 6.8 Hz, H-10). Inspection of the ^{13}C NMR (Table S1 and Figure S8, Supporting Information) spectrum indicated a total number of 14 carbons, including two carbonyl groups both of which were α, β, γ, δ-unsaturated with resonances at δ_C 202.8 (C-6) and 167.4 (C-1) attributable to a ketone and an amide group, respectively. An HMBC (Table S1 and Figure S11, Supporting Information) correlation observed between the signal at δ_H 2.68 (H-7) with a carbonyl group at δ_C 202.8 allowed the placement of the alkyl chain at C-6. The position of the alkyl chain was further confirmed from HMBC correlations between the signal at δ_H 6.53 (H-5) with carbons at δ_C 202.8 (C-6) and 41.5 (C-7). The *N*-isobutyl substituent was placed at C-1 on the basis of the HMBC correlation between signal at δ_H 3.12 (H-1′) with the

carbonyl resonance at δ_C 167.4 (C-1). Based on these spectroscopic and spectrometric data, and comparison with literature values compound **1** was unambiguously characterised as (2*E*,4*E*)-6-oxo-*N*-isobutyldeca-2,4-dienamide.

Preliminary bioactive potencies of the root extract (10 μg/mL) and isolated compounds (10 μM) from *Z. paracanthum* were screened against the drug-sensitive CCRF-CEM leukemia cell line using resazurin reduction assay. As shown in Table S2 (Supporting Information), only compounds **3**, **4** and **6** were active as they exhibited cell inhibition of > 70% (Nyaboke et al. 2018). Based on the preliminary screening results, the crude extract and the active compounds (**3**, **4** and **6**) were further screened to determine their half maximal inhibitory concentration (IC$_{50}$) values on CCRF-CEM and CEM/ADR5000 cancer cell lines (Table S3, Supporting Information). The crude extract showed low cytotoxic effect against CCRF-CEM (IC$_{50}$ = 51.11 ± 14.22 μM) and CEM/ADR5000 (IC$_{50}$ = 66.46 ± 8.15 μM) (Kuete and Efferth 2015). For the active compounds, strong activity towards CCRF-CEM was observed for compounds **3** (IC$_{50}$ = 2.00 ± 0.33 μM) and **4** (IC$_{50}$ = 2.31 ± 0.20 μM), while low and moderate activities towards CEM/ADR5000 for compounds **3** (IC$_{50}$ = 55.56 ± 0.69 μM) and **4** (IC$_{50}$ = 35.00 ± 2.35 μM), respectively (Kuete and Efferth 2015). Excellent activity was observed for compound **6** against CCRF-CEM (IC$_{50}$ = 0.11 ± 0.04 μM) and CEM/ADR5000 (IC$_{50}$ = 2.34 ± 0.39 μM). This finding concurs with previous studies which have reported increased potency and selectivity of dimeric natural products owing to their ability to bind two distinct individual binding sites on a single receptor (Hadden and Blagg 2008). In conclusion, the results of the present study for **3**, **4** and **6** corroborates the previous research findings for related benzophenanthridines which have been shown to exhibit anti-cancer properties against several cancer cell lines (Vrba et al. 2008; Deng& Qin 2010; Qing et al. 2018).

3. Experimental

3.1. General experimental procedures

The general experimental procedures were conducted according to our previous described method (Mukavi et al. 2020; Nchiozem-Ngnitedem et al. 2020a, 2021).

3.2. Plant material

The root of *Z. paracanthum* was collected in March 2018 GPS (S 04°29′17.4′′E 039°15′19.8′′136 m) at Mrima forest in the coastal region of Kenya. The plant material was identified by a botanist at the School of Biological Sciences (SBS), University of Nairobi under the voucher specimen NNA 2018/008.

3.3. Extraction and isolation

The root of *Z. paracanthum* was air-dried and ground into fine powder (3.5 Kg) and exhaustively extracted with CH$_3$OH/CH$_2$Cl$_2$ (1:1) (3 × 9 L, 24 h each) at room temperature. The extracts were combined and concentrated under reduced pressure using a rotary evaporator to give 160 g of crude extract. The extract was subjected to silica gel

column chromatography eluting with cyclohexane- EtOAc (from 10:0, 8:2, 7:3, 1:1 and 0:10) followed by EtOAc-MeOH (from 10:0, 8.5:1.5 and 0:10) resulting in 70 fractions of 500 mL each which were further combined based on their TLC and LC-MS similarities into 7 main subfractions (Fr$_{A-G}$).

Compound **3** (90.0 mg) crystalised from Fr$_A$ (cyclohexane-EtOAc (10:0)). Fr$_B$ (cyclohexane-EtOAc (8:2)) was further chromatographed on a silica gel column eluting with a gradient of cyclohexane-EtOAc (from 10:0 to 0:10) to give compound **10** (500 mg). Fr$_C$ (cyclohexane-EtOAc (7:3) was separated on a silica gel column chromatography and isocratically eluted with a ternary system of cyclohexane-EtOAc-MeOH (6.5:3:0.5) to give three subfractions Fr$_{C1-3}$. These subfractions (Fr$_{C1-3}$) were further purified into semi-prep HPLC using an isocratic mixture of MeOH-H$_2$O (1:1) (0.1% HCOOH, flow rate, 4 mL/min) for 20 min to yield compounds **1** (3.0 mg), **4** (5.7 mg) and **11** (22.0 mg), respectively. Fr$_D$ (cyclohexane-EtOAc (1:1)) was further purified using column chromatography (CC) in silica gel eluting with cyclohexane-EtOAc-MeOH (6.5:3:0.5) to afford compounds **5** (11.0 mg) and **7** (435.0 mg). Fr$_E$ (cyclohexane-EtOAc (0:10)) was purified by semi-prep HPLC using an isocratic mixture of MeOH-H$_2$O (1:1) (0.1% HCOOH, flow rate, 4 mL/min) for 25 min to give compound **9** (1.7 mg). Fr$_F$ (cyclohexane-EtOAc (8.5:1.5)) was loaded onto a silica gel column and eluted with cyclohexane-EtOAc-MeOH (6.5:3:0.5) to obtain 34 fractions of 100 mL each which were pooled based on their TLC and LC-MS profiles into 4 subfractions (Fr$_{1-4}$). Subfractions Fr$_{1 \text{ and } 2}$ were further purified by semi-prep HPLC using an isocratic mixture of MeOH-H$_2$O (1:1) (0.1% HCOOH, flow rate, 4 mL/min) for 25 min to yield compounds **2** (16.0 mg) and **8** (4.1 mg), respectively. Further purification of subfraction Fr$_4$ on silica gel CC as solid matrix eluting with cyclohexane-EtOAc-MeOH (6.5:3:0.5) afforded compound **6** (17.0 mg).

(2*E*,4*E*)-6-oxo-*N*-isobutyldeca-2,4-dienamide (**1**). White amorphous solid, LC-UV [MeOH-H$_2$O (0.1% formic acid)] λ_{max} 274 nm; IR (neat)ν_{max} 3316, 2957, 2929, 1650, 1549, 1249, 1060 cm^{-1}; ^{1}H NMR (CD$_3$OD, 600 MHz) and ^{13}C NMR (CD$_3$OD, 150 MHz) data, see Table S1; HRESIMS *m/z* 238.1803 [M + H]$^+$ (calcd for [M + H]$^+$ C$_{14}$H$_{24}$NO$_2$: 238.1762).

3.4. Cytotoxicity assays

The experiments were carried out in accordance to protocols published in our recent studies (Nyaboke et al., 2018). Resazurin reduction assay (O'Brien et al., 2000) was carried to evaluate the cytotoxic potency of the crude extract and the isolated compounds against drug-sensitive CCRF-CEM and multi-drug resistant CEM/ADR5000 leukemia cells. The assay is based on reduction of the oxidised non-fluorescent blue dye, resazurin, to the pink highly fluorescent resorufin by metabolically viable cells. Non-viable cells quickly loose the metabolic ability to reduce resazurin and, therefore, produce no fluorescent signal.

4. Conclusion

Phytochemical study of the root of *Z. paracanthum* led to the isolation of eleven secondary metabolites among which (2*E*,4*E*)-6-oxo-*N*-isobutyldeca-2,4-dienamide (**1**) is

new. Using resazurin reduction assay, the cytotoxicity of the isolated compounds was evaluated. Excellent activity was observed for compound **6** against both drug-sensitive (CCRF-CEM) and its multidrug-resistant counterpart (CEM/ADR5000) leukemia cell lines. While compounds **3** and **4** displayed strong to moderate activities against both cancer cell lines.

Acknowledgements

Disclosure statement

The authors declare no conflict of interest.

Funding

ORCID

Leonidah Kerubo Omosa http://orcid.org/0000-0002-5821-8307
Vaderament-A Nchiozem-Ngnitedem http://orcid.org/0000-0001-8337-9260
Justus Mukavi http://orcid.org/0000-0001-9638-3250
Ibrahim Hashim http://orcid.org/0000-0002-0900-3334

References

Appelhans MS, Reichelt N, Groppo M, Paetzold C, Wen J. 2018. Phylogeny and biogeography of the pantropical genus *Zanthoxylum* and its closest relatives in the proto-Rutaceae group (Rutaceae). Mol Phylogenet Evol. 126:31–44.

Beentje HJ. 1994. Kenya trees, shrubs and lianas. 6th ed. Nairobi Kenya: National Museums of Kenya; p. 1–722.

Chen IS, Chen TL, Lin WY, Tsai IL, Chen YC. 1999. Isobutylamides from the fruit of *Zanthoxylum integrifoliolum*. Phytochemistry. 52(2):357–360.

Dalitz C, Dalitz H, Musila W, Masinde S. 2011. Illustrated field guide to the common woody plants of Kakamega forest. Inst Für Landschafts- Und Pflanzenökologie. 24:1–615.

Deng A-J, Qin HL. 2010. Cytotoxic dihydrobenzophenanthridine alkaloids from the roots of *Macleaya microcarpa*. Phytochemistry. 71(7):816–822.

Devkota KP, Wilson JA, Henrich CJ, McMahon JB, Reilly KM, Beutler JA. 2014. Compounds from *Simarouba berteroana* which inhibit proliferation of NF1-defective cancer cells. Phytochem Lett. 7:42–45.

Hadden M, Blagg B. 2008. Dimeric approaches to anti-cancer chemotherapeutics. Anticancer Agents Med Chem. 8(7):807–816.

Kaigongi MM, Lukhoba CW, Yaouba S, Makunga NP, Githiomi J, Yenesew A. 2020. *In vitro* anti-microbial and antiproliferative activities of the root bark extract and isolated chemical constituents of *Zanthoxylum paracanthum* kokwaro (Rutaceae). Plants. 9(7):920.

Khalid SA, Waterman PG. 1985. 6-Hydroxymethyldihydronitidine from *Fagaropsis angolensis*. J Nat Prod. 48(1):118–119.

Kuete V, Efferth T. 2015. African flora has the potential to fight multidrug resistance of cancer. Biomed Res Int. 2015:914813.

Lin CL, Kao CL, Li WJ, Li HT, Chen CY. 2018. Secondary metabolites from the stems of *Cinnamomum kanehirai*. Chem Nat Compd. 54(4):762–763.

Makong YS, Fotso GW, Mouthe GH, Lenta B, Rennert R, Sewald N, Arnold N, Wansi JD, Ngadjui BT. 2019. Bruceadysentoside A, a new pregnane glycoside and others secondary metabolites with cytotoxic activity from *brucea antidysenterica* J. F. Mill. (simaroubaceae). Nat Prod Res. :1–7.

Mukavi J, Omosa LK, Nchiozem-Ngnitedem VA, Nyaga J, Omole R, Bitchagno GTM, Spiteller M. 2020. Anti-inflammatory norhopanes from the root bark of *Fagaropsis angolensis* (Engl.) H.M.Gardner. Fitoterapia. 146:104690.

Nchiozem-Ngnitedem VA, Omosa LK, Bedane KG, Derese S, Brieger L, Strohmann C, Spiteller M. 2020a. Anti-inflammatory steroidal sapogenins and a conjugated chalcone-stilbene from *Dracaena usambarensis* Engl. Fitoterapia. 146:104717.

Nchiozem-Ngnitedem VA, Omosa LK, Bedane KG, Derese S, Spiteller M. 2021. Inhibition of proinflammatory cytokine release by flavones and flavanones from the leaves of *Dracaena steudneri* Engl. Planta Med. 87(3):209–217.

Nchiozem-Ngnitedem VA, Omosa LK, Derese S, Tane P, Heydenreich M, Spiteller M, Seo EJ, Efferth T. 2020b. Two new flavonoids from *Dracaena usambarensis* Engl. Phytochem Lett. 36: 80–85.

Ngoc PB, Pham TB, Nguyen HD, Tran TT, Chu HH, Chau VM, Lee JH, Nguyen TD. 2016. A new anti-inflammatory β-carboline alkaloid from the hairy-root cultures of *Eurycoma longifolia*. Nat Prod Res. 30(12):1360–1365.

Nguta JM, Mbaria JM, Gakuya DW, Gathumbi PK, Kiama SG. 2010. Antimalarial herbal remedies of Msambweni, Kenya. J Ethnopharmacol. 128(2):424–432.

Nyaboke OH, Moraa M, Omosa KL, Mbaveng AT, Nchiozem-Nchiozem V-A, Masila V, Okemwa E, Heydenreich M, Efferth T, Kuete V. 2018. Cytotoxicity of lupeol from the stem bark of *Zanthoxylum gilletii* against multi-factorial drug resistant cancer cell lines. Invest. Med. Chem. Pharmacol. 1(1):1–6.

O'brien J, Wilson I, Orton T, Pognan F. 2000. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. Eur. J. Biochem. 267(17): 5421–5426.

Ochieng CO, Nyongesa DW, Yamo KO, Onyango JO, Langat MK, Manguro LAO. 2020. α-Amylase and α-glucosidase inhibitors from *Zanthoxylum chalybeum* Engl. root bark. Fitoterapia. 146: 104719.

Omosa LK, Mbogo GM, Korir E, Omole R, Seo EJ, Yenesew A, Heydenreich M, Midiwo JO, Efferth T. 2019. Cytotoxicity of fagaramide derivative and canthin-6-one from *Zanthoxylum* (Rutaceae) species against multidrug resistant leukemia cells. Nat Prod Res. 35(4):1–8.

Omosa LK, Okemwa EK. 2017. Antiplasmodial activities of the stem bark extract and compounds of *Zanthoxylum gilletii* (De wild). PC. 7(1):41–46.

Qing Z-X, Huang J-L, Yang X-Y, Liu J-H, Cao H-L, Xiang F, Cheng P, Zeng J-G. 2018. Anticancer and reversing multidrug resistance activities of natural isoquinoline alkaloids and their structure-activity relationship. Curr Med Chem. 25(38):5088–5114.

Rodríguez-Guzmán R, Johansmann FLC, Radwan MM, Burandt CL, Ross SA. 2011. Chemical constituents, antimicrobial and antimalarial activities of *Zanthoxylum monophyllum*. Planta Med. 77(13):1542–1544.

Samita FN, Sandjo LP, Ndiege IO, Hassanali A, Lwande W. 2013. Zanthoxoaporphines A-C: Three new larvicidal dibenzo[de,g]quinolin-7-one alkaloids from *Zanthoxylum paracanthum* (Rutaceae). Beilstein J Org Chem. 9:447–452.

Takaku N, Choi DH, Mikame K, Okunishi T, Suzuki S, Ohashi H, Umezawa T, Shimada M. 2001. Lignans of *Chamaecyparis obtusa*. J Wood Sci. 47(6):476–482.

Vrba J, Doležel P, Vičar J, Modrianský M, Ulrichová J. 2008. Chelerythrine and dihydrochelerythrine induce G1 phase arrest and bimodal cell death in human leukemia HL-60 cells. Toxicol in Vitro. 22(4):1008–1017.

Yang C, Cheng M, Lee S, Cheng-Wei Y. 2009a. Secondary metabolites and cytotoxic activities from the stem bark of *Zanthoxylum nitidum* Molucca Islands, New Guinea, South China, Ryukyus, and at low altitudes throughout. Helv Chim Acta. 6:846–857.

Yang CL, Chik SC, Li JC, Cheung BK, Lau AS. 2009b. Identification of the bioactive constituent and its mechanisms of action in mediating the anti-inflammatory effects of Black Cohosh and related *Cimicifuga* species on human primary blood macrophages. J Med Chem. 52(21): 6707–6715.

Zhang Y, Liu J, Wang M, Sun C, Li X. 2020. Five new compounds from *Hosta plantaginea* flowers and their anti-inflammatory activities. Bioorg Chem. 95:103494.