Heliyon 6 (2020) e05412

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Botanicals from the leaves of *Acacia sieberiana* had better cytotoxic effects than isolated phytochemicals towards MDR cancer cells lines

Carine M.N. Ngaffo [a], Rodrigue S.V. Tchangna [b], Armelle T. Mbaveng [a], Justin Kamga [b,c], Freya M. Harvey [c], Bonaventure T. Ngadjui [b], Christian G. Bochet [c], Victor Kuete [a,*]

[a] Department of Biochemistry, Faculty of Science, University of Dschang, Dschang, Cameroon
[b] Department of Chemistry, Faculty of Science, University of Yaounde I, Yaounde, Cameroon
[c] University of Fribourg, Faculty of Science and Medicine, Department of Chemistry, Chemin du Musée 9, CH-1700 Fribourg, Switzerland

ARTICLE INFO

Keywords:
Acacia sieberiana
Apoptosis
Cytotoxicity
Fabaceae
Multi-drug resistance
Phytochemicals
Metabolite
Pharmaceutical science
Cancer research
Toxicology
Pharmacology

ABSTRACT

The efficiency of cancer chemotherapy is seriously hampered by the development of resistance of neoplastic cells to cytotoxic agents. In the present investigation, the cytotoxicity of the dichloromethane-methanol (1:1) extract of *Acacia sieberiana* (ASL), fractions (ASLa-c) from the leaves and isolated compounds: chrysoeriol-7-*O*-rutinoside (**1**), luteolin-7-*O*-rutinoside (**2**), chrysoeriol-7-*O*-β-D-glucopyranoside (**3**), Apigenin-7-*O*-β-D-glucopyranoside (**4**), luteolin-3′,4′-dimethoxylether-7-*O*-β-D-glucoside (**5**) and luteolin (**6**) was investigated. The study was extended to the assessment of the mode of induction of apoptosis by ASL. The resazurin reduction assay (RRA) was used for cytotoxicity studies. Assessments of cell cycle distribution, apoptosis, and reactive oxygen species (ROS) were performed by flow cytometry. A caspase-Glo assay was used to evaluate caspase activities. Botanicals ASL, ASLb and ASLc as well as doxorubicin displayed observable IC_{50} values towards the nine tested cancer cell lines while ASLa and compounds **1–7** had selective activities. The IC_{50} values ranged from 13.45 μg/mL (in CCRF-CEM leukemia cells) to 33.20 μg/mL (against MDA-MB-231-*BCRP* breast adenocarcinoma cells) for ASL, from 16.42 μg/mL (in CCRF-CEM cells) to 29.64 μg/mL (against MDA-MB-231-*pcDNA* cells) for ASLc, and from 22.94 μg/mL (in MDA-MB-231-*BCRP* cells) to 40.19 μg/mL (against HCT116 (*p53−/−*) colon adenocarcinoma cells) for ASLb (Table 1), and from 0.02 μM (against CCRF-CEM cells) to 122.96 μM (against CEM/ADR5000 cells) for doxorubicin. ASL induced apoptosis in CCRF-CEM cells, mediated by ROS production. *Acacia sieberiana* is a good cytotoxic plant and should be further explored to develop an anticancer phytomedicine to combat both sensitive and drug resistant phenotypes.

1. Introduction

The effectiveness of herbal medicines in the treatment of cancer is well established. Plants have been the source of many established anticancer drugs. Clinically used secondary metabolites in cancer chemotherapy include combretastatins isolated from the Combretaceae plant, *Combretum caffrum* (Eckl. & Zeyh.) Kuntze. [1], vinblastine and vincristine isolated from Madagascar periwinkle, *Catharanthus roseus* (L.) G. Don., (Apocynaceae) [2], camptothecin from *Camptotheca acuminata* Decne (Nyssaceae) [3], paclitaxel isolated from a tree of the Taxaceae family, *Taxus brevifolia* Spjut [4], or homoharringtonine isolated from *Cephalotaxus harringtonia* (Knight ex Forbes) K. Koch (Cephalotaxaceae) [3]. Regarding the high diversity of phytochemicals in the plant kingdom, medicinal plants still constitute an undeniable source of novel anticancer agents. In the last decade, intensive studies have been performed on the anticancer potential of African medicinal plants. Considering the rapid development of resistance in cancer cell lines to cytotoxic drugs, several botanicals from African flora were successfully screened against multi-drug resistant phenotypes. A number of these botanicals showed a good potential to fight recalcitrant cancers. Some of such cytotoxic plants include *Pachypodanthium staudtii* Engl. & Diels (Annonaceae) [5], *Albizia adianthifolia* (Schumach.) W. Wight (Fabaceae) [6], *Dichrostachys cinerea* (L.) Wight & Arn. (Fabaceae) [7], *Fagara tessmannii* Engl. (Rutaceae) [8], *Withania obtusifolia* L. Dunal (Solanaceae), *Jasonia candicans* (Delile) Botsch. (Asteraceae), *Centaurea lippii* L (Asteraceae) and *Pulicaria undulata* (Forssk.) Hook.f. ex Benth., Oliv. & Hiern (Asteraceae) [9]. In the continuation of our research program aimed at discovering new

* Corresponding author.
E-mail address: kuetevictor@yahoo.fr (V. Kuete).

https://doi.org/10.1016/j.heliyon.2020.e05412
Received 16 September 2020; Received in revised form 8 October 2020; Accepted 29 October 2020

botanicals to combat cancer multidrug resistance (MDR), the present work was designed to assess the cytotoxicity of the dichloromethane-methanol (1:1) extract and fractions from the leaves of *Acacia sieberiana* var Woodii (Fabaceae). This study was extended to the evaluation of possible cellular modes of action of the crude extract (ASL). *Acacia sieberiana* is traditionally used to treat bilharzia, tapeworm, heamorrhage, rheumatism, gonorrhea, syphilis, ophtalmia, stomach-ache, urethral diseases, oedema, and diarrhea [10]. The rationale of the present study comes to from fact that, there are recommendations that, ethnopharmacological usages such as immune and skin disorders, inflammatory diseases as well as infectious, parasitic and viral diseases should be taken into account when selecting plants that treat cancer; this is because these reflect disease states bearing relevance to cancer or cancer-like symptoms [11, 12, 13]. Previous phytochemical studies from the leaves of *Acacia sieberiana* harvested from Saudi Arabia afforded seven phenolics, namely 6,7,8-trihydroxy-3,4′-dimethoxy dihydroflavone, ellagic acid, gallic acid, isoferulic acid, quercetin, kaempferol, quercetin 3-*O*-β-$_D$-glucoside and kaempferol 3-α-$_L$-arabinoside [14]. The cytotoxicity of *Acacia sieberiana* is being reported for the first time.

2. Materials and methods

2.1. General procedure

Optical rotations were taken with a Polarimeter (Perkin-Elmer) using a sodium lamp operating at 589 nm. Infrared (IR) spectra (KBr disc) were recorded on a Perkin-Elmer 881 spectrometer. UV spectra were obtained on a Kontron model spectrophotometer. The nuclear magnetic resonance (NMR) spectra were performed on a Bruker DRX-400 MHz (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR) with tetramethylsilane (TMS) as internal reference. High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was carried out in the positive-ion mode. Thin Layer Chromatography (TLC) employed precoated silica gel plates $60F_{254}$ (Merck). For flavonoids, the TLC solvent system $CHCl_3$–MeOH–H_2O (80-20-02 to 70-30-03) was used. Detection by UV at $\lambda = 254$ and 365 nm was followed by spraying with H_2SO_4–H_2O (80-20). Column chromatography (CC) and Flash cartridge (Isolera) were carried out with SiO_2 gel (63–200, 60 Å) in normal phase, and DIAION HP-20.

2.2. Chemicals

The tested phytochemicals were: chrysoeriol-7-*O*-rutinoside (**1**), luteolin-7-*O*-rutinoside (**2**), chrysoeriol-7-*O*-β-$_D$-glucopyranoside (**3**), Apigenin-7-*O*-β-$_D$-glucopyranoside (**4**), luteolin-3′,4′-dimethoxylether-7-*O*-β-$_D$-glucoside (**5**) and luteolin (**6**) (Figure 1). They were isolated from the dichloromethane (CH_2Cl_2): methanol (MeOH) (1:1) extract of the leaves of *Acacia sieberiana* var Woodii (Fabaceae) as described below. Doxorubicin (purity: 98.0%) purchased from Sigma-Aldrich (Munich, Germany) was obtained from the Johannes Gutenberg University Medical Center (Mainz, Germany). Geneticin >98% (used at 800 ng/mL and 400 μg/mL in culture media for MDA-MB-231 overexpressing breast cancer resistance protein *(BCRP),* U87MG with deleted epidermal growth factor receptor *(EGFR)* and HCT116 *p53*$^{-/-}$ respectively) was obtained from Sigma-Aldrich and stored at 72.18 mM. Hydrogen peroxide (H_2O_2) was purchased from Sigma-Aldrich (Taufkirchen, Germany). DMSO (Sigma-Aldrich) was used to dissolved the test samples and the final concentration in all experiments was not greater than 0.1%.

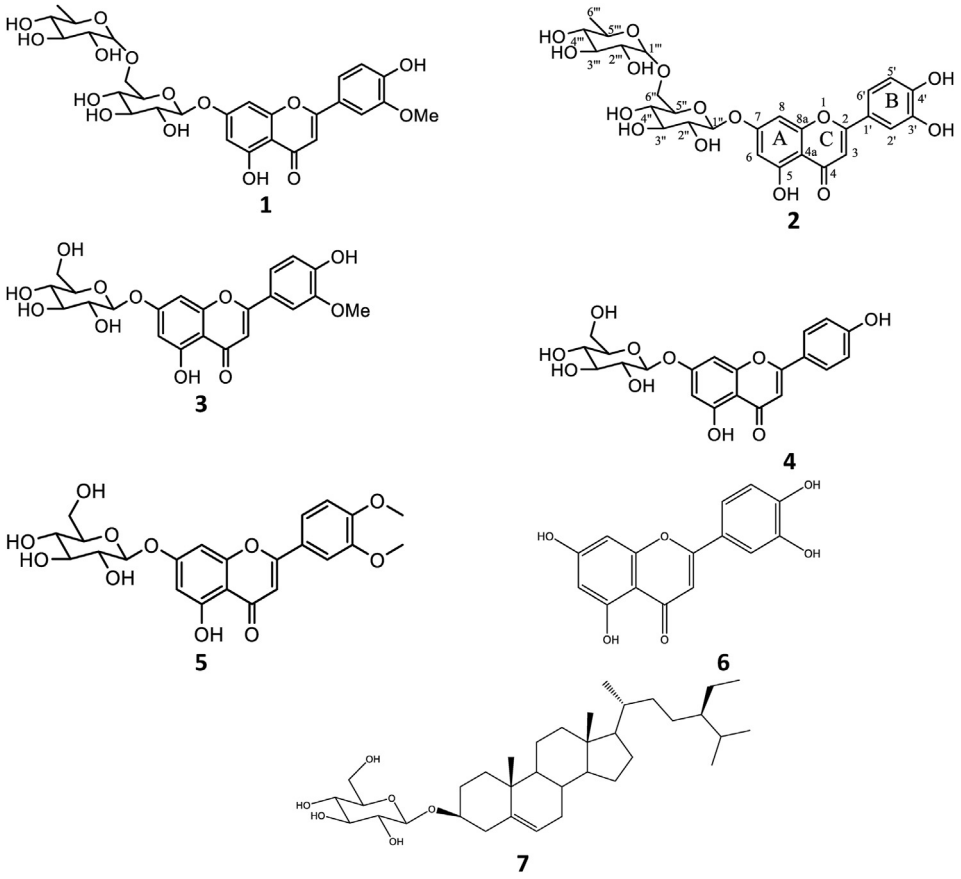

Figure 1. Chemical structure of phytochemicals isolated from the leaves of *Acacia sieberiana*. Chrysoeriol-7-*O*-rutinoside (**1**), luteolin-7-*O*-rutinoside (**2**), chrysoeriol-7-*O*-β-$_D$-glucopyranoside (**3**), apigenin-7-*O*-β-$_D$-glucopyranoside (**4**), luteolin-3′,4′-dimethoxylether-7-*O*-β-$_D$-glucoside (**5**), luteolin (**6**), sitosterol-3-*O*-β-$_D$-glucoside (**7**).

2.3. Plant material

The leaves of *Acacia sieberiana* var Woodii (Fabaceae) were collected in February 2017 at Kaélé (Far North Region, Cameroon; 10°6′00″N 14°27′00″E) and were identified by the botanist, Nana Victor of the National Herbarium of Cameroon, where a voucher specimen has been deposited under the registration number 49882/HNC.

2.4. Extraction and isolation of phytochemicals from the leaves of Acacia sieberiana

The leaves of *Acacia sieberiana* were reduced to a fine powder (930 g) and extracted by maceration in a mixture of dichloromethane (CH_2Cl_2): methanol (MeOH) (1:1) (8 L for 48 h) at room temperature. The solution was filtered and the solvents were evaporated *in vacuo* to afford a green residue that constituted the crude extract (ASL; 66 g). Part of ASL (60 g) was dissolved in water (H_2O; 300 mL) and sucessively partitioned with *n*-hexane (Hex; 200 mL), ethyl acetate (EtOAc; 200 mL) and water-satured n-butanol (n-BuOH; 200 mL) to afford three fractions, ASLa, ASLb and ASLc, respectively. ASLc and ASLb displayed the best cytotoxic activities and were further processed to isolate their active phytochemicals.

For instance, ASLc (18.512 g) was fractionated with DIAION HP-20, using respectively H_2O (100%), MeOH:H_2O (1:1) and MeOH (100%). The MeOH sub-fraction (sub-fr; 7.64 g) was further chromatographed using the Flash cartridge (Isolera) with silica gel (63–200, 60 Å) eluted with $CHCl_3$:MeOH:H_2O (90:10:01 to 70:30:03). 115 new sub-fractions (frs) of 25 mL each were collected as follows: $CHCl_3$:MeOH:H_2O (90:10:01): sub-frs 1 to 71; $CHCl_3$:MeOH:H_2O (80:20:02): sub-frs 72 to 91; $CHCl_3$:MeOH:H_2O (70:30:03): sub-frs 92 to 115. They were further pooled into six sub-frs on the basis of their thin layer chromatography (TLC) profiles as follows: ASLc1 (sub-frs 1 to 21), ASLc2 (sub-frs 22 to 70), ASLc3 (sub-frs 71 to 83), ASLc4 (sub-frs 84 to 91), ASLc5 (sub-frs 92 to 108) and ASLc6 (sub-frs 109 to 115). Sub-fraction ASLc6 was further column chromatographed similarly to the methanol sub-fr above, eluting with $CHCl_3$:MeOH:H_2O (70:30:03); 120 fractions (frs) of 20 mL each were collected as follows: $CHCl_3$:MeOH:H_2O (85: 15 : 1.5): frs 1 to 40; $CHCl_3$:MeOH:H_2O (80 : 20: 02): frs 41 to 87; $CHCl_3$:MeOH:H_2O (70 : 30: 01): frs 88 to 120; Sub-fr eluted with $CHCl_3$:MeOH:H_2O (80:20:02) yielded compound **2** (603 mg) whilst compound **1** (27 mg) was obtained from the eluted with $CHCl_3$:MeOH:H_2O (80:20:02). The mixture of sub-fractions 1 to 87 was eluted with $CHCl_3$:MeOH:H_2O (90:10:01 to 80:20:02); 90 new sub-frs of 10 mL each were collected as follows: $CHCl_3$:MeOH:H_2O (90: 10: 01): frs 1 to 60; $CHCl_3$:MeOH:H_2O (85: 15 : 1.5): frs 61 to 90. This afforded compounds **3** (14 mg; sub-frs 78 to 86), **4** (8 mg; sub-frs 59 to 67), **5** (11 mg; sub-frs 28 to 33) and **7** (16 mg; sub-frs 14 to 22).

Fraction ASLb (23.217 g) was further column chromatographed using the Flash cartridge (Isolera) with silica gel (63–200, 60 Å) eluted with $CHCl_3$:MeOH:H_2O (90:10:01 to 80:20:02); 81 new sub-frs of 20 mL each were collected as follows: $CHCl_3$:MeOH:H_2O (90 : 10: 01): sub-frs 1 to 32; $CHCl_3$:MeOH:H_2O (85 : 15: 1.5): sub-frs 33 to 61; $CHCl_3$:MeOH:H_2O (80 : 20: 02): sub-frs 62 to 81. They were further pooled into two main sub-frs based on their TLC profiles as follows: ASLb1 (sub-frs 1 to 47) and ASLb2 (sub-frs 48 to 81). Sub-fraction ASLb2 was further column chromatographed similarly to the methanol sub-fr above, eluting with $CHCl_3$:MeOH:H_2O (80:20:02). 34 new sub-frs of 10 mL each were collected. Compound **6** (15 mg) was obtained from sub-frs 22 to 30 whilst sub-frs 8 to 17 afforded compound **7** (12 mg).

2.5. Investigated cell lines

The total of 10 cell lines (9 cancer cells a normal AML12 hepatocyte) used in this study. Previous reports provided their origins and their characteristics; both drug-sensitive CCRF-CEM leukemia cells and its multidrug-resistant P-glycoprotein-overexpressing subline CEM/ADR5000 cells [15, 16, 17], HCT116 $p53^{+/+}$ colon cancer cells and its knockout clone HCT116 $p53^{-/-}$, U87.MG glioblastoma cells and its EGFR-transfected U87.MGΔ*EGFR* subline, MDA-MB-231-pcDNA3 breast cancer cells and *BCRP*-transfected, multidrug-resistant MDA-MB-231-*BCRP* clone 23 cells [18] were investigated. Besides HepG2 liver cancer cells [19], and normal AML12 hepatocytes were also used; AML12 hepatocytes were used to compare with HepG2 cells [19].

2.6. Cytotoxicity assay

The cytotoxicity of botanicals (ASL, ASLa-ASLc), phytochemicals (**1–6**) and doxorubicin was determined using the well-described resazurin reduction assay (RRA) [20, 21] at identical experimental conditions as documented earlier [22, 23]. After 72 h incubation in the standard culture condition (humidified 5% CO_2 atmosphere at 37 °C), the fluorescence was measured with Infinite M2000 Pro™ plate reader (Tecan, Crailsheim, Germany) at 544 nm as excitation wavelength and 590 nm as detection wavelength. The IC_{50} was defined as the concentrations of botanicals, phytochemicals or doxorubicin required to inhibit 50% of the cell proliferation, and were determined by linear regression using Microsoft Excel 2007 [24]. If both sensitive cells and their corresponding resistant counterparts were tested, the degree of resistance (D.R.) against the tested samples was determined as the ratio of the IC_{50} value of the resistant cell line divided by that of the corresponding parental sensitive cell line. The selectivity index (S.I.) was determined as the ratio of the IC_{50} value of the normal AML12 hepatocyte divided by that of HepG2 hepatocarcinoma cells.

2.7. Analysis of cell cycle distribution and detection of apoptotic cells by flow cytometry

The distribution of cycle phases of CCRF-CEM cells after application of the crude extract (ASL) or doxorubicin was analyzed by the flow cytometry [25, 26]. We followed the methods of Mbaveng et al. [23]. Additionally, flow cytometry was also applied with annexin V/propidium iodide (PI) staining to detect apoptotic cells as previously described [25, 26]. Briefly, CCRF-CEM cells (1×10^6 cells) were treated with various concentrations of ASL, doxorubicin or DMSO (solvent control). After 24 h incubation in the standard culture condition described above, the cell cycle was analyzed using a BD Accury C6 Flow Cytometer (BD Biosciences, Heidelberg, Germany) by measuring the propidium iodide fluorescence of individual nuclei. For the annexin V/PI staining, apoptosis was also assessed after 24 h incubation using fluorescein isothiocynate (FITC)-conjugated annexin V/PI assay kit (eBioscience™ Annexin V; Invitogen, San Diego, USA) and BD Accury C6 Flow Cytometer (BD Biosciences). Cells were stained with 5 μL FITC-conjugated annexin V (10 mg/mL) and 10 μl PI (50 mg/mL) and incubated for 15 min in the dark at room temperature (RT) and then analyzed using BD Accury C6 Flow Cytometer (BD Biosciences) [25, 26]. At least three independent experiments with three repetitions each were done.

2.8. Detection of caspases activation

Caspases activity in CCRF-CEM cells treated with ASL was determined by measuring the luminescence using spectrophotometric method. After application of ASL to the cells, followed by 6 h incubation under standard culture conditions, the activities of caspases were evaluated using Caspase-Glo 3/7, 8 and 9 Assay kits (Promega, Mannheim, Germany) with an Infinite M2000 ProTM plate reader (Tecan) as previously reported [19]. The protocol was followed as described by Mbaveng et al. [23].

2.9. Flow cytometric analysis of ROS production

The production of reactive oxygen species (ROS) in CCRF-CEM cells after application of ASL was detwemined by the flow cytometry. For instance, CCRF-CEM cells were treated with ASL, DMSO (solvent

control), or hydrogen peroxide (H_2O_2; positive control). After 24 h incubation in standard culture conditions, cells were resuspended in Phosphate Buffer Saline (PBS; Sigma-Aldrich) and the production of ROS was evaluated using 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFH-DA) (Sigma-Aldrich) staining as earlier described [27, 28, 29]. The protocol was followed as described by Mbaveng et al. [23].

2.10. Statistics

Graph pad prism 5 software was used for statistical analyses. Representative data from three independent experiments are shown as mean value ±S.E.M. One-way Analysis Variance (ANOVA) followed by post hoc Tukey's test was used to determine the significance of the difference between mean values relative to the control. The *p*-value was calculated to determine significant differences (p-value < 0.05).

3. Results

3.1. Phytochemistry

The phytochemical investigations of the leaves of *Acacia sieberiana* led to the isolation of six phenolic including one flavone: luteolin (**6**) and five flavone glycosides: chrysoeriol-7-*O*-rutinoside (**1**), luteolin-7-*O*-rutinoside (**2**), chrysoeriol-7-*O*-β-$_D$-glucopyranoside (**3**), apigenin-7-*O*-β-$_D$-glucopyranoside (**4**), luteolin-3′,4′-dimethoxylether-7-*O*-β-$_D$-glucoside (**5**) and a terpenoid, the sterol glycoside identified as sitosterol-3-*O*-β-$_D$-glucoside (**7**) (Figure 1).

3.2. Cytotoxicity of botanicals and phytochemicals

The cytotoxicity of the crude extract, ASL and its fractions ASLa to ASLc was first performed against a panel of 9 cancer cells lines and the normal AML12 hepatocytes. The results are summarized in Table 1. Botanicals ASL, ASLb and ASLc displayed observable IC_{50} values towards all tested cancer cell lines while ASLa had selective activity. The recorded IC_{50} values ranged from 13.45 μg/mL (in CCRF-CEM leukemia cells) to 33.20 μg/mL (against the resistant MDA-MB-231-*BCRP* breast adenocarcinoma cells) for ASL, from 16.42 μg/mL (in CCRF-CEM cells) to 29.64 μg/mL (against MDA-MB-231-*pcDNA* breast adenocarcinoma cells) for ASLc, and from 22.94 μg/mL (in MDA-MB-231-*BCRP* cells) to 40.19 μg/mL (against the resistant HCT116 ($p53^{-/-}$) colon adenocarcinoma cells) for ASLb (Table 1), and from 0.02 μM (against CCRF-CEM cells) to 122.96 μM (against CEM/ADR5000 leukemia cells) for doxorubicin (Table 2). Hypersensitivity of HCT116 $p53^{-/-}$ cells (D.R. of 0.91) and U87MG.*ΔEGFR* glioblastoma cells (D.R. of 0.86) to ASL compared to their sensitive counterparts HCT116 $p53^{+/+}$ cells and U87MG cells was observed meanwhile that of the MDR MDA-MB-231-*BCRP* cells and U87MG.*ΔEGFR* cells to ASLb (D.R. of 0.72 and 0.74, respectively) and ASLc (D.R. of 0.68 and 0.75, respectively) compared to their respective sensitive counterparts was also noted (Table 1). Observable IC_{50} values were obtained with ASLa on 6/9 cancer cell lines tested. This fraction (ASLa) was less active than ASLb and ASLc and was no further processed for the isolation of its active constituents. The purification of ASLc led to phytochemicals **1–5** and **7** while that of ASLb afforded compounds **6** and **7**. The cytotoxicity of phytochemicals **1–6** was further evaluated on the tested panel of cancer cell lines and AML12 hepatocytes. The results summarized in Table 2 show that all the tested compounds had selective cytotoxic effects, with obervable IC_{50} values obtained in 7/9, 7/9, 6/9, 3/9, 3/9 and 1/9 for **3, 6, 5, 1, 2** and **5**, respectively. It is worthnoting that the hypersensitivity CEM/ADR5000 leukemia cells (D.R. of 0.90), HCT116 $p53^{-/-}$ cells (D.R. of 0.93) and U87MG.*ΔEGFR* cells (D.R. of 0.85) to compound **3** compared with their respective sensitive counterparts was also acheived (Table 2). From the cytotoxicity data, it appears that the best activity was obtained with the crude extract, ASL. Consequently, the cellular mode of action of ASL was investigated towards the most sensitive CCRF-CEM cells.

3.3. Cell cycle distribution and apoptosis

The effects of botanical ASL and doxorubicin on the distribution of the cell cycle of CCRF-CEM cells after 24 h treatment are depicted in Figure 2. ASL and doxorubicin modified the cell cycles' distribution in concentration-dependent manner. ASL induced cycle arrest in the G0/G1 phase in CCRF-CEM cells, whilst doxorubicin induced S and G2/M phase arrest (Figure 2). ASL also increased significantly the amount of cells in the sub-G0/G1 phase in a range from 3.35% (3.36 μg/mL) to 20.05%

Table 1. IC_{50} values botanicals from the leaves of *Acacia sieberiana* towards of drug sensitive and MDR cancer cells lines after 72 h incubation.

Cell lines	Samples, IC_{50} values in μg/mL and degrees of resistance* or selectivity index**			
	Crude extract	Fractions		
	ASL	ASLa	ASLb	ASLc
CCRF-CEM	**13.45 ± 2.12**	44.17 ± 3.58	23.08 ± 1.72	**16.42 ± 1.33**
CEM/ADR5000	**14.26 ± 0.88**	>80	26.55 ± 3.22	20.17 ± 1.17
Degree of resistance*	1.06	>1.81	1.15	1.23
MDA-MB-231-*pcDNA*	27.76 ± 4.09	33.61 ± 1.83	31.82 ± 1.72	29.64 ± 2.55
MDA-MB-231-*BCRP*	33.20 ± 3.13	38.12 ± 2.40	22.94 ± 3.01	20.27 ± 1.16
Degree of resistance	1.20	1.13	0.72	0.68
HCT116 ($p53^{+/+}$)	**18.85 ± 1.10**	28.14 ± 0.96	34.52 ± 2.37	**16.71 ± 1.84**
HCT116 ($p53^{-/-}$)	**17.23 ± 2.01**	>80	40.19 ± 3.51	23.87 ± 1.76
Degree of resistance	0.91	>2.84	1.16	1.41
U87MG	**19.44 ± 1.89**	44.16 ± 4.78	32.12 ± 2.46	28.09 ± 1.81
U87MG.Δ*EGFR*	**16.78 ± 1.72**	27.30 ± 3.28	23.66 ± 1.19	21.11 ± 1.97
Degree of resistance	0.86	0.62	0.74	0.75
HepG2	21.65 ± 2.09	>80	38.95 ± 2.87	23.19 ± 2.08
AML12	67.53 ± 4.76	>80 nd	>80	67.86 ± 4.91
Selectivity index**	3.12		2.05	2.93

(*): The degree of resistance was determined as the ratio of IC_{50} value in the resistant divided by the IC_{50} in the sensitive cell line; CEM/ADR5000, MDA-MB-231-*BCRP*, HCT116 ($p53^{-/-}$) and U87MG.Δ*EGFR* were used as the corresponding resistant counterpart for CCRF-CEM, MDA-MB-231-*pcDNA*, HCT116 ($p53^{+/+}$), U87MG respectively; (**): The selectivity index was determined as the ratio of IC_{50} value in the normal AML12 hepacytes divided by the IC_{50} in HepG2 hepatocarcinoma cells; In bold: Significant cytotoxic effect [30, 34, 35]; nd: not determined; ASL: crude CH2Cl2:MeOH (1:1) extract from the leaves of *Acacia sieberiana*, ASLa: Hexane fractions from ASL; ASLb: ethyl acetate fraction from ASL; ASLc: n-butanol fraction from ASL; nd: not determined; The data for doxorubicin used as positive control in similar experimental conditions are shown in Table 2.

Table 2. IC_{50} values phytochemicals from the leaves of *Acacia sieberiana* and doxorubicin towards of drug sensitive and MDR cancer cells lines after 72 h incubation.

Cell lines	Samples, IC_{50} values in μM and degrees of resistance* or selectivity index**						
	1	2	3	4	5	6	Doxorubicin
CCRF-CEM	51.63 ± 4.23	64.65 ± 4.89	20.80 ± 1.76	**10.40 ± 1.17**	58.52 ± 5.28	**10.97 ± 0.97**	**0.02 ± 0.00**
CEM/ADR5000 Degree of resistance*	>100	>100	18.68 ± 1.25 0.90	>100 >9.62	18.68 ± 0.64 0.32	27.04 ± 1.29 2.46	122.96 ± 10.94 6,683.00
MDA-MB-231-*pcDNA*	>100	>100	>100	>100	>100	>100	**0.13 ± 0.01**
MDA-MB-231-*BCRP* Degree of resistance	>100 nd	>100 nd	>100 nd	>100 nd	>100 nd	>100 nd	**0.79 ± 0.08** 6.14
HCT116 ($p53^{+/+}$)	>100	>100	30.69 ± 4.04	>100	>100	28.32 ± 1.07	**0.48 ± 0.06**
HCT116 ($p53^{-/-}$) Degree of resistance	>100	>100	28.43 ± 1.72 0.93	>100	27.98 ± 1.43 <0.28	30.35 ± 2.55 1.07	**1.78 ± 0.08** 3.73
U87MG	42.48 ± 3.12	46.71 ± 2.98	24.92 ± 2.19	>100	33.54 ± 2.09	22.71 ± 2.20	**0.26 ± 0.03**
U87MG.Δ*EGFR* Degree of resistance	38.12 ± 3.48 0.90	48.22 ± 4.51 1.03	21.08 ± 0.92 0.85	>100	27.34 ± 3.12 0.82	18.95 ± 1.06 0.83	**0.98 ± 0.07** 3.79
HepG2	>100	>100	34.25 ± 2.86	>100	27.93 ± 1.57	28.98 ± 1.77	**4.56 ± 0.48**
AML12 Selectivity index**	>100 nd	>100 nd	>100 >2.92	>100 nd	>100 >3.58	>100 >3.45	52.90 ± 4.09 11.59

(*): The degree of resistance was determined as the ratio of IC_{50} value in the resistant divided by the IC_{50} in the sensitive cell line; CEM/ADR5000, MDA-MB-231-*BCRP*, HCT116 ($p53^{-/-}$) and U87MG.Δ*EGFR* were used as the corresponding resistant counterpart for CCRF-CEM, MDA-MB-231-*pcDNA*, HCT116 ($p53^{+/+}$), U87MG respectively; (**): The selectivity index was determined as the ratio of IC_{50} value in the normal AML12 hepacytes divided by the IC_{50} in HepG2 hepatocarcinoma cells; In bold: Significant cytotoxic effect [30, 34, 35], The cytotoxicity of compound **7** sitosterol-3-*O*-β-$_D$-glucoside) shown as non-active on these cell lines was previous reported [8] and this compound was no more tested in this study, Chrysoeriol-7-*O*-rutinoside (**1**), luteolin-7-*O*-rutinoside (**2**), chrysoeriol-7-*O*-β-$_D$-glucopyranoside (**3**), Apigenin-7-*O*-β-$_D$-glucopyranoside (**4**), luteolin-3′,4′-dimethoxylether-7-*O*-β-$_D$-glucoside (**5**), luteolin (**6**); nd: not determined.

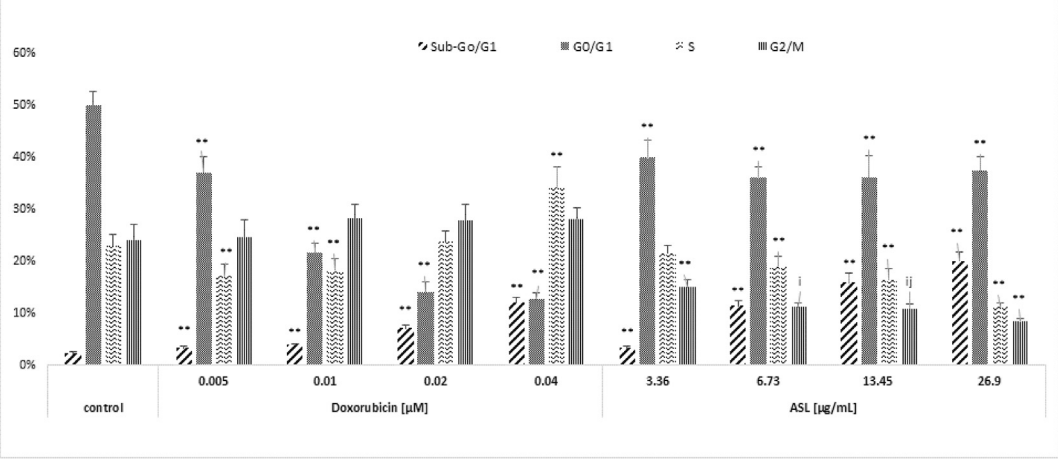

Figure 2. Effects of the crude extract (ASL) and doxorubicin on the distribution of CCRF-CEM cells' cycle after 24 h treatment. IC_{50} values were 13.45 μg/mL for ASL and 0.02 μM for doxorubicin. Control cells were treated with DMSO to a final concentration of 0.1%. (**): values are significantly different to that of untreated control ($P < 0.05$).

(26.9 μg/mL). The corresponding significant increase for doxorubicin was from 3.28% (0.005 μM) to 12.05% (0.04 μM). The induction of apoptosis by CCRF-CEM cells by ASL and doxorubicin was later confirmed by using annexin V/PI staining as shown in Figure 3. It can be observed that the treatment of CCRF-CEM cells with 26.9 μg/mL of ASL induced late apoptosis with 17.6% annexin V (+)/PI (+) cells *vs.* 6.1% in non-treated control cells (Figure 3).

3.4. Activation of caspases

The effects of ASL on the activation of caspases in CCRF-CEM cells was also investigated. The results depicted in Figure 4 show that, though some significant differences were observed, treatment of cells with ASL does not importantly regulate the activity of caspases, with only 1.02-fold, 1.11-fold and 1.03-fold increases, respectively, for caspases 3/7, 8 and 9 at 26.9 μg/mL. Hence, ASL is not an intrinsic (caspase 3/7 and 9) nor extrinsic (caspase 8) caspases modulator.

3.5. Production of ROS

After treatment of CCRF-CEM cells with ASL or H_2O_2, increase of ROS level was observed as compared to cells treated with solvent control, DMSO (Figure 5). Botanical ASL significantly enhanced the production of ROS in the range of 3.36% (3.36 μg/mL) to 26.9% (26.9 μg/mL). Treatment of the cells with the reference compound, H_2O_2, increased the ROS levels to 94.30% at 50 μM, while ROS production in non-treated cells was 0.6%.

4. Discussion

Recalcitrant cancers are the major cause of treatment failures in cancer patients suffering from malignant diseases. Therefore, the use of resistant cell lines when searching for novel cytotoxic drugs is an interesting strategy. In the present study, several models of MDR cancer cell lines were used. They included a transfectant cell line harboring a

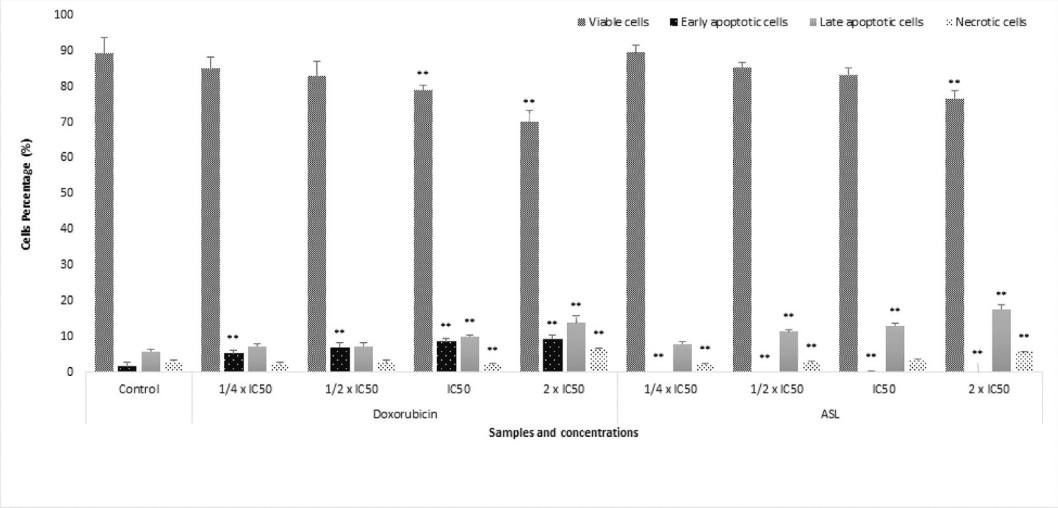

Figure 3. Apoptotic effect of the crude extract (ASL) and doxorubicin as determined by the annexin V/PI assay in CCRF-CEM cells after 24 h. Flow cytometric measurements were performed after annexin V-PI double staining. IC_{50} values were 13.45 μg/mL for ASL and 0.02 μM for doxorubicin. Shown are mean values ±SD of three independent experiments. Control cells were treated with DMSO to a final concentration of 0.1%.

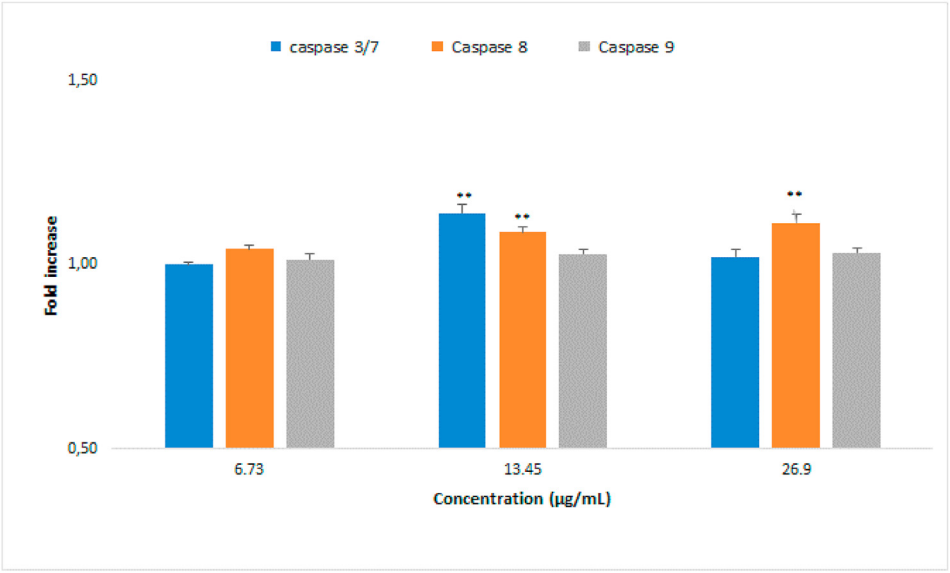

Figure 4. Effects of the crude extract (ASL) on caspase activities in CCRF-CEM cells treated for 6 h. Caspase activity is expressed as percentage (%) compared to untreated cells. Shown are mean values ±SD of three independent experiments. Untreated cells were treated only with DMSO to a final concentration of 0.1%. (**): values are significantly different to that of untreated control ($P < 0.05$).

mutation-activated EGFR gene (ΔEGFR), p53 knockout cell line, breast cancer resistance protein (ABCG2/BCRP) and ATP-binding cassette (ABC)-transporter-overexpressing MDR-mediating-P-glycoprotein (P-gp; ABCB1/MDR1). The cross-resistance profile of CEM/ADR5000 cells to *Vinca* alkaloids, anthracyclines, epipodophyllotoxins or taxanes, has been demonstrated [30]; that of the MDA-MB-231-*BCRP* has been shown towards doxorubicin, daunorubicin, mitoxantrone and topotecan [18, 22]; cross-resistance of HCT-116 $p53^{-/-}$ was reported towards 5-fluorouracil, 6-thioguanine, gemcitabine, cisplatin, oxaliplatin, doxorubicin, etoposide, irinotecan paclitaxel, bleomycin, bortezomib [31, 32]; The resistance of U87MG.*ΔEGFR* cells was reported towards doxorubicin, cisplatin, erlotinib, and homoharringtonine [32]. Interestingly, collateral sensitivity or hypersensitivity of HCT116 $p53^{-/-}$ colon adenocarcinoma cells and U87MG.*ΔEGFR* cells to ASL as well as that of MDA-MB-231-*BCRP* breast adenocarcinoma cells and U87MG.*ΔEGFR* glioblastoma cells to ASLb and were observed (Table 1). These data clearly indicate that these botanicals can be exploited in the fight against refractory tumors. More importantly, IC_{50} values below 20 μg/mL were obtained with ASL against 6/9 tested cancer cells lines and with ASLc against 2/9 cancer cell lines (Table 1). In effect, the cutoff point for good botanicals has been set below 20 μg/mL upon 48 h or 72 h incubation by the National Cancer Institute USA (NCI) [33]. It can therefore be confirmed that the crude extract ASL, and in lesser extend fraction ASLc are interesting cytotoxic agents. These data also show that fractions ASLb and had better cytotoxic activity than ASLa, explaining why they were selected for further isolation of their bioactive constituents. The purification of ASLc afforded phytochemicals **1–5** while that of ASLb led to **6** and **7**. Unfortunately, none of these compounds had a good cytotoxic activity, as they generally displayed IC_{50} values above 10 μM (Table 2). In effect, the cytotoxicity of phytochemicals is considered significant if the recorded IC_{50} value is below 10 μM [7, 33, 34]. This might be indication that the good activity of the crude extract and in lesser extent that of the

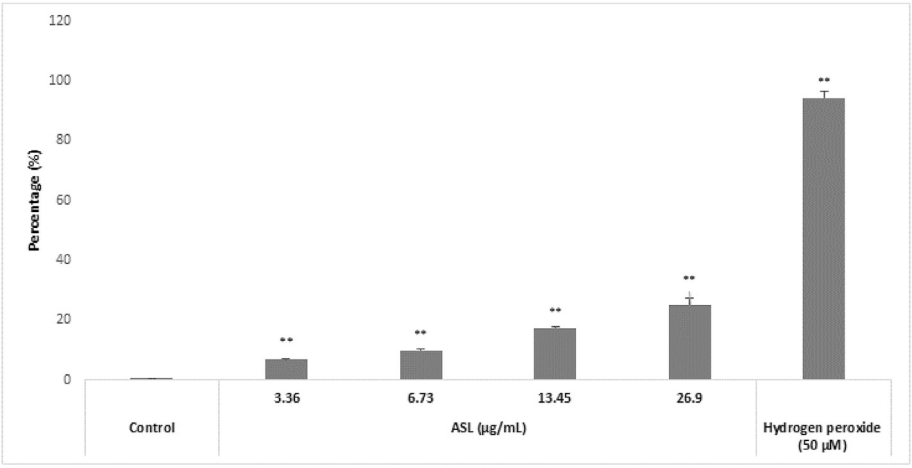

Figure 5. Effects of the crude extract (ASL) and hydrogen peroxide (H_2O_2) on the production of reactive oxygen species in CCRF-CEM cells treated for 24 h. IC_{50} value of 13.45 µg/mL for ASL; Shown are mean values ±SD of three independent experiments. Control cells were treated with DMSO to a final concentration of 0.1%. (**): values are significantly different to that of control ($P < 0.05$).

fractions ASLc and ASLb could be due the synergistic effects of their various constituents, rather than to the prominent inhibitory activity of individual compounds. It is important to note that several flavonoids such chrysoeriol-7-*O*-rutinoside (**1**), luteolin-7-*O*-rutinoside (**2**), chrysoeriol-7-*O*-β-D-glucopyranoside (**3**), apigenin-7-*O*-β-D-glucopyranoside (**4**), luteolin-3′,4′-dimethoxylether-7-*O*-β-D-glucoside (**5**) and luteolin (**6**) were isolated from ASL. Their modes of action were not evaluated in the presenst study, in regards to their low or moderate activity towards the tested cancer cell lines. However, various flavone aglycones such artocarpesin gancaonin Q, 6-prenylapigenin or 6,8-diprenyleriodictyol have earlier been shown to induce apoptosis in CCRF-CEM, through caspase activation, MMP alteration or ROS production [35, 36]. Luteolin has also been reported to induce apoptosis in SMMC-7721 human liver cancer cells [37], and A375 human melanoma cells *via* suppression of MMP-2 and MMP-9 through the PI3K/AKT pathway [38]. Chrysoeriol also had cytotoxic effects towards A549 human lung cancer cells *via* activation of autophagy, sub-G1/G0 cell cycle arrest, cell migration and invasion inhibition, and modulation of MAPK/ERK signalling pathway [39]. Apigenin induced apoptosis and autophagy in HepG2 cells through inhibition of PI3K/Akt/mTOR pathway [40]. It is worth noting that sitosterol-3-*O*-β-D-glucoside (**7**) was not tested in the present study, because a previous work had documented its poor cytotoxicity towards the cancer cell lines tested herein, with all IC_{50} values above 100 µM [8]. Consequently, only the crude extract, which had the best cytotoxic activity, was further selected for mechanistic studies. The cellular modes of action of ASL, including cell cycle distribution, apoptosis, caspases activation and ROS level evaluations, were determined towards the most sensitive CCRF-CEM leukemia cell line.

Apoptosis induction in cancer cells has been documented as a major mode of cellular death provoked by cytotoxic drugs including botanicals and phytochemicals [23, 25, 41, 42]. In the present study, it was found that ASL induced apoptosis in CCRF-CEM cells and provoked cycle arrest in the G0/G1 phase (Figure 2) and this was later confirmed by the annexin V/PI results (Figure 3). ASL did not activate neither intrinsic caspase 3/7 and 9 nor the extrinsic caspase 8 (Figure 4), suggesting a poor involvement of intrinsic and extrinsic pathways in the apoptotic process induced by this botanical. The increase production of ROS has also been reported in the apoptotic process induced by various botanicals and phytochemicals in cancer cells [8, 41, 43, 44]. Treatment of CCRF-CEM cells with the crude extract, ASL, led to important increased in ROS level (Figure 5). This suggests that ROS production contributes to ASL-induced cell death. Finally, the present study shows that ASL extract induces apoptosis and increases the production of ROS, which is closely related to apoptosis, but is not caspase-dependent. Such phenomenon have peviously been identified for several botanicals [29, 44, 45]. Caspase-independent pathways have also been reported in cadmium-induced apoptosis renal in proximal tubule (RPT) cells [46]; In effect, caspase-independent apoptotic pathway, known as the apoptosis-inducing factor (AIF)/Endo G pathway has earlier been discussed. Caspase-independent apoptosis is activated by Bcl-2/adenovirus E1B 19-kDa interacting protein 3 (BNIP-3), which induces mitochondrial AIF release; Endo G acts as a modulator. Forced BNIP-3 expression by plasmid transfection results in mitochondrial Endo G release and nuclear translocation, leading to cell death [47].

5. Conclusion

The results of the present investigation demonstrate that *Acacia sieberiana* is a good cytotoxic plant that can help to fight cancers, including MDR phenotypes. Its active constituents: chrysoeriol-7-*O*-rutinoside, luteolin-7-*O*-rutinoside, chrysoeriol-7-*O*-β-D-glucopyranoside, apigenin-7-*O*-β-D-glucopyranoside, luteolin-3′,4′-dimethoxylether-7-*O*-β-D-glucoside, luteolin and sitosterol-3-*O*-β-D-glucoside may act synergically to contribute to the cytotoxicity of the plant. The crude extract induced apoptosis in CCRF-CEM cells through increased ROS production. This extract should be further explored to develop a new drug to fight cancers.

Declarations

Author contribution statement

V. Kuete: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

C.M.N. Ngaffo: Performed the experiments; Contributed reagents, materials, analysis tools or data.

R.S.V. Tchangna: Performed the experiments.

A.T. Mbaveng: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

J. Kamga: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

F.M. Harvey and C.G. Bochet: Contributed reagents, materials, analysis tools or data.

B.T. Ngadjui: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Funding statement

This work was supported by the Alexander von Humboldt (AvH) foundation (Grant no.: CMR 1163890 GF-E to ATM) and the Swiss Government Excellence Scholarship (Grant no.: 2017.1063 to JK).

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2020.e05412.

Acknowledgements

All authors are also thankful to Prof. Dr. Thomas Efferth for providing facilities for cytotoxic studies.

References

[1] A. Cirla, J. Mann, Combretastatins: from natural products to drug discovery, Nat. Prod. Rep. 20 (2003) 558–564.
[2] N.P. Gullett, A.R. Ruhul Amin, S. Bayraktar, J.M. Pezzuto, D.M. Shin, F.R. Khuri, B.B. Aggarwal, Y.J. Surh, O. Kucuk, Cancer prevention with natural compounds, Semin. Oncol. 37 (2010) 258–281.
[3] A.M. Aboul-Enein, S.M. Shanab, E.A. Shalaby, M.M. Zahran, D.A. Lightfoot, H.A. El-Shemy, Cytotoxic and antioxidant properties of active principals isolated from water hyacinth against four cancer cells lines, BMC Compl. Alternative Med. 14 (2014) 397.
[4] R.F. Luduena, Multiple forms of tubulin: different gene products and covalent modifications, Int. Rev. Cytol. 178 (1998) 207–275.
[5] V. Kuete, J.K. Dzotam, I.K. Voukeng, A.G. Fankam, T. Efferth, Cytotoxicity of methanol extracts of *Annona muricata, Passiflora edulis* and nine other Cameroonian medicinal plants towards multi-factorial drug-resistant cancer cell lines, SpringerPlus 5 (2016) 1666.
[6] V. Kuete, C.F. Tchinda, F.T. Mambe, V.P. Beng, T. Efferth, Cytotoxicity of methanol extracts of 10 Cameroonian medicinal plants towards multi-factorial drug-resistant cancer cell lines, BMC Compl. Alternative Med. 16 (2016) 267.
[7] A.T. Mbaveng, F. Damen, J.D. Simo Mpetga, M.D. Awouafack, P. Tane, V. Kuete, T. Efferth, Cytotoxicity of crude extract and isolated constituents of the *Dichrostachys cinerea* bark towards multifactorial drug-resistant cancer cells, Evid. Based Compl. Alternat. Med. 2019 (2019) 8450158.
[8] A.T. Mbaveng, F. Damen, I. Celik, P. Tane, V. Kuete, T. Efferth, Cytotoxicity of the crude extract and constituents of the bark of *Fagara tessmannii* towards multi-factorial drug resistant cancer cells, J. Ethnopharmacol. 235 (2019) 28–37.
[9] M.F. Hegazy, S. Abdelfatah, A.R. Hamed, T.A. Mohamed, A.A. Elshamy, I.A. Saleh, E.H. Reda, N.S. Abdel-Azim, K.A. Shams, M. Sakr, Y. Sugimoto, P.W. Pare, T. Efferth, Cytotoxicity of 40 Egyptian plant extracts targeting mechanisms of drug-resistant cancer cells, Phytomedicine 59 (2019) 152771.
[10] C. Orwa, A. Mutua, R. Kindt, R. Jamnadass, S. Anthony, Agroforestree Database: a Tree Reference and Selection Guide Version 4.0, World Agroforestry Centre, Nairobi: Kenya, 2003.
[11] G. Cordell, C. Beecher, J. Pezzut, Can ethnopharmacology contribute to development of new anti-cancer? J. Ethnopharmacol. 32 (1991) 117–133.
[12] J. Popoca, A. Aguilar, D. Alonso, M. Villarreal, Cytotoxic activity of selected plants used as antitumorals in Mexican traditional medicine, J. Ethnopharmacol. 59 (1998) 173–177.
[13] V. Kuete, B. Krusche, M. Youns, I. Voukeng, A.G. Fankam, S. Tankeo, S. Lacmata, T. Efferth, Cytotoxicity of some Cameroonian spices and selected medicinal plant extracts, J. Ethnopharmacol. 134 (2011) 803–812.
[14] M.I.S. Abdelhady, A novel polyphenolic compound isolated from Acacia sieberiana, Org. Chem. Indian J. 9 (2013) 236–238.
[15] A. Kimmig, V. Gekeler, M. Neumann, G. Frese, R. Handgretinger, G. Kardos, H. Diddens, D. Niethammer, Susceptibility of multidrug-resistant human leukemia cell lines to human interleukin 2-activated killer cells, Canc. Res. 50 (1990) 6793–6799.
[16] T. Efferth, A. Sauerbrey, A. Olbrich, E. Gebhart, P. Rauch, H.O. Weber, J.G. Hengstler, M.E. Halatsch, M. Volm, K.D. Tew, D.D. Ross, J.O. Funk, Molecular modes of action of artesunate in tumor cell lines, Mol. Pharmacol. 64 (2003) 382–394.
[17] J. Gillet, T. Efferth, D. Steinbach, J. Hamels, F. de Longueville, V. Bertholet, J. Remacle, Microarray-based detection of multidrug resistance in human tumor cells by expression profiling of ATP-binding cassette transporter genes, Canc. Res. 64 (2004) 8987–8993.
[18] L.A. Doyle, W. Yang, L.V. Abruzzo, T. Krogmann, Y. Gao, A.K. Rishi, D.D. Ross, A multidrug resistance transporter from human MCF-7 breast cancer cells, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 15665–15670.
[19] V. Kuete, L.P. Sandjo, D.E. Djeussi, M. Zeino, G.M. Kwamou, B. Ngadjui, T. Efferth, Cytotoxic flavonoids and isoflavonoids from *Erythrina sigmoidea* towards multi-factorial drug resistant cancer cells, Invest. N. Drugs 32 (2014) 1053–1062.
[20] J. O'Brien, I. Wilson, T. Orton, F. Pognan, Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity, Eur. J. Biochem. 267 (2000) 5421–5426.
[21] A.T. Mbaveng, G.T.M. Bitchagno, V. Kuete, P. Tane, T. Efferth, Cytotoxicity of ungeremine towards multi-factorial drug resistant cancer cells and induction of apoptosis, ferroptosis, necroptosis and autophagy, Phytomedicine 60 (2019) 152832.
[22] V. Kuete, A.T. Mbaveng, E.C. Nono, C.C. Simo, M. Zeino, A.E. Nkengfack, T. Efferth, Cytotoxicity of seven naturally occurring phenolic compounds towards multi-factorial drug-resistant cancer cells, Phytomedicine 23 (2016) 856–863.
[23] F.A. Adem, A.T. Mbaveng, V. Kuete, M. Heydenreich, A. Ndakala, B. Irungu, A. Yenesew, T. Efferth, Cytotoxicity of isoflavones and biflavonoids from *Ormocarpum kirkii* towards multi-factorial drug resistant cancer, Phytomedicine 58 (2019) 152853.
[24] V. Kuete, A.H.L. Nkuete, A.T. Mbaveng, B. Wiench, H.K. Wabo, P. Tane, T. Efferth, Cytotoxicity and modes of action of 4′-hydroxy-2′,6′-dimethoxychalcone and other flavonoids toward drug-sensitive and multidrug-resistant cancer cell lines, Phytomedicine 21 (2014) 1651–1657.
[25] A.T. Mbaveng, G.W. Fotso, D. Ngnintedo, V. Kuete, B.T. Ngadjui, F. Keumedjio, K. Andrae-Marobela, T. Efferth, Cytotoxicity of epunctanone and four other phytochemicals isolated from the medicinal plants *Garcinia epunctata* and *Ptycholobium contortum* towards multi-factorial drug resistant cancer cells, Phytomedicine 48 (2018) 112–119.
[26] A.T. Mbaveng, B.L. Ndontsa, V. Kuete, Y.M.M. Nguekeu, I. Celik, R. Mbouangouere, P. Tane, T. Efferth, A naturally occuring triterpene saponin ardisiacrispin B displayed cytotoxic effects in multi-factorial drug resistant cancer cells via ferroptotic and apoptotic cell death, Phytomedicine 43 (2018) 78–85.
[27] D.A. Bass, J.W. Parce, L.R. Dechatelet, P. Szejda, M.C. Seeds, M. Thomas, Flow cytometric studies of oxidative product formation by neutrophils: a graded response to membrane stimulation, J. Immunol. 130 (1983) 1910–1917.
[28] A. Cossarizza, R. Ferraresi, L. Troiano, E. Roat, L. Gibellini, L. Bertoncelli, M. Nasi, M. Pinti, Simultaneous analysis of reactive oxygen species and reduced glutathione content in living cells by polychromatic flow cytometry, Nat. Protoc. 4 (2009) 1790–1797.
[29] V. Kuete, S.B. Tankeo, M.E. Saeed, B. Wiench, P. Tane, T. Efferth, Cytotoxicity and modes of action of five Cameroonian medicinal plants against multi-factorial drug resistance of tumor cells, J. Ethnopharmacol. 153 (2014) 207–219.
[30] T. Efferth, V.B. Konkimalla, Y.F. Wang, A. Sauerbrey, S. Meinhardt, F. Zintl, J. Mattern, M. Volm, Prediction of broad spectrum resistance of tumors towards anticancer drugs, Clin Cancer Res 14 (2008) 2405–2412.
[31] V. Kuete, P.D. Tchakam, B. Wiench, B. Ngameni, H.K. Wabo, M.F. Tala, M.L. Moungang, B.T. Ngadjui, T. Murayama, T. Efferth, Cytotoxicity and modes of action of four naturally occuring benzophenones: 2,2',5,6'-tetrahydroxybenzophenone, guttiferone E, isogarcinol and isoxanthochymol, Phytomedicine 20 (2013) 528–536.
[32] A.T. Mbaveng, G.F. Chi, I.N. Bonsoub, S. Abdelfataha, A.N. Tamfud, E.M.O. Yeboahe, V. Kuete, T. Efferth, *N*-acetylglycoside of oleanolic acid (aridanin) displayed impressive cytotoxicity towards human and animal cancer cells, inducing apoptotic, ferroptotic and necroptotic cell death, Phytomedicine 76 (2020) 153261.
[33] J. Boik, Natural Compounds in Cancer Therapy, Oregon Medical Press, Minnesota USA, 2001.
[34] G. Brahemi, F.R. Kona, A. Fiasella, D. Buac, J. Soukupova, A. Brancale, A.M. Burger, A.D. Westwell, Exploring the structural requirements for inhibition of the ubiquitin E3 ligase breast cancer associated protein 2 (BCA2) as a treatment for breast cancer, J. Med. Chem. 53 (2010) 2757–2765.
[35] V. Kuete, A.T. Mbaveng, M. Zeino, C.D. Fozing, B. Ngameni, G.D. Kapche, B.T. Ngadjui, T. Efferth, Cytotoxicity of three naturally occurring flavonoid derived compounds (artocarpesin, cycloartocarpesin and isobavachalcone) towards multi-factorial drug-resistant cancer cells, Phytomedicine 22 (2015) 1096–1102.
[36] V. Kuete, B. Ngameni, B. Wiench, B. Krusche, C. Horwedel, B.T. Ngadjui, T. Efferth, Cytotoxicity and mode of action of four naturally occuring flavonoids from the genus *Dorstenia*: gancaonin Q, 4-hydroxylonchocarpin, 6-prenylapigenin, and 6,8-diprenyleriodictyol, Planta Med. 77 (2011) 1984–1989.
[37] Z. Cao, H. Zhang, X. Cai, W. Fang, D. Chai, Y. Wen, H. Chen, F. Chu, Y. Zhang, Luteolin promotes cell apoptosis by inducing autophagy in hepatocellular carcinoma, Cell. Physiol. Biochem. 43 (2017) 1803–1812.
[38] X. Yao, W. Jiang, D. Yu, Z. Yan, Luteolin inhibits proliferation and induces apoptosis of human melanoma cells in vivo and in vitro by suppressing MMP-2 and MMP-9 through the PI3K/AKT pathway, Food Funct. 10 (2019) 703–712.
[39] W. Wei, J. He, H. Ruan, Y. Wang, *In vitro* and *in vivo* cytotoxic effects of chrysoeriol in human lung carcinoma are facilitated through activation of autophagy, sub-G1/G0 cell cycle arrest, cell migration and invasion inhibition and modulation of MAPK/ERK signalling pathway, J. BUON 24 (2019) 936–942.
[40] J. Yang, C. Pi, G. Wang, Inhibition of PI3K/Akt/mTOR pathway by apigenin induces apoptosis and autophagy in hepatocellular carcinoma cells, Biomed. Pharmacother. 103 (2018) 699–707.
[41] V. Kuete, D.E. Djeussi, A.T. Mbaveng, M. Zeino, T. Efferth, Cytotoxicity of 15 Cameroonian medicinal plants against drug sensitive and multi-drug resistant cancer cells, J. Ethnopharmacol. 186 (2016) 196–204.
[42] V. Kuete, A.T. Mbaveng, L.P. Sandjo, M. Zeino, T. Efferth, Cytotoxicity and mode of action of a naturally occurring naphthoquinone, 2-acetyl-7-methoxynaphtho[2,3-b]furan-4,9-quinone towards multi-factorial drug-resistant cancer cells, Phytomedicine 33 (2017) 62–68.

[43] A.T. Mbaveng, V. Kuete, T. Efferth, Potential of Central, Eastern and Western Africa medicinal plants for cancer therapy: spotlight on resistant cells and molecular targets, Front. Pharmacol. 8 (2017) 343.
[44] V. Kuete, T. Efferth, African flora has the potential to fight multidrug resistance of cancer, BioMed Res. Int. 2015 (2015) 914813.
[45] V. Kuete, L.P. Sandjo, B. Wiench, T. Efferth, Cytotoxicity and modes of action of four Cameroonian dietary spices ethno-medically used to treat Cancers: *Echinops giganteus, Xylopia aethiopica, Imperata cylindrica* and *Piper capense*, J. Ethnopharmacol. 149 (2013) 245–253.
[46] G. Liu, H. Zou, T. Luo, M. Long, J. Bian, X. Liu, J. Gu, Y. Yuan, R. Song, Y. Wang, J. Zhu, Z. Liu, Caspase-dependent and caspase-independent pathways are Involved in iadmium-induced apoptosis in primary rat proximal tubular cell culture, PloS One 11 (2016), e0166823.
[47] Z. Zhang, X. Yang, S. Zhang, X. Ma, J. Kong, BNIP3 upregulation and EndoG translocation in delayed neuronal death in stroke and in hypoxia, Stroke 38 (2007) 1606–1613.