Phytomedicine Plus 2 (2022) 100234

Contents lists available at ScienceDirect

Phytomedicine Plus

journal homepage: www.sciencedirect.com/journal/phytomedicine-plus

Cytotoxic flavonoids from the seeds of *Dracaena steudneri* Engl against leukemia cancer cell lines☆

Vaderament-A Nchiozem-Ngnitedem [a,c,*], Leonidah Kerubo Omosa [a], Solomon Derese [a], Thomas Efferth [b], Michael Spiteller [c]

[a] Department of Chemistry, University of Nairobi, P. O. Box 30197-00100, Nairobi, Kenya
[b] Department of Pharmaceutical Biology, Institute of Pharmaceutical and Biomedical Sciences, Johannes Gutenberg University, Staudinger Weg 5, Mainz 55128, Germany
[c] Department of Chemistry and Chemical Biology, Chair of Environmental Chemistry and Analytical Chemistry, Institute of Environmental Research (INFU), TU Dortmund, Otto-Hahn-Straße 6, Dortmund 44221, Germany

ARTICLE INFO

Keywords:
Asparagaceae
Cytotoxicity
Dracaena steudneri
Flavonoids
Leukemia
Multidrug resistance

ABSTRACT

Background: Leukemia is the most common type of childhood cancer. Numerous flavonoids isolated from plants have been reported as potential chemotherapeutic agents against malignant growth while taking care of healthy cells.
Purpose: To discover new anticancer agents from the seeds of *Dracaena steudneri* Engl for their potential uses as candidate compounds against leukemia cell lines.
Methods: A panel of chromatography techniques (CC, Sephadex LH-20 and semi-preparative HPLC) were used to isolate these compounds from the MeOH/CH_2Cl_2 (1:1) crude extract of the seeds of *D. steudneri.* Their structure elucidation was achieved based on spectral evidence (UV, NMR and HRESIMS). Resazurin reduction assays were performed to assess the cytotoxicity of the crude extract and isolates.
Results: From the seeds of *D. steudneri* 8 compounds were isolated (**1** – **8**). Quercetin derivatives: 3,3′-di-*O*-methylquercetin-4′-*O*-*β*-D-glucoside (**5**) and 3,3′-di-*O*-methylquercetin (**7**) displayed significant cytotoxicity against the two leukemia cell lines tested with IC_{50} < 10 μM. Doxorubicin (reference drug) exhibited strong cytotoxic potency; IC_{50} of 0.01 μM (against CCRF-CEM cells) and moderate activity; IC_{50} of 26.78 μM (towards CEM/ADR5000 cells). To the best of our knowledge, this is the first report of flavonoids glycosides from the genus *Dracaena*.
Conclusion: The results obtained in this study showed that flavonoids isolated from *Dracaena steudneri* are promising candidates for cancer chemotherapy. The mode of action and the cytotoxicity of the most active compounds (**5** and **7**) should be further investigated.

Introduction

Flavonoids represents a wide range of natural or synthetic compounds belonging to the family of polyphenols (Panche et al., 2016). These substances are responsible for the yellow, orange and red colors in numerous plant species. From a structural point of view, flavonoids have a common biosynthetic origin and, therefore, share the same scaffold consisting of 15 carbon atoms (Rauter et al., 2018). They are well known for their antioxidant properties to protect plants against UV radiation. For humans, several health beneficial properties of dietary flavonoids have been described, which may protect the body from chronic inflammation, cancer and other diseases (Górniak et al., 2019; Bisol et al., 2020).

Cancer is a critical problem affecting global health with 19.3 million new cases and 10.0 million deaths reported in 2020 (Sung et al., 2021). Cancer is the second leading cause of death after cardiovascular diseases and the morbidity and mortality rates associated with this infliction has increased globally. Human leukemia is among the top 20 malignant diseases in human beings with annually 437,033 new cases and 309,006 deaths (Bray et al., 2018). Most vulnerable are children under 15 years of age accounting for 80% of all leukemia cases (Terwilliger and

☆ Given his role as Editor-in-Chief Prof. Thomas Efferth had no involvement in the peer-review of this article and has no access to information regarding its peer-review. Full responsibility for the peer-review process for this article was delegated to one of the Associate Editors.

* Corresponding author at: Department of Chemistry, University of Nairobi, P. O. Box 30197-00100, Nairobi, Kenya.
E-mail address: n.vaderamentalexe@gmail.com (V.-A. Nchiozem-Ngnitedem).

https://doi.org/10.1016/j.phyplu.2022.100234
Received 23 May 2021; Received in revised form 13 December 2021; Accepted 14 December 2021
Available online 3 February 2022

Abbreviations

1	isorhamnetin 3-*O*-rungioside
2	kaempferol 3-*O*-rungioside
3	quercetin-3-*O*-*β*-D-glucoside
4	isorhamnetin 3-*O*-*β*-D-glucopyranoside
5	3,3′-di-*O*-methylquercetin-4′-*O*-*β*-D-glucoside
6	quercetin
7	3,3′-di-*O*-methylquercetin
8	4-(2'-formyl-1'-pyrrolyl)butanoic acid
P-gp	P-glycoprotein
D	*Dracaena*
CCRF-CEM	drug-sensitive leukemia cells
CEM/ADR5000	multidrug-resistant P-glycoprotein-overexpressing leukemia cells
NMR	nuclear magnetic resonance
HRESIMS	high resolution electrospray ionization mass spectrometry
CD_3OD	deuterated Methanol
HPLC	high performance liquid chromatography
MeOH	methanol
H_2O	water
TLC	thin layer chromatography
CH_2Cl_2	dichoromethane
EtOAc	ethylacetate
LC-MS	liquid chromatography–mass spectrometry
t_R	retention time
LC-UV	liquid chromatography-ultraviolet
MHz	megahertz
ddH_2O	double-distilled water
IC_{50}	half-maximal inhibitory concentration
ATP	adenosine triphosphate
MRP1	multidrug resistance protein 1
MMP	mitochondrial membrane potential
ROS	reactive oxygen species

Abdul-Hay, 2017). Chemotherapy which remains the method of choice is associated with undesirable effects. Further, the appearance of multidrug resistance (MDR) of cancer cells to chemotherapy remains a serious problem in the treatment and management of the disease. This phenomenon considerably reduces the efficacy of antiproliferative drugs, leading to increased numbers of therapeutic failure (Gottesman et al., 2009). As a result, there is need to continuously search for new drugs to fight drug-resistant cancer particularly from plants, as they have proved to be good candidates for anticancer drugs (Efferth et al., 2020a).

The genus *Dracaena* (Asparagaceae family) comprises >100 species that are widely distributed in the tropical and subtropical regions of the world (Lu and Morden, 2010). Out of 100 species found in this genus, 8 have been reported in Kenya including *Dracaena steudneri* Engl. Traditionally, the extract from the leaves of *D. steudneri* is used indigenously for the treatment of splenomegaly, hernia, asthma and chest problems in Tanzania (Moshi et al., 2012) and in Rwanda to treat liver diseases (Mukazayire et al., 2011). In Kenya, the decoction from the stem is drunk for the management of hepatic liver ailments, treatment of measles and reducing pain during childbirth (Kokwaro, 2009). Besides the traditional aspects, species of this genus exhibit a wide range of biological activities, such as cytotoxicity (Teponno et al., 2017) as well as antimicrobial (Zhu et al., 2007) and anti-inflammatory effects (Nchiozem-Ngnitedem et al., 2020a, 2020b). Previous phytochemical studies of *Dracaena* species have indicated the presence of saponins (Shen et al., 2014), flavonoids (Nchiozem-Ngnitedem et al., 2020b), homoisoflavonoids (Nchiozem-Ngnitedem et al., 2020c) and polymeric flavonoids (Pang et al., 2016).

Hence, this study aimed to investigate the cytotoxicity of flavonoids isolated from the seeds of *Dracaena steudneri* against two leukemia cancer cell lines including drug-sensitive CCRF-CEM cells and its multidrug-resistant P-glycoprotein overexpressing subline CEM/ADR5000.

Materials and methods

General experimental procedures

NMR experiments were carried out using Bruker spectrometer operating at 600 MHz (Avance III). All spectra were processed using MestReNova-9.0.1 software. ^1H ($\delta = 3.31$) and ^{13}C ($\delta = 49.0$) NMR for CD_3OD solvent peaks were used as references. HRESIMS was conducted on a LTQ Orbitrap spectrometer (Thermo Scientific, USA) equipped with a HESI-II source. Data were processed by Xcalibur Software. For column chromatography, Silica gel (0.063 – 0.2 mm, Macherey-Nagel, Germany) and Sephadex LH-20 (18 – 111 μm, GE Healthcare, Germany) were used as a solid matrix.

Preparative HPLC was performed on a Shimadzu LC-20AP system equipped with DGU 20A5R degassing unit, an SPD-M20A detector, SIL-20ACHT autosamplers, and a Nucleodur Polartec 5 μm RP column (10 × 125 mm) using LabSolution software system. The mobile phase was composed of MeOH (solvent B) and H_2O (solvent A, containing 0.1 % formic acid). TLC was carried out on pre-coated silica gel 60 plates (0.20 mm; Macherey-Nagel, Germany). Fluorescence in the cytotoxicity assay was measured on an Infinite 200 Pro-TECAN plate reader. The general experimental procedures has been published (Mukavi et al., 2020).

Plant material

The seeds of *D. steudneri* were collected in November 2018 from Riverside drive, Nairobi, Kenya (about 2 km from Nairobi Central Business District). The plant material was identified by a taxonomist from the University of Nairobi Herbarium, Faculty of Science and Technology (FST), where a voucher specimen (NNA 2018/003) has been deposited for reference.

Extraction and isolation of chemical constituents from the seeds of Dracaena steudneri

The seeds of *D. steudneri* were dried under shade and then ground to yield 2.9 kg of dried material. The obtained powder was macerated in equal volume of MeOH in CH_2Cl_2 (3 L, 24 h × 3) affording 640 g (22.1% yield) of oily residue. Part of the crude extract (300 g) was defatted using flash column chromatography with silica gel as stationary phase. The mobile phase was composed of pure cyclohexane followed by a gradient elution of cyclohexane/EtOAc (9:1, 1:1 and 0:10) and EtOAc/MeOH (9:1, 1:1 and 0:10). A total of 100 fractions of 500 mL each were collected and pooled based on their TLC and LC-MS profile into four sub-fractions; Fr_A (cyclohexane/EtOAc (10:0 – 9:1)), Fr_B (cyclohexane/EtOAc (1:1 – 0:10)), Fr_C (EtOAc/MeOH (9:1 – 1:1)) and Fr_D (MeOH (neat)).

Sub-fraction Fr_C was further purified through semi-preparative HPLC set as follows–Gradient elution started at MeOH/H_2O (1:9) up to neat MeOH for 20.5 min and thereafter isocratic elution for 10 min using pure MeOH, the solvent system MeOH/H_2O returned to the initial concentration within an interval of 0.5 min and was constant for 9.0 min to afford compounds **1** (2.3 mg, t_R 15.2 min), **2** (0.7 mg, t_R 15.4 min), **3** (2.5 mg, t_R 15.8 min), **4** (1.5 mg, t_R 16.8 min), **5** (2.0 mg, t_R 17.6 min), **6** (1.1 mg, t_R 18.3 min), **7** (1.6 mg, t_R 19.6 min) and **8** (0.6 mg, t_R 13.5 min).

Isorhamnetin 3-O-rungioside (**1**). Yellow amorphous solid, LC-UV (MeOH-H_2O [0.1% formic acid]) λ_{max} 356 and 254 nm; ^1H (CD_3OD, 600 MHz) and ^{13}C (CD_3OD, 150 MHz) NMR data, see Table 1S and Figs. 2S –4S, see Supporting Information; HRESIMS *m/z* 625.1760 $[M + H]^+$ (calcd for $[M + H]^+$ $C_{28}H_{33}O_{16}$, 625.1724).

Kaempferol 3-O-rungioside (**2**). Yellow amorphous solid, LC-UV (MeOH-H_2O [0.1% formic acid]) λ_{max} 350, 266 and 232 nm; ^1H (CD_3OD, 600 MHz) and ^{13}C (CD_3OD, 150 MHz) NMR data, see Table S1 and Figs. 5S –7S, see Supporting Information; HRESIMS *m/z* 595.1655 $[M + H]^+$ (calcd for $[M + H]^+$ $C_{27}H_{31}O_{15}$, 595.1618).

Quercetin-3-O-β-D-glucoside (**3**). Yellow amorphous solid, LC-UV (MeOH-H_2O [0.1% formic acid]) λ_{max} 354 and 260 nm; ^1H (CD_3OD, 600 MHz) and ^{13}C (CD_3OD, 150 MHz) NMR data, see Table S2 and Figs. 8S –10S, see Supporting Information; HRESIMS *m/z* 465.1023 $[M + H]^+$ (calcd for $[M + H]^+$ $C_{21}H_{21}O_{12}$, 465.0988).

Isorhamnetin 3-O-β-D-glucopyranoside (**4**). Yellow amorphous solid, LC-UV (MeOH-H_2O [0.1% formic acid]) λ_{max} 356 and 264 nm; ^1H (CD_3OD, 600 MHz) and ^{13}C (CD_3OD, 150 MHz) NMR data, see Table S2 and Figs. 11S –13S, see Supporting Information; HRESIMS *m/z* 479.1179 $[M + H]^+$ (calcd for $[M + H]^+$ $C_{22}H_{23}O_{12}$, 479.1145).

3,3′-Di-O-methylquercetin 4′-O-β-D-glucoside (**5**). Yellow amorphous solid, LC-UV (MeOH-H_2O [0.1% formic acid]) λ_{max} 350 and 268 nm; ^1H (CD_3OD, 600 MHz) and ^{13}C (CD_3OD, 150 MHz) NMR data, see Table S3 and Figs. 14S–16S, see Supporting Information; HRESIMS *m/z* 493.1333 $[M + H]^+$ (calcd for $[M + H]^+$ $C_{23}H_{25}O_{12}$, 493.1301).

Quercetin (**6**). Yellow amorphous solid, LC-UV (MeOH-H_2O [0.1% formic acid]) λ_{max} 372, 256 and 232 nm; ^1H (CD_3OD, 600 MHz) and ^{13}C (CD_3OD, 150 MHz) NMR data, see Table S3 and Figs. 17S –19S, see Supporting Information; HRESIMS *m/z* 303.0501 $[M + H]^+$ (calcd for $[M + H]^+$ $C_{15}H_{11}O_7$, 303.0460).

3,3′-Di-O-methyl quercetin (**7**). Yellow amorphous solid, LC-UV (MeOH-H_2O [0.1% formic acid]) λ_{max} 357 and 255 nm; ^1H (CD_3OD, 600 MHz) and ^{13}C (CD_3OD, 150 MHz) NMR data, see Table S4 and Figs. 20S –22S, see Supporting Information; HRESIMS *m/z* 331.0812 $[M + H]^+$ (calcd for $[M + H]^+$ $C_{17}H_{15}O_7$, 331.0773).

4-(2′-Formyl-1′-pyrrolyl)butanoic acid (**8**). Yellow amorphous solid, LC-UV (MeOH-H_2O [0.1% formic acid]) λ_{max} 218 nm; ^1H (CD_3OD, 600 MHz) and ^{13}C (CD_3OD, 150 MHz) NMR data, see Table S5 and Figs. 23S –25S, see Supporting Information; HRESIMS *m/z* 182.0811 $[M + H]^+$ (calcd for $[M + H]^+$ $C_9H_{12}O_3N$, 182.0772).

Cell lines and cultures

The cell lines used in the current work, their origin, culturing, and resistance development were previously reported (Kimmig et al., 1990; Efferth et al., 2003; Kadioglu et al., 2016; Nyaboke et al., 2018, Omosa et al., 2021). Two leukemia cancer cell lines including drug-sensitive CCRF-CEM leukemia and its multidrug-resistant P-glycoprotein-over-expressing subline CEM/ADR5000 were used. The cell lines were cultured under standard conditions (RPMI 1640 medium, 10 % fetal calf serum, 1 % penicillin/streptomycin; Invitrogen, Eggenstein, Germany) in an incubator (SteriCult, Thermo Fisher Scientific GmbH, Dreieich, Germany) at 37 °C and 5% CO_2. The multidrug resistance phenotype of the CEM/ADR5000 cells has been maintained by treatement with 5 μg/mL doxorubicin from 24 h every other week. The experiments were performed using cells in the logarithmic growth phase.

Cytotoxicity of botanical, isolates and doxorubicin by resazurin reduction assay

Resazurin reduction assay was performed to assess the cytotoxicity of the studied samples toward the drug-sensitive and resistant leukemia cell lines as described earlier (Mahmoud et al., 2020; Mbaveng et al., 2020). The assay is based on reduction of the indicator dye, resazurin, to the highly fluorescent resorufin by viable cells. Non-viable cells rapidly loose the metabolic capacity to reduce resazurin and, thus, produce no fluorescent signal (O'Brien et al., 2000). Aliquots of 2×10^4 cells per well were seeded in 96-well-plates in a total volume of 100 μL. The studied sample was immediately added in varying concentrations in an additional 100 μL of culture medium to obtain a total volume of 200 μL/well. After 72 h, 20 μL resazurin (Sigma-Aldrich, Taufkirchen, Germany) 0.01 % w/v in ddH_2O was added to each well and the plates were incubated at 37 °C for 4 h. The fluorescence was measured on an Infinite M2000 Pro™ plate reader (Tecan, Crailsheim, Germany) using an excitation wavelength of 544 nm and an emission wavelength of 590 nm. Each assay was done at least two times, with six replicate each. The viability was evaluated based on a comparison with untreated cells. IC_{50} values represent samples' concentrations required to inhibit 50% of cell proliferation and were calculated from a calibration curve by linear regression using Microsoft Excel.

Results

Compounds isolated from the seeds of Dracaena steudneri

Systematic phytochemical investigation of the MeOH/CH_2Cl_2 (1:1) crude extract of the seeds of *D. steudneri* afforded 8 known compounds, Fig. 1. The chemical structures of all analogues were elucidated by comprehensive spectroscopic and spectrometry methods such nuclear magnetic resonance spectroscopy (NMR), high resolution electrospray mass spectrometry (HRESIMS) as well as by comparison with data reported in the literature. The proposed structures included 7 flavonoids out of which 5 were glycosylated bearing a mono or disaccharide units at C-3 (**1** – **4**) or C-4′ (**5**) positions in C- and B-rings, respectively. These compounds were identified as isorhamnetin 3-*O*-rungioside (**1**) (Ahmad et al., 2010), kaempferol 3-*O*-rungioside (**2**) (Seshadri and Vydeeswaran, 1972), quercetin-3-*O*-*β*-D-glucoside (**3**) (Kwon and Bae, 2011), isorhamnetin 3-*O*-*β*-D-glucopyranoside (**4**) (Touil et al., 2006), 3, 3′-di-*O*-methylquercetin 4′-*O*-*β*-D-glucoside (**5**) (Sick et al., 1983), quercetin (**6**) (Teponno et al., 2006), 3,3′-di-*O*-methyl quercetin (**7**) (Wang et al., 2012). Compound **8**, with a heterocyclic aromatic architecture was identified as 4-(2'-formyl-1'-pyrrolyl)butanoic acid (**8**) (Tressl et al., 1993). To the best of our knowledge compound **8** was previously described without ^{13}C NMR data. Herein, the ^{13}C NMR data of this naturally isolate is reported (Table S5 and Figs. 23S –25S, see Supporting Information). The NMR and HRESIMS spectra of these heterocyclic organic compounds are provided as supporting information (Figs. 2S –34S).

Cytotoxicity of compounds isolated on leukemia cell lines

At the end of phytochemical study, the crude extract obtained from the seeds of *Dracaena steudneri* and isolates (**1** – **8**) were preliminarily screened for their cytotoxic potencies against the most sensitive leukemia cell line (CCRF-CEM). The extract and some isolates except **5** and **7** were not cytotoxic at the tested concentration as they displayed less than 70% of cell inhibition in accordance to established criteria, Fig. 2 (Table 6S, see Supporting Information) (Nchiozem-Ngnitedem et al., 2020c). Based on the resulted obtained from the preliminary screening, compounds **5** and **7** which showed cell inhibition rates of 82.46% and 76.98%, respectively were selected and tested further against the multidrug-resistant (CEM/ADR5000) leukemia cell line in order to calculate their half inhibitory concentration (IC_{50}). The results for these two isolates recorded as IC_{50} values, degree of resistance (D.R) are reported in Table 1. The IC_{50} values of these compounds (**5** and **7**) ranged from 3.31 μM (against CEM/ADR5000) to 8.81 μM (towards CCRF-CEM). Compounds **5** and **7** act upon 2/2 (100%) inhibition with an $IC_{50} < 10$ μM against the two cancer cell lines. More specifically, compound **5** displayed cytotoxic activity with IC_{50} values of 8.81 ± 0.75 μM and 3.31 ± 0.36 μM against CCRF-CEM and CEM/ADR5000, respectively. Compound **7** showed similar inhibition with IC_{50} values of 7.89 ± 0.76 μM and 5.29 ± 0.85 μM towards CCRF-CEM and

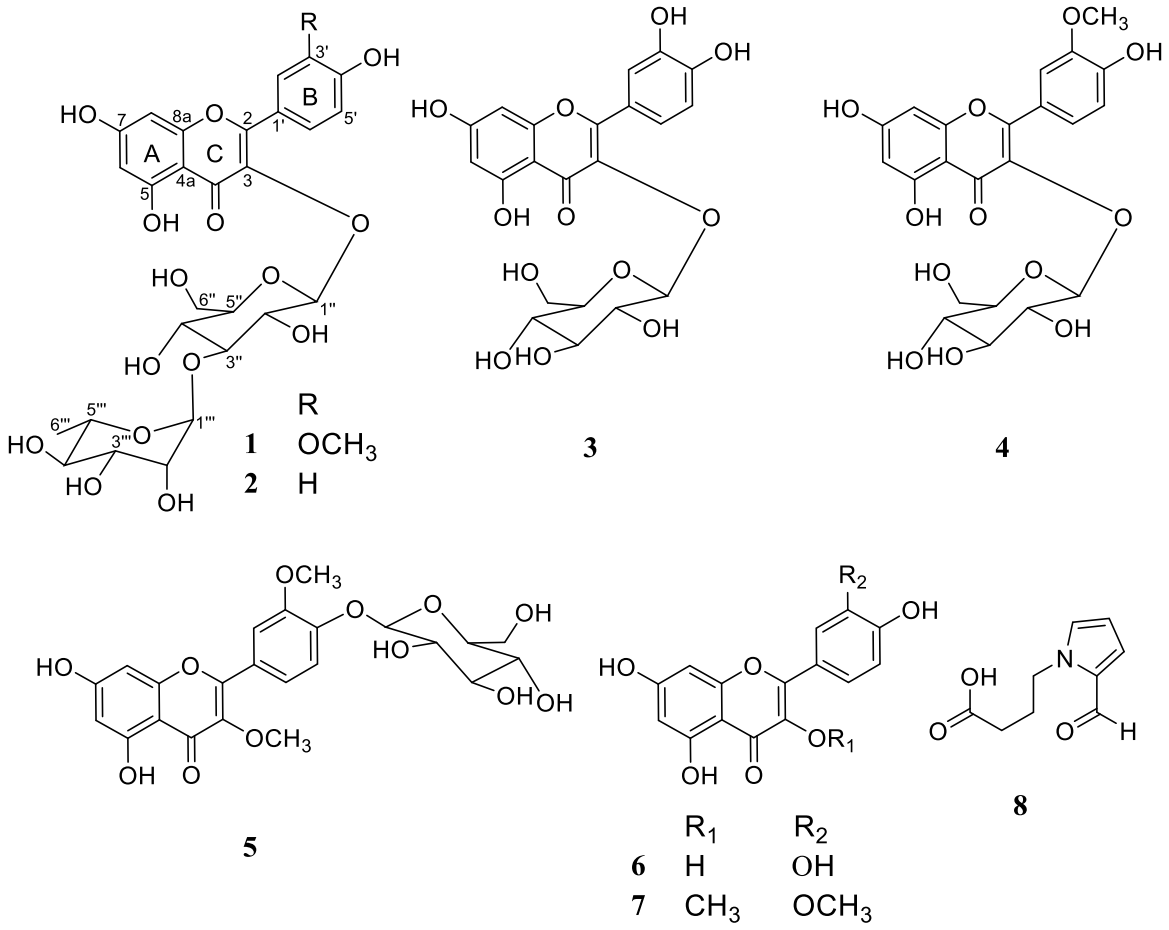

Fig. 1. Chemical structures of compounds isolated from the seeds of *Dracaena steudneri* Engl Isorhamnetin 3-*O*-rungioside (**1**), Kaempferol 3-*O*-rungioside (**2**), Quercetin-3-*O*-*β*-D-glucoside (**3**), Isorhamnetin 3-*O*-*β*-D-glucopyranoside (**4**), 3,3′-di-*O*-Methylquercetin-4′-*O*-*β*-D-glucoside (**5**), Quercetin (**6**), 3,3′-di-*O*-Methylquercetin (**7**) and 4-(2'-formyl-1'-pyrrolyl)Butanoic acid (**8**).

CEM/ADR5000, respectively. Doxorubicin (standard drug) displayed selective activity against CCRF-CEM with IC_{50} value of 0.01 ± 0.14 μM. Both isolates, **5** and **7** were 8- and 5-folds more potent than doxorubicin (IC_{50} = 26.78 ± 3.30 μM) against CEM/ADR5000 cell line. Hypersensitivity or collateral sensitivity (degree of resistance (D.R) < 1) of CEM/ADR5000 cells compared to CCRF-CEM cells was observed for 3, 3′-di-*O*-methylquercetin-4′-*O*-*β*-D-glucoside (**5**) and 3,3′-di-*O*-methylquercetin (**7**) implying that these compounds might have inhibitory effect on P-glycoprotein's expression (Mbaveng et al., 2017).

Discussion

The continuous development of drug resistance especially multidrug resistance (MDR) to chemotherapeutic agents remains a major concern for the treatment and management of cancer. Numerous mechanisms of drug resistance have been documented including the overexpression of adenosine triphosphate (ATP)-binding cassette (ABC) membrane proteins that function as drug efflux pumps (Ho et al., 2008). Among them, the permeability-glycoprotein (P-gp), and multidrug resistance protein 1 (MRP1) are relevant for acute myeloid leukemia (Van et al., 2002). Hence, the search of new secondary plant metabolites with better activity against MDR cancer cells than established anticancer drugs is of utmost importance. Numerous flavonoids isolated from plants have fueled the pipeline for cytotoxic agents against a panel of cancer cell lines (Watanabe et al., 2011; Šmejkal, 2014; Taleghani and Tayarani-Najaran, 2019). In the present study, flavonol derivatives were assessed for their anticancer potencies against drug-sensitive CCRF-CEM leukemia cells and their multidrug-resistant P-glycoprotein-overexpressing subline CEM/ADR5000 (Kimmig et al., 1990; Efferth et al., 2004).

Between incubation times of 48 and 72 h, the cytotoxicity for fractions (and isolated compounds) can be considered as strong at IC_{50} < 4 μg/mL (or IC_{50} < 10 μM), as moderate at 4 μg/mL < IC_{50} < 20 μg/mL (or 10 μM < IC_{50} < 50 μM) and as low at 20 μg/mL < IC_{50} < 100 μg/mL (or 50 μM <IC_{50} < 250 μM). No cytotoxicity can be assumed at IC_{50} > 100 μg/mL (or IC_{50} > 250 μM) (Kuete and Efferth, 2015). Based on these thresholds, the flavon-3-ol derivatives (**5** and **7**) displayed strong cytotoxicity against both leukemia cancer cell lines with IC_{50} values < 10 μM. We have chosen leukemia cells, because leukemia cells have been routinely used at the National Cancer Institute, USA, before establishment of the NCI60 panel and leukemia cells are more frequently sensitive to cytotoxic agents than other tumor types. Therefore, they are better suited for initial compound screenings than tumor cell lines from solid tumor origin. Some isolates revealed reduced cytotoxicity in the presence of a more polar groups (-OH, sugar unit) at C-3 of the flavon-3-ol scaffold in compounds **1 – 4** and **6**, instead of a more hydrophobic methyl substituent as in **5** and **7**. Compounds **1** – **4** shared more or less the same substitution pattern in A- and B-rings with the only difference being in C-ring. The presence of the sugar moiety at C-3 position in **4** had virtually no effect, since the cell inhibition was less than 10%. The same trend can be observed in **1** (no inhibition against CCRF-CEM). Compound **5** (a glucoside) was more sensitive/active on the drug-resistant leukemia cells than compound **7**. This could be explained by the fact that compound **5** with 4′-*O*-glycosyl could play a more important role in P-gp inhibition than their 4′-OH counterpart.

To the best of our knowledge, the cytotoxicity of quercetin

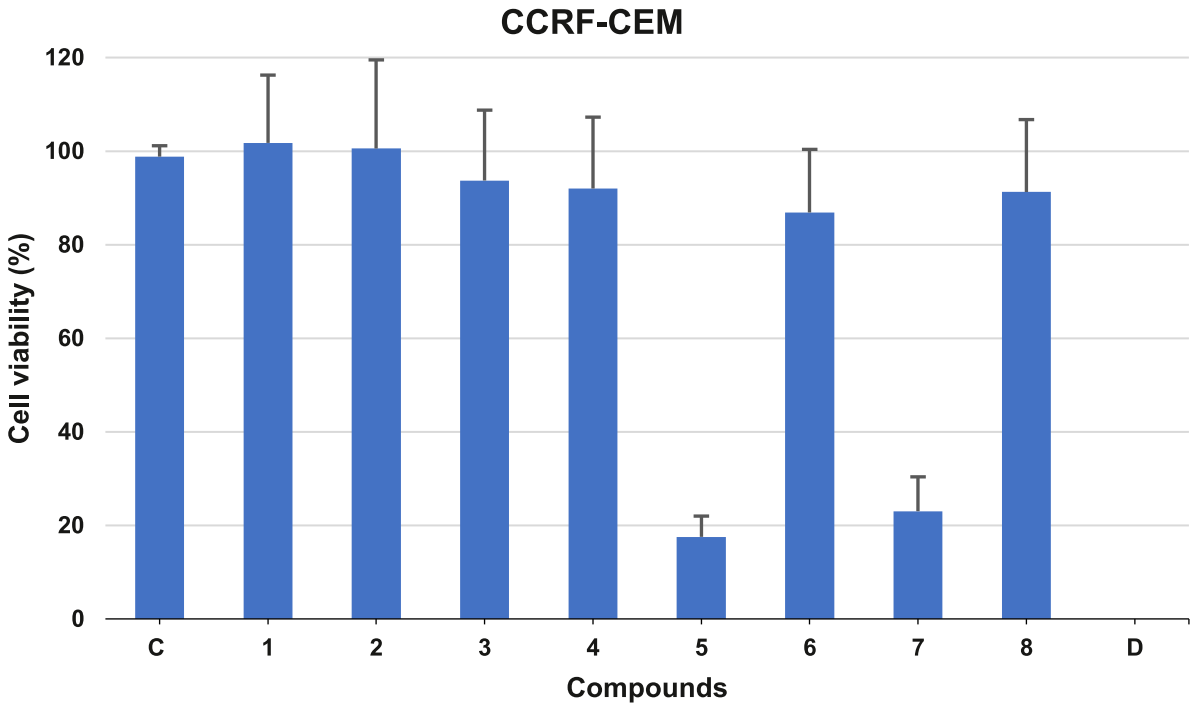

Fig. 2. Cell viability (%) of crude extract (**C**), Isorhamnetin 3-*O*-rungioside (**1**), Kaempferol 3-*O*-rungioside (**2**), Quercetin-3-*O*-*β*-D-glucoside (**3**), Isorhamnetin 3-*O*-*β*-D-glucopyranoside (**4**), 3,3′-di-*O*-Methylquercetin-4′-*O*-*β*-D-glucoside (**5**), Quercetin (**6**), 3,3′-di-*O*-Methylquercetin (**7**), 4-(2'-formyl-1'-pyrrolyl) Butanoic acid (**8**) and Doxorubicin (**D**) against drug-sensitive CCRF-CEM leukemia cell (mean ± SD of three independent experiments with each 6 parallel measurements).

Table 1
Cytotoxicity of 3,3′-di-*O*-methylquercetin-4′-*O*-*β*-D-glucoside (**5**), 3,3′-di-*O*-methylquercetin (**7**) and doxorubicin against CCRF-CEM and CEM/ADR5000 cells as determined by the resazurin reduction assay.

Compounds	CCRF-CEM IC_{50} in μM	CEM/ADR5000 IC_{50} in μM	Degree of resistance[a]
5	8.81 ± 0.75	3.31 ± 0.36	0.38
7	7.89 ± 0.76	5.29 ± 0.85	0.67
Doxorubicin	0.01 ± 0.14	26.78 ± 3.30	2678

Shown are mean ± SD of three independent experiments with each 6 parallel measurements.

[a] The degree of resistance was calculated as the ratio of IC_{50} value in multi-drug- resistant CEM/ADR5000 cells divided by the IC_{50} in sensitive CCRF-CEM cells.

derivatives **5** and **7** against leukemia cell lines including both drug-sensitive and -resistant parental sublime is documented here for the first time. The cytotoxicity of 3,3′-di-*O*-methylquercetin (**7**) confirmed similar findings reported by Talib et al. (2012) against MCF-7; IC_{50} value of 10.11 μg/mL. Compound (**7**) exerted its antiproliferative effect by inducing apoptosis as indicated by the presence of DNA fragmentation, nuclear condensation, and formation of apoptotic bodies in treated cancer cells. In contrast, quercetin-3-*O*-*β*-D-glucoside (**3**) was not cytotoxic against the cell lines tested, but previous studies showed that quercetin-3-*O*-*β*-D-glucoside (**3**) displayed minor cytotoxicity against Caco-2 and HepG2 cell lines with IC_{50} of 79 and 150 μg/mL, respectively (Maiyo et al., 2016). Although it is widely known that the antioxidant flavonoid, quercetin (**6**) play a pivotal role in apoptosis, various studies revealed that the opposite can occur depending on the cell type (Lee et al., 2003; Nicole Cotelle, 2005). In the present study, quercetin (**6**) did not displayed activity at 10 μM confirming its poor cytotoxicity against leukemia cell lines. However, literature showed, compound (**6**) was more active on Jurkat cells (IC_{50} = 8.4 μM), but was ineffective against PC-3, HepG2 and Colon 205 tumor cells (IC_{50} > 200 μM) (Rao et al., 2007).

Compounds **5** and **7** are quercetin derivatives bearing a methoxy substituent at C-3. Their activities are in good agreement with related compounds reported in the literature (Beutler et al., 1998; Díaz et al., 2003). On the basis of the results obtained in this study, the basic requirement for a flavon-3-ol hydroxylated at C-5 and C-7 position in the A-ring for cytotoxic activity seems to be methylation at C-3, whereas in the B-ring, the requirement for activity is 3′-methoxy-4′-hydroxy substitution, which facilitated cellular uptake. Multidrug-resistant CEM/ADR5000 leukemia cells line are resistant towards quite number of chemotherapeutic agents, including anthracyclines, taxanes, *Vinca* alkaloids, epipodophyllotoxons and many others (Efferth et al., 2008). Interestingly, the most active isolates were more potent than the standard drug against CEM/ADR5000 leukemia cell line with IC_{50} values < 10 μM compared to that of doxorubicin (IC_{50} = 26.78 μM). An inspection of the degree of resistance (D.R.) doxorubicin as reference anticancer agent (D.R > 1) compared to those of compounds **5** and **7** (D.R < 1) clearly indicated that the latter can be further investigated as possible cytotoxic agents against the drug-resistant cell lines. It is remarkable that CEM/ADR5000 were 2678-fold more resistant to doxorubicin than CCRF/CEM cells, while CEM/ADR5000 were more sensitive to compounds **5** and **7** than CCRF-CEM cells. This phenomenon is termed collateral sensitivity, and compound with this feature may be exquisitely suited to kill multidrug-resistant cancer cells (Efferth et al., 2020b).

Conclusion

The results obtained in this study show that quercetin derivatives (**5** and **7**) are potential anticancer agents against human leukemia cells. The cytotoxicity of these isolates can be investigated against a panel of cancer cell lines including drug-sensitive and -resistant phenotypes. Further their mode of action includes ferroptosis, necroptosis, autophagy as well as apoptosis mediated by caspases activation, MMP alteration and increase ROS production can be established.

CRediT author statement/author contribution

V-A.N-N: Data curation, TE: Data curation (resazurin assays); Writing - original draft; V-A.N-N, LKO, SD: Formal analysis; LKO, SD, TE and MS: Conceptualization, Supervision, Writing - review & editing manuscript. All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy

Supplementary data

The NMR and HRESIMS spectra of compounds **1** – **8** can be found in the supporting information.

CRediT authorship contribution statement

Vaderament-A Nchiozem-Ngnitedem: Data curation, Formal analysis, Writing – original draft. **Leonidah Kerubo Omosa:** Conceptualization, Supervision, Writing – review & editing, Formal analysis. **Solomon Derese:** Conceptualization, Supervision, Writing – review & editing, Formal analysis. **Thomas Efferth:** Conceptualization, Supervision, Writing – review & editing, Data curation, Writing – original draft. **Michael Spiteller:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Acknowledgments

Nchiozem-Ngnitedem V-Alexe is grateful for the financial support of the German Academic Exchange Service (DAAD) through the NAPRECA network (Grant number: 91672460). Special thanks to Mr. Patrick Mutiso from University of Nairobi for authentication of the plant material. We acknowledged the financial support of the International Science Program, Uppsala University, Sweden (ISP) through the KEN-02 project. We are thankful to Dr. Wolf Hiller and Mrs Eva Maria Wieczorek from TU, Dortmund, Germany for acquisitions of NMR and HRESIMS analysis, respectively.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phyplu.2022.100234.

References

Ahmad, S., Riaz, N., Saleem, M., Jabbar, A., Nisar-Ur-Rehman, Ashraf, M., 2010. Antioxidant flavonoids from *Alhagi maurorum*. J. Asian Nat. Prod. Res. 12, 138–143.
Beutler, J.A., Hamel, E., Vlietinck, A.J., Haemers, A., Rajan, P., Roitman, J.N., Cardellina, J.H., Boyd, M.R., 1998. Structure-activity requirements for flavone cytotoxicity and binding to tubulin. J. Med. Chem. 41, 2333–2338.
Bisol, A., de Campos, P.S., Lamers, M.L., 2020. Flavonoids as anticancer therapies–A systematic review of clinical trials. Phyther. Res. 34, 568–582.
Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A., Jemal, A., 2018. Global cancer statistics 2018–GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 68, 394–424.
Díaz, F., Chávez, D., Lee, D., Mi, Q., Chai, H.B., Tan, G.T., Kardono, L.B.S., Riswan, S., Fairchild, C.R., Wild, R., Farnsworth, N.R., Cordell, G.A., Pezzuto, J.M., Kinghorn, A. D., 2003. Cytotoxic flavone analogues of vitexicarpin, a constituent of the leaves of *Vitex negundo*. J. Nat. Prod. 66, 865–867.
Efferth, T., Hamels, J., Remacle, J., Longeuville, F., Bertholet, V., Steinbach, D., Gillet, J.-P., 2004. Microarray-based detection of multidrug resistance in human tumor cells by expression profiling of ATP-binding cassette transporter genes. Cancer Res. 64, 8987–8993.
Efferth, T., Kadioglu, O., Saeed, M.E.M., Seo, E.J., Mbaveng, A.T., Kuete, V., 2020a. Medicinal plants and phytochemicals against multidrug-resistant tumor cells expressing ABCB1, ABCG2, or ABCB5–A synopsis of 2 decades. Phytochem. Rev. 20, 7–53.
Efferth, T., Konkimalla, V.B., Wang, Y.F., Sauerbrey, A., Meinhardt, S., Zintl, F., Mattern, J., Volm, M., 2008. Prediction of broad spectrum resistance of tumors towards anticancer drugs. Clin. Cancer Res. 14, 2405–2412.
Efferth, T., Saeed, M.E.M., Kadioglu, O., Seo, E.J., Shirooie, S., Mbaveng, A.T., Nabavi, S. M., Kuete, V., 2020b. Collateral sensitivity of natural products in drug-resistant cancer cells. Biotechnol. Adv. 38, 107342.
Efferth, T., Sauerbrey, A., Olbrich, A., Gebhart, E., Rauch, P., Weber, H.O., Hengstler, J. G., Halatsch, M.E., Volm, M., Tew, K.D., Ross, D.D., Funk, J.O., 2003. Molecular modes of action of artesunate in tumor cell lines. Mol. Pharmacol. 64, 382–394.
Górniak, I., Bartoszewski, R., Króliczewski, J., 2019. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem. Rev. 18, 241–272.
Gottesman, M.M., Ludwig, J., Xia, D., Szakacs, G., 2009. Defeating drug resistance in cancer. Discov. Med. 6, 18–23.
Ho, M.M., Hogge, D.E., Ling, V., 2008. MDR1 and BCRP1 expression in leukemic progenitors correlates with chemotherapy response in acute myeloid leukemia. Exp. Hematol. 36, 433–442.
Kadioglu, O., Cao, J., Kosyakova, N., Mrasek, K., Liehr, T., Efferth, T., 2016. Genomic and transcriptomic profiling of resistant CEM/ADR-5000 and sensitive CCRF-CEM leukaemia cells for unravelling the full complexity of multi-factorial multidrug resistance. Sci. Rep. 6, 1–18.
Kimmig, A., Gekeler, V., Neumann, M., Frese, G., Handgretinger, R., Niethammer, D., Kardos, G., 1990. Susceptibility of multidrug-resistant human leukemia cell lines to human interleukin 2-activated killer cells. Cancer Res. 50, 6793–6800.
Kokwaro, J.O., 2009. Medicinal Plant of East Africa, 3rd edition. Univertsity of Nairobi Press, Nairobi, pp. 1–478.
Kuete, V., Efferth, T., 2015. African flora has the potential to fight multidrug resistance of cancer. Biomed. Res. Int., 914813
Kwon, D.-J., Bae, Y.-S., 2011. Chemical constituents from the stem bark of *Acer barbinerve*. Chem. Nat. Compd. 47, 636–638.
Lee, J.C., Kim, J., Park, J.K., Chung, G.H., Jang, Y.S., 2003. The antioxidant, rather than prooxidant, activities of quercetin on normal cells–Quercetin protects mouse thymocytes from glucose oxidase-mediated apoptosis. Exp. Cell Res. 291, 386–397.
Lu, P.-L., Morden, C., 2010. Phylogenetics of the plant genera *Dracaena* and *Pleomele* (Asparagaceae). Bot. Orient. 7, 64–72.
Mahmoud, N., Saeed, M.E.M., Sugimoto, Y., Klinger, A., Fleischer, E., Efferth, T., 2020. Putative molecular determinants mediating sensitivity or resistance towards carnosic acid tumor cell responses. Phytomedicine 77, 153271.
Maiyo, F., Moodley, R., In, M.S.-A.-C.A., 2016. U., 2016. Cytotoxicity, antioxidant and apoptosis studies of quercetin-3-O glucoside and 4-(β-D-glucopyranosyl-1→ 4-α-L-rhamnopyranosyloxy)-benzyl isothiocyanate from. Anticancer Agents Med. Chem. 16, 648–656.
Mbaveng, A.T., Damen, F., Guefack, M.G.F., Tankeo, S.B., Abdelfatah, S., Bitchagno, G.T. M., Çelik, İ., Kuete, V., Efferth, T., 2020. 8,8-bis-(Dihydroconiferyl)-diferulate displayed impressive cytotoxicity towards a panel of human and animal cancer cells. Phytomedicine 70, 153215.
Mbaveng, A.T., Kuete, V., Efferth, T., 2017. Potential of central, Eastern and Western Africa medicinal plants for cancer therapy–Spotlight on resistant cells and molecular targets. Front. Pharmacol. 8, 343.
Moshi, M.J., Otieno, D.F., Weisheit, A., 2012. Ethnomedicine of the Kagera Region, north western Tanzania. Part 3–Plants used in traditional medicine in Kikuku village, Muleba District. J. Ethnobiol. Ethnomed. 8, 1–11.
Mukavi, J., Omosa, L.K., Nchiozem-Ngnitedem, V-A., Nyaga, J., Omole, R., Bitchagno, G. T.M., Spiteller, M., 2020. Anti-inflammatory norhopanes from the root bark of *Fagaropsis angolensis* (Engl.) H.M.Gardner. Fitoterapia 146, 104690.
Mukazayire, M.J., Minani, V., Ruffo, C.K., Bizuru, E., Stévigny, C., Duez, P., 2011. Traditional phytotherapy remedies used in Southern Rwanda for the treatment of liver diseases. J. Ethnopharmacol. 138, 415–431.
Nchiozem-Ngnitedem, V-A., Omosa, L.K., Bedane, K.G., Derese, S., Brieger, L., Strohmann, C., Spiteller, M., 2020a. Anti-inflammatory steroidal sapogenins and a conjugated chalcone-stilbene from *Dracaena usambarensis* Engl. Fitoterapia 104717.
Nchiozem-Ngnitedem, V-A., Omosa, L.K., Bedane, K.G., Derese, S., Spiteller, M., 2020b. Inhibition of proinflammatory cytokine release by flavones and flavanones from the leaves of *Dracaena steudneri* Engl. Planta Med. 87, 209–217.
Nchiozem-Ngnitedem, V-A., Omosa, L.K., Derese, S., Tane, P., Heydenreich, M., Spiteller, M., Seo, E.J., Efferth, T., 2020c. Two new flavonoids from *Dracaena usambarensis* Engl. Phytochem. Lett. 36, 80–85.
Nicole Cotelle, B.S.P., 2005. Role of flavonoids in oxidative stress. Curr. Top. Med. Chem. 1, 569–590.
Nyaboke, O.H., Moraa, M., Omosa, L.K., Mbaveng, A.T., Nchiozem-Ngnitedem, 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 lLines. Investig. Med. Chem. Pharmacol. 1, 10.
O'Brien, J., Wilson, I., Orton, T., Pognan, Ë., 2000. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. Eur. J. Biochem. 267, 5421–5426.
Omosa, L.K., Nchiozem-Ngnitedem, V-A., Mukavi, J., Atieno, O.B., Nyaboke, O.H., Hashim, I., Obegi, M.J., Efferth, T., Spiteller, M., 2021. Cytotoxic alkaloids from the root of *Zanthoxylum paracanthum* (mildbr) Kokwaro. Nat. Prod. Res. 1–8.
Panche, A.N., Diwan, A.D., Chandra, S.R., 2016. Flavonoids–An overview. J. Nutr. Sci. 5, 1–15.
Pang, D., Su, X., Zhu, Z., Sun, J., Li, Y., Song, Y., Zhao, Y., 2016. Flavonoid dimers from the total phenolic extract of Chinese dragon's blood, the red resin of *Dracaena cochinchinensis*. Fitoterapia 115, 135–141.
Rao, Y.K., Geethangili, M., Fang, S.H., Tzeng, Y.M., 2007. Antioxidant and cytotoxic activities of naturally occurring phenolic and related compounds–A comparative study. Food Chem. Toxicol. 45, 1770–1776.

Rauter, A.P., Ennis, M., Hellwich, K.H., Herold, B.J., Horton, D., Moss, G.P., Schomburg, I., 2018. Nomenclature of flavonoids (IUPAC Recommendations 2017). Pure Appl. Chem.

Seshadri, T.R., Vydeeswaran, S., 1972. Chrysoeriol glycosides and other flavonoids of *Rungia repens* flowers. Phytochemistry 11, 803–806.

Shen, H., Zuo, W., Wang, H., Zhao, Y., Guo, Z., Luo, Y., Li, X., Dai, H., Mei, W., 2014. Steroidal saponins from dragon's blood of *Dracaena cambodiana*. Fitoterapia 94, 94–101.

Sick Woo, W., Sue Choi, J., Sik Kang, S., 1983. A flavonol glucoside from *Typha latifolia*. Phytochemistry 22, 2881–2882.

Šmejkal, K., 2014. Cytotoxic potential of C-prenylated flavonoids. Phytochem. Rev. 13, 245–275.

Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F., 2021. Global cancer statistics 2020–GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 71, 209–249.

Taleghani, A., Tayarani-Najaran, Z., 2019. Potent cytotoxic natural flavonoids–The limits of perspective. Curr. Pharm. Des. 24, 5555–5579.

Talib, W.H., Abu Zarga, M.H., Mahasneh, A.M., 2012. Antiproliferative, antimicrobial and apoptosis inducing effects of compounds isolated from *Inula viscosa*. Molecules 17, 3291–3303.

Teponno, R.B., Dzoyem, J.P., Ngansop, R., Kauhl, U., Sandjo, L.P., Tapondjou, L.A., Bakowsky, U., Opatz, T., 2017. Cytotoxicity of secondary metabolites from *Dracaena viridiflora* Engl & Krause and their semisynthetic analogues. Rec. Nat. Prod. 5, 421–430.

Teponno, Remy Bertrand, Leon, Tapondjou Azefack, Desire, Djoukeng Jules, Eliane, Abou-Mansour, Raphael, Tabacci, Tane Pierre, L.D., 2006. Isolation and NMR assignment of a pennogenin glycoside from *Dioscorea bulbifera* L. var *sativa*. Nat. Prod. Sci. 12, 62–66.

Terwilliger, T., Abdul-Hay, M., 2017. Acute lymphoblastic leukemia–A comprehensive review and 2017 update. Blood Cancer J. 7, e577.

Touil, A., Rhouati, S., Creche, J., 2006. Flavonoid glycosides from *Pituranthos chloranthus*. Chem. Nat. Compd. 42, 104–105.

Tressl, R., Kersten, E., Rewicki, D., 1993. Formation of pyrroles, 2-pyrrolidones, and pyridones by heating of 4-aminobutyric acid and reducing sugars. J. Agric. Food Chem. 41, 2125–2130.

Van Der Kolk, D.M., De Vries, E.G.E., Müller, M., Vellenga, E., 2002. The role of drug efflux pumps in acute myeloid leukemia. Leuk. Lymphoma 43, 685–701.

Wang, J., Lou, J., Luo, C., Zhou, L., Wang, M., Wang, L., 2012. Phenolic compounds from *Halimodendron halodendron* (Pall.) voss and their antimicrobial and antioxidant activities. Int. J. Mol. Sci. 13, 11349–11364.

Watanabe, M.A.E., Amarante, M.K., Conti, B.J., Sforcin, J.M., 2011. Cytotoxic constituents of propolis inducing anticancer effects–A review. J. Pharm. Pharmacol. 63, 1378–1386.

Zhu, Y., Zhang, P., Yu, H., Li, J., Wang, M.W., Zhao, W., 2007. Anti-*Helicobacter pylori* and thrombin inhibitory components from Chinese dragon's blood, *Dracaena cochinchinensis*. J. Nat. Prod. 70, 1570–1577.