Natural Product Research

Formerly Natural Product Letters

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

Cytotoxicity of fagaramide derivative and canthin-6-one from *Zanthoxylum* (Rutaceae) species against multidrug resistant leukemia cells

Leonidah Kerubo Omosa, Gacheru Martin Mbogo, Eric Korir, Ruth Omole, Ean-Jeong Seo, Abiy Yenesew, Matthias Heydenreich, Jacob Ogweno Midiwo & Thomas Efferth

To cite this article: Leonidah Kerubo Omosa, Gacheru Martin Mbogo, Eric Korir, Ruth Omole, Ean-Jeong Seo, Abiy Yenesew, Matthias Heydenreich, Jacob Ogweno Midiwo & Thomas Efferth (2019): Cytotoxicity of fagaramide derivative and canthin-6-one from *Zanthoxylum* (Rutaceae) species against multidrug resistant leukemia cells, Natural Product Research, DOI: 10.1080/14786419.2019.1587424

To link to this article: https://doi.org/10.1080/14786419.2019.1587424

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2019.1587424

Taylor & Francis
Taylor & Francis Group

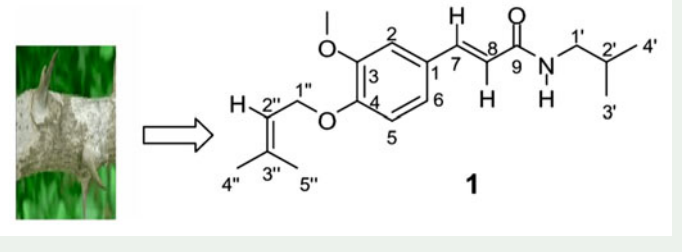
Cytotoxicity of fagaramide derivative and canthin-6-one from *Zanthoxylum* (Rutaceae) species against multidrug resistant leukemia cells

Leonidah Kerubo Omosa[a], Gacheru Martin Mbogo[a], Eric Korir[a], Ruth Omole[d], Ean-Jeong Seo[b], Abiy Yenesew[a], Matthias Heydenreich[c], Jacob Ogweno Midiwo[a] and Thomas Efferth[b]

[a]Department of Chemistry, University of Nairobi, Nairobi, Kenya; [b]Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, Johannes Gutenberg University, Mainz, Germany; [c]Institute of Chemistry, University of Potsdam, Potsdam, Germany; [d]Department of Chemical Science and Technology, Technical University of Kenya, Nairobi, Kenya

ABSTRACT

In our continuous search for cytotoxic compounds from the genus *Zanthoxylum,* chromatographic separation of the MeOH/CH_2Cl_2 (1:1) extract of *Z. chalybeum* yielded one new alkamide; 4-(isoprenyloxy)-3-methoxy-3,4-deoxymethylenedioxyfagaramide (**1**) and a known one; fagaramide (**2**). Similarly, from the MeOH/CH_2Cl_2 (1:1) extract of the stem bark of *Z. parachanthum* four known compounds; canthin-6-one (**3**), dihydrochelerythrine (**4**), lupeol (**5**) and sesamin (**6**) were isolated. Characterization of the structures of these compounds was achieved using spectroscopic techniques (NMR and MS). Using resazurin reduction assay **1**, **3** and **6** displayed moderate cytotoxicity with IC_{50} values below 50 μM against the drug sensitive CCRF-CEM and multidrug-resistant CEM/ADR5000 leukemia cell lines. It is interesting to note that **3** was more active than the standard drug, doxorubicin against CEM/ADR5000 leukemia cells. Compounds **3** and **6** showed good selectivity on leukemia cells than normal cells. In future studies **3** should be tested against a panel of drug resistant human cells.



ARTICLE HISTORY
Received 18 May 2018
Accepted 21 February 2019

KEYWORDS
Cytotoxicity; 4-(isoprenyloxy)-3-methoxy-3,4-deoxymethylenedioxyfagaramide (**1**); canthin-6-one (3); multi-drug resistant leukemia cells; selectivity; *Zanthoxylum chalybeum*; Rutaceae

CONTACT Leonidah Kerubo Omosa leoomosa@gmail.com, lkerubo@uonbi.ac.ke
Supplemental data for this article is available online at https://doi.org/10.1080/14786419.2019.1587424.

1. Introduction

Cancer is a broad group of various diseases typified by unregulated cell growth. In Kenya, cancer is estimated to be the third leading cause of death after infectious and cardiovascular diseases accounting for 7% of overall national mortality after cardiovascular diseases (WHO 2014).

Although chemotherapy is one of the major cancer treatment modalities besides surgery and radiotherapy, it has many challenges, including severe side effects to patients as well as the emergence of multidrug resistance (Efferth et al. 2017). Consequently, complementary and alternative therapies such as herbal therapies are believed to exert less adverse effects and are increasingly used to counteract the resistance problem (Boonyong et al. 2017). In Africa and most specifically Kenya, different communities use herbal remedies to manage numerous ailments, including tumors and related disorders (Ochwang'i et al. 2014). Incidentally, a high percentage of these ethnomedicinal trees and shrubs, including the plants used in the current study; *Zanthoxylum chalybeum* Engl. and *Zanthoxylum parachanthum* Kokwaro., have been merely investigated scientifically to support their traditional uses.

The major morphological difference between the two plants is the characteristic trunk in *Z. chalybeum* which is furrowed, with corky ridges crowned with spines (Beentje 1994). The genus *Zanthoxylum* is distributed worldwide, mainly in tropical and temperate regions (Epifano et al. 2011). Different parts of *Z. chalybeum* are used to treat various ailments in traditional African medicine (Beentje 1994; Kokwaro 2009). The leaf decoction is used for the treatment of oedema in kwashiakor, while the root bark is used for the treatment of chest pains, malaria, colds, coughs and dizziness, for joint haemorrhagic septicaemia and helminthiansis in livestock. The fruits are used for chest pain, fever and sore throat, the leaves for snake bite (Kokwaro 2009). On the other hand, *Z. paracanthum* is rarely used in traditional Kenyan medicine. However, previous phytochemical studies on a number of *Zanthoxylum* species have revealed a diversity of interesting alkaloids including; benzo[*c*]phenanthridine, protoberberines, bishordeninyls, aporphines some with interesting biological activities including, cytotoxicity against a panel of cancer cell lines (Mansoor et al. 2013; Omosa and Okemwa 2017; Nyaboke et al. 2018; Chakthong et al. 2018). Other bioactive classes of compounds from this genus include; coumarins, lignans and quinolones (Chakthong et al. 2018). The antimicrobial activity of extracts of *Z. elephantiasis* Macf. against a panel of bacteria has been attributed to canthin-6-one (**3**), which was characterized from *Z. chalybeum* in the current study (Anderson et al. 1983).

In the present project, the cytotoxicity of compounds from *Z. chalybeum* and *Z. parachanthum* against drug sensitive, multidrug-resistant cancer cell lines and normal, peripheral blood cells in order to determine their selectivity indices

2. Results and discussion

2.1. Structure elucidation of 1

The structures of known compounds; fagaramide (**2**) (Omosa and Okemwa 2017), canthin-6-one (**3**) (Bröckelmann et al. 2004), dihydrochelerythrine (**4**) (Omosa and

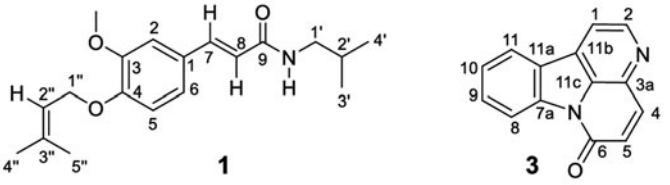


Figure 1. Structures of 4-(isoprenyloxy)-3-methoxy-3,4-deoxymethylenedioxyfagaramide (1) and canthin-6-one (3) isolated from *Z. chalybeum* and *Z. paracanthum*, respectively.

Okemwa 2017), lupeol (**5**) (Reynolds et al. 1986) and sesamin (**6**) (Mbaze et al. 2009) (Figure S1) were identified by comparison of the spectroscopic data obtained with those reported in literature.

The new compound, **1** (Figure 1) was isolated as white crystals which were UV_{254} active. The molecular formula of this compound ($C_{19}H_{27}O_3N$) was confirmed from its HRESIMS (*m/z* 317.1979, calculated 317.1991) (Figure S17). The ^{1}H NMR spectrum (Table S1, Figure S6 to S8), of **1** showed signals for three olefinic protons at δ 7.58 (*d*, *J* = 15.5 Hz, 1H, H-7), δ 6.35 (*d*, *J* = 15.5 Hz, 1H, H-8) and δ 5.52 (*m*, 1H, H-2″). The large coupling constant of *J* = 15.5 Hz is characteristic of an olefinic bond flanked by two protons in a *trans*-configuration (Okorie 1976; Kubo et al. 1984). The analysis of this spectrum also made it possible to highlight the presence of three mutually coupled aromatic protons with an ABX spin system at δ 7.08 (*d*, *J* = 8.2 Hz, 1H, H-2), δ 7.10 (*dd*, *J* = 8.2, 2.0 Hz, 1H, H-6) and δ 6.89 (*d*, *J* = 8.2 Hz, 1H, H-5), of a *tri*-substituted benzene ring. The ^{1}H NMR (Table S1, Figure S6, S8, S9) further displayed signals at δ 3.21 (-CH_2, *t*, *J* = 6.4 Hz, 2H, H-1′) resulting from the H-1′ protons coupling with N-H and H-2′ as observed from the COSY spectrum, δ 1.86 (*m*, 1H), 0.98 (CH_3 × 2, *d*, *J* = 6.7 Hz) assigned to C-1′, C-2′ and C-3′/4′ indicating the presence of an isobutyl amide functional group (Kubo et al. 1984). There were two characteristic resonances for oxygenated carbons of which one was assigned to a methoxy groups at δ 3.89 (-OCH_3, *s*) assigned to C-3 with the other being placed to a prenyloxy group at C-4. Furthermore, the ^{1}H NMR spectrum (Table S1, Figure S6, S8, S9) showed two shielded singlets characteristic of methyl groups appearing at δ 1.78 (CH_3 *s*) and 1.83 (CH_3 *s*). ^{1}H NMR showed a downfield shifted resonance integrating for two protons at δ 4.59 (*d*, *J* = 6.8 Hz, 2H, H-1″) that were placed at C-1″ by virtue of their strong ^{1}H, ^{1}H COSY correlation (Figure S11) with the methyne proton at δ 5.52 (*br s* 1H, H-2″) together with ^2J and ^3J long range correlation of these methyl protons with C-2″ and C-3″ (δ 138.4), respectively. Similarly, the placement of two set of the methyl groups at C-4″ and C-5″ was confirmed from the ^2J and ^3J long range correlation of these methyl protons with C-3″ and C-2″, respectively, (Table S1, Figure S13, S15).

Analysis of ^{13}C NMR spectrum (Table S1, Figure S10) confirmed the presence of 19 carbon signals which were attributed to five methyls (including one methoxy group), two methylenes, seven methynes (including six sp^2 hybridized ones) from HSQC spectrum (Figure S12) together with 5 quaternary carbons. One of this quaternary carbons was characteristic of the carbonyl group δ 165.9 assigned to C-9. This carbonyl group was attached to an amide functional group and an olefinic hydrocarbon C-8 and hence the observed chemical shift value (Table S1, Figure 1, Figure S10). There was a signal at δ 47.0 assigned to -CH_2 that was bonded to a nitrogen atom while that at δ

65.4 was due to oxygenated methylene group. There was one carbon resonance integrating for six protons at δ 19.8 assigned to two identical methyl groups. Furthermore, there were two resonances for two methyl groups at δ 17.9 and 25.5 (Table S1, Figure S10). The placement of the methyl groups resonating at δ 19.8 at 3′ and 4′ was confirmed from the observed long range correlations of the two carbon atoms with H-1′ (δ 3.21). Similarly, the methyl groups at δ 17.9 and 25.5 were assigned to C-4″ and 5″, respectively based on their long range correlation with the olefinic proton at H-2″ (Table S1, Figure S13, S15).

The placement of the methoxy group at C-3 and not C-4 was due to the ^{3}J long range correlation of the methoxy protons (δ 3.89) with C-3 (δ 149.5). The prenyloxy group was placed at C-4 (δ 149.9) as was confirmed from the ^{2}J and ^{3}J long range correlations of H-6 (δ 7.10), H-5 (δ 6.89) and H-2 (δ 7.08) with C4 (δ 149.9) and not with C-3 (δ 149.5) (Table S1, Figure S13, S14, S15). The placement of the prenyloxy group at C-3 was confirmed further from the NOESY cross peak of H-1″ with the aromatic H-5 (Figure 16S). Based on these spectral data and comparison with chemical shift values of known compounds, **1** was characterized as 4-(isoprenyloxy)-3-methoxy-3,4-deoxymethylenedioxyfagaramide (**1**), this compound has not been identified in nature before.

2.2. In vitro cytotoxicity results

The isolated compounds were tested for their potential to inhibit drug-sensitive (CCRF-CEM), multidrug-resistant cell lines (CEM/ADR5000) and normal human peripheral blood mononuclear cells with doxorubicin as the reference drug.

4-(isoprenyloxy)-3-methoxy-3,4-deoxymethylenedioxyfagaramide (**1**), canthin-6-one (**3**) and sesamin (**6**) displayed moderate cytotoxicity with IC_{50} values below 50 μM against the two tested cell lines (Table S2, Figure 1, Figures S1–S4) (Kuete and Efferth 2015). The values were as follows; 29.13 ± 2.54 (towards drug sensitive CCRF-CEM) and 31 ± 4.74 (towards multidrug-resistant CEM/ADR5000 leukemia cell line) (Table S2, Figure S2) for **1**; 15.82 ± 1.26 μM (towards CCRF-CEM) and 10.52 ± 0.05 μM (towards CEM/ADR5000) (Table S2, Figure S3) for **3** and 40.74 ± 4.41 μM (towards CCRF-CEM), 30.70 ± 2.89 μM (towards CEM/ADR5000) (Table S2, Figure S4) for **6**. It is important to note that **3** was more active than the standard drug, doxorubicin against CEM/ADR5000 leukemia cell lines and therefore would be considered for development into a drug prototype especially against the drug resistant cancer cells (Table S2, Figure S5). Compounds **3** and **6** were more active towards the two leukemia cells as compared to the normal cells, PBMCs with selectivity indices of 1.27 (towards CCRF-CEM) and 1.97 (towards CEM/ADR5000) and 2.03 (towards CCRF-CEM) and 2.70 (towards CEM/ADR5000) (Table S2, Figure S3, S4), respectively and therefore relatively safer on normal cells. However, **1** which exhibited selectivity indices of <1 was more active on the normal cells (Table S2, Figure S2). The results against the drug sensitive leukemia cell lines concurs with previous studies on canthin-6-one (**3**) which exhibited an IC_{50} value of 1.11 μg/mL against KB tissue culture studies (Anderson et al. 1983). Follow up studies (Fukamiya et al. 1987) showed that hydroxylation and methylation of canthin-6-one (**3**) at C-10/C-11 were the structural requirement for good activity in KB tissue

culture cells in contrast to hydroxylation and methylation at C-1 and C-5 which led to insignificant effect on cytotoxicity. Recent studies on canthin-6-one (**3**) and derivatives have focused on evaluating their cytotoxicity against mainly drug sensitive cell lines (Jiang and Zhou 2008; Miyake et al. 2010). However, there is scanty or no information on the cytotoxic activity of canthin-6-one (**3**) against the drug resistant and normal cell lines, therefore the importance of the current study. It has been proven from previous investigations that structure modification of canthin-6-one (**3**) substantially improved on its cytotoxicity against various cancer cells. This therefore should be one of the motivations for future research on extensive chemical modifications to obtain analogues probably with higher potencies against different cancer cell lines. Furthermore, this compound should be tested on a panel of drug resistant cell lines in order to establish its degree of resistance. Compounds **3** and **6** showed higher cytotoxic activities against drug resistant as compared to the drug sensitive cells making them better candidates to fight multi-drug resistance in different cancer cells than doxorubicin. Fagaramide (**2**) was inactive as it exhibited IC_{50} values $>100\,\mu M$ against all the cell lines tested (Table S2) (Kuete and Efferth 2015).

3. Experimental

3.1. General experimental procedures

Merck silica gel 60 (70–230 mesh) and Sephadex LH–20 were used as stationary phases for column chromatography (CC). Preparative thin layer chromatography (PTLC) (1.0 mm, 20×20 cm) were prepared using Merck silica gel 60 (PF254 + 366); factory made analytical aluminium TLC plates (silica gel 60 F254, Merck) were used to monitor the purity of the isolates by visualizing the spots under UV light at 254 or 366 nm for UV active compounds, followed by placing the plate in an iodine tank and spraying with Dragendorff's reagent for both the non–UV active and alkaloid compound tests, respectively. The ^{1}H and ^{13}C NMR spectra were recorded using Varian–Mercury 200 MHz and Bruker–Avance 500 and 600 MHz spectrometers. The Homo Nuclear Correlation Spectroscopy (COSY), Hetero Nuclear Single Quantum Coherence (HSQC) and Hetero Nuclear Multiple Bond Connectivity (HMBC) spectra were obtained using standard Bruker software. Chemical shifts were measured in ppm relative to the internal standard tetramethylsilane (TMS). The solvents used for chromatography were *n*–hexane (*n*–C_6H_{14}), dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and methanol (MeOH).

3.2. Plant material

The stem barks of *Z. paracanthum* and *Z. chalybeum* were collected from Msambweni in Kwale County, which is approximately 37 km from Mombasa city in the Coastal region of Kenya on 25 August 2016. The two plants were authenticated at the University of Nairobi herbarium, School of Biological Science (SBS), where voucher specimen (GMM2016/09/ZC and GMM/2016/09/ZP) are deposited.

3.3. Extraction and isolation

The dried and ground stem bark of *Z. chalybeum* (900 g) and *Z. parachanthum* (1000 g) were each extracted at room temperature with sufficient volumes of 50% methanol (MeOH) in dichloromethane (CH_2Cl_2) (Omosa and Okemwa 2017). The resultant extracts were concentrated *in vacuo* using a rotary evaporator resulting into residues of 84 and 97 g for *Z. chalybeum* and *Z. parachanthum,* respectively. The total extract of *Z. chalybeum* (84 g) were purified using column chromatography using silica gel matrix and eluting with solvent systems of varying polarities; initially with 1% ethyl acetate (EtOAc) in *n*-C_6H_{14} and then increasing upto 100% EtOAc. The fraction eluted with 15 to 20% EtOAc in *n*-C_6H_{14} were further purified through preparative TLC with multiple development (10% EtOAc in *n*-C_6H_{14}) affording white crystals of the previously undescribed compound; 4-(isoprenyloxy)-3-methoxy-3,4-deoxymethylenedioxyfagaramide (**1**,50 mg). The fractions eluted with 20% and 30%; were pulled, loaded onto Sephadex LH 20 (50% MeOH in CH_2Cl_2); affording creamy crystals of fagaramide (**2,** 28 mg). Similarly, the residue from *Z. parachanthum* stem bark (97 g) loaded onto a silica gel column (1 kg) initially in 1% EtOAc in *n*-hexane (*n*-C_6H_{14}) and then increasing the polarity up to 100% EtOAc. The fractions of the main column eluted with 7-10% EtOAc in *n*-C_6H_{14} yielded white amorphous solids of sesamin (**6**, 60 mg) while subsequent fractions eluted with 10-15% EtOAc in *n*-C_6H_{14} formed colorless crystals of dihydrochelerythrine (**4**, 22 mg). The fractions eluted with 20% EtOAc in *n*-C_6H_{14} yielded white amorphous solids of lupeol (**5**, 80 mg). Purification of the fraction of the major column eluted with 30 to 50% EtOAc in *n*-C_6H_{14} by size exclusion using Sephadex-LH 20 eluting with 50% MeOH in CH_2Cl_2 solvent system resulted to white amorphous solids of the indolonaphthyridine alkaloid, canthin-6-one (**3,** 20 mg).

3.4. Leukemia cell lines

The cell lines used were drug-sensitive leukemia CCRF-CEM and multidrug-resistant P-glycoprotein-over-expressing subline CEM/ADR5000 cells. Their origin, maintenance and characterization were described (Efferth et al. 2003; Kuete et al. 2016). The cell lines were tested for *Mycoplasma* with a *Mycoplasma* Stain Kit (Sigma-Aldrich, St Louis, MO, USA) and found to be free from contamination. Cells were not passaged more than 20 times.

3.5. Normal cells line

The normal human peripheral blood mononuclear cells (PBMC)s were isolated from fresh blood samples of a healthy donor using Histopaque® (Sigma-Aldrich, St. Louis, MO, USA). Briefly, three mL of blood was layered onto the Histopaque® and centrifuged (400 × g) for 30 mins at 20 °C. The buffy coat interface, including lymphocytes and other mononuclear cells, was transferred into a new tube and washed several times with phosphate buffered saline solution. Obtained PBMCs were stored in Panserin 413 medium (PAN-Biotech, Aidenbach, Germany) supplemented with 2% phytohemagglutinin M (PHA-M, Life Technologies, Darmstadt, Germany) and directly used for the resazurin assay. The resazurin assay was performed as described below.

3.6. Cytotoxicity assays

Resazurin reduction assay was applied as previously described (O'Brien et al. 2000) and used to evaluate the cytotoxicity of samples. All experimental conditions were similar to those previously reported (Kuete et al. 2016). Controls used were doxorubicin (drug or positive control) and dimethylsulfoxide (DMSO; solvent or negative control). The highest concentration of DMSO was less than 0.4%. Assays were performed at least twice, with six replicate each. The fluorescence was measured after 72 h incubation with Infinite M2000 Pro^{TM} plate reader (Tecan, Crailsheim, Germany) using an excitation wavelength of 544 nm and an emission wavelength of 590 nm.

Acknowledgement

The authors would like to thank the International Science Programme, Uppsala University, Sweden (ISP) through the KEN-02 project for providing funds for reagents for the isolation process. The authors wish to thank Mr. Patrick Chalo Mutiso for the identification and collection of the plant materials from which the compounds were isolated.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

Funds for this work was provided by the International Science Programme, Uppsala University, Sweden (ISP) through the KEN-02 project.

References

Anderson LA, Harris A, Phillipson JD. 1983. Production of cytotoxic canthin-6-one alkaloids by *Ailanthus altissima* plant cell cultures. J Nat Prod. 46(3):374–378.

Beentje H. 1994. Kenyan, trees, shrubs and lianas. Nairobi (Kenya): National Museums of Kenya; p. 373–374.

Boonyong C, Pattamadilok C, Suttisri R, Jianmongkol S. 2017. Benzophenones and xanthone derivatives from *Garcinia schomburgkiana*-induced P-glycoprotein overexpression in human colorectal Caco-2 cells via oxidative stress-mediated mechanisms. Phytomedicine. 27:8–14.

Bröckelmann MG, Dasenbrock J, Steffan B, Steglich W, Wang Y, Raabe G, Fleischhauer J. 2004. An unusual series of thiomethylated canthin-6-ones from the North American mushroom *Boletus curtisii*. Eur J Org Chem. 2004(23):4856–4863.

Chakthong S, Ampaprom R, Inparn S, Phetkul U, Chusri S, Limsuwan S, Voravuthikunchai SP. 2018. New alkylamide from the stems of *Zanthoxylum nitidum*. Nat Prod Res. 17:1–9.

Efferth T, Saeed MEM, Mirghani E, Alim A, Yassin Z, Saeed E, Khalid HE, Daak S. 2017. Integration of phytochemicals and phytotherapy into cancer precision medicine. Oncotarget. 8(30): 50284–50304.

Efferth T, Sauerbrey A, Olbrich A, Gebhart E, Rauch P, Weber HO, Hengstler JG, Halatsch ME, Volm M, Tew KD, et al. 2003. Molecular modes of action of artesunate in tumor cell lines. Mol Pharmacol. 64(2):382–394.

Epifano F, Curini M, Marcotullio MC, Genovese S. 2011. Searching for novel cancer chemopreventive plants and their products: the genus *Zanthoxylum*. Curr Drug Targets. 12(13): 1895–1902.

Fukamiya N, Okano M, Aratani T, Negoro K, Lin YM, Lee KH. 1987. Antitumor agents: LXXXVII. Cytotoxic antileukemic canthin-6-one alkaloids from *Brucea antidysenterica* and the structure activity relationships of their related derivatives1. Planta Med. 53(02):140–143.

Jiang MX, Zhou YJ. 2008. Canthin-6-one alkaloids from *Picrasma quassioides* and their cytotoxic activity. J Asian Nat Prod Res. 10(11–12):1009–1012.

Kokwaro JO. 2009. Medicinal plants of East Africa. 3rd ed. Nairobi (Kenya): University of Nairobi Press; p. 256.

Kubo I, Matsumoto T, Klocke JA, Kamikawa T. 1984. Molluscicidal and insecticidal activities of isobutylamides isolated from *Fagara macrophylla*. Cell Mol Life Sci. 40(4):340–341.

Kuete V, Mbaveng AT, Nono EC, Simo CC, Zeino M, Nkengfack AE, Efferth T. 2016. Cytotoxicity of seven naturally occurring phenolic compounds towards multi-factorial drug-resistant cancer cells. Phytomedicine. 23(8):856–863.

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

Mansoor TA, Borralho PM, Luo X, Mulhovo S, Rodrigues CMP, Ferreira M-JU. 2013. Apoptosis inducing activity of benzophenanthridine-type alkaloids and 2-arylbenzofuranneolignans in HCT116 colon carcinoma cells. Phytomedicine. 20(10):923–929.

Mbaze LM, Lado JA, Wansi JD, Shiao TC, Chiozem DD, Mesaik MA, Choudhary MI, Lacaille-Dubois MA, Wandji J, Roy R, Sewald N. 2009. Oxidative burst inhibitory and cytotoxic amides and lignans from the stem bark of *Fagara heitzii* (Rutaceae). Phytochemistry. 70(11–12):1442–1447.

Miyake K, Tezuka Y, Awale S, Li F, Kadota S. 2010. Canthin-6-one alkaloids and a tirucallanoid from *Eurycoma longifolia* and their cytotoxic activity against a human HT-1080 fibrosarcoma cell line. Nat Prod Commun. 5(1):17–22.

Nyaboke HO, Moraa M, Omosa LK, Mbaveng AT, Vaderament-Alexe NN, 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):10.

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.

Ochwang'i DO, Kimwele CN, Oduma JA, Gathumbi PK, Mbaria JM, Kiama SG. 2014. Medicinal plants used in treatment and management of cancer in Kakamega County, Kenya. J Ethnopharmacol. 151(3):1040–1055.

Okorie DA. 1976. A new phthalide and xanthones from *Anthocleista djalonensis* and *Anthocleista vogelli*. Phytochemistry. 15(11):1799–1800.

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

Reynolds WF, McLean S, Poplawski J, Enriquez RG, Escobar LI, Leon I. 1986. Total assignment of ^{13}C and ^{1}H spectra of three isomeric triterpenol derivatives by 2D NMR: an investigation of the potential utility of 1H chemical shifts in structural investigations of complex natural products. Tetrahedron. 42(13):3419–3428.

World Health Organization (WHO). 2014. Noncommunicable diseases country profiles 2014.