Cytotoxicity of Crude Extract and Isolated Constituents of the *Dichrostachys cinerea* Bark towards Multifactorial Drug-Resistant Cancer Cells

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1. Introduction

Recent data from the World Health Organization revealed that most countries still face an increase in cancer incidences [1]. The global cancer burden reached 18.1 million new cases in 2018, with one in eight men and one in 11 women dying in developing countries [1]. Worldwide, the five-year prevalence of cancer is estimated at 43.8 million people [1]. The effectiveness of anticancer chemotherapy is greatly impeded by the resistance of malignant cells to cytotoxic drugs [2]. The search for new antiproliferative drugs should therefore take into consideration the ability of cancer cells to develop resistant phenotypes. Natural products are well recognized as source of cytotoxic molecules [3]. Various studies have previously documented the effectiveness of botanicals and phytochemicals from the flora of Africa to fight cancer multidrug resistance (MDR) [4, 5]. However, research should be intensified to increase the library of cytotoxic plants and molecules available in the African flora, in order to have better chances of achieving clinically exploitable drugs in the future. The present study was hence designed to evaluate the cytotoxicity of crude extract and compounds from the bark of *Dichrostachys cinerea* (L.) Wight & Arn. (Fabaceae) towards a panel of drug-sensitive...
and drug-resistant cancer cell lines. The mode of induction of apoptosis of crude extract and compound I was further investigated. *Dichrostachys cinerea*, also known as sicklebush, Bell mimosa, Chinese lantern tree, or Kalahari Christmas tree, is a fast growing tree of up to 7 m height, traditionally used as laxative, diuretic and to treat dysentery, elephantiasis, gonorrhoea, boils, headache, syphilis, sore, worms [6, 7], inflammation, and cancer [8]. Previous phytochemical analysis of *Dichrostachys cinerea* led to the identification of a triterpenoid β-amyrin glucoside, apigenin-7-O-apioside (1—2) glucoside, chrysoeriol-7-O-apioside (1—2) glucoside, clavonic acid, quercetin-3-O-rhamnosyranoside, quercetin-3-O-glucopyranoside, myricetin-3-O-rhamnosyranoside, myricetin-3-O-glucopyranoside, myricetin, apigenin, and kaempferol from the leaves [6, 9] as well as the meroterpene derivatives, dichrostachines A-R from the bark and roots [10]. Preliminary cytotoxicity investigations of this plant were reported towards DU145 and 22Rv1 prostate cancer cells and HeLa cervical cancer cells [7]. This is the first intensive study on the potential of *Dichrostachys cinerea* and some of its constituents against MDR cancer cell lines.

2. Materials and Methods

2.1. Plant Material and Extraction. *Dichrostachys cinerea* barks were collected in February 2017 in Bazou (5° 4’ 0” N, 10° 28’ 0” E) in the West Region of Cameroon. The plant was identified at the National Herbarium of Cameroon (Yaoundé), where voucher is available under number 34028/HNC. The bark of *D. cinerea* was air-dried and powdered (2000 g) and then macerated in 20 l of ethanol for 48 h. The solvent was evaporated in vacuum under reduced pressure to give the crude extract (170 g; DCB).

2.2. Isolation of Compounds from the Bark of *Dichrostachys cinerea*. An aliquot of DCB (160 g) was treated with ethyl acetate (EtOAc) to give two subextracts: the EtOAc extract (DCA, 85 g) and the methanol (MeOH) extract (DCB, 75 g). An aliquot of DCB (160 g) was treated with ethyl acetate (EtOAc) to give two subextracts: the EtOAc extract (DCA, 85 g) and the methanol (MeOH) extract (DCB, 75 g). The solvent was evaporated in vacuum under reduced pressure to give the crude extract (170 g; DCB).

2.3. General Procedure. All general chemistry procedures (mass spectral data, 1H and 13C nuclear magnetic resonance (NMR) spectra) and CC were performed with the same apparatus and reagents, and in similar experimental conditions as reported earlier [13].

2.4. Cell Cultures. Drug-sensitive and drug-resistant cancer cell lines of previously reported origin were used in this study. These included drug-sensitive CCRF-CEM leukemia cells and its multidrug-resistant P-glycoprotein-overexpressing subline CEM/ADR5000 cells [14–16], MDA-MB-231-pDNA breast cancer cells and their resistant subline MDA-MB-231-BCRP clone 23 cells [17], HCT116 p53+/− colon cancer cells and their knockdown clone HCT116 p53−/− cells, and U87MG glioblastoma cells and their resistant subline U87MGΔEGFR cells [18, 19]. Normal AML12 hepatocytes were used and compared with HepG2 hepatocarcinoma cells [18, 19].

2.5. Cytotoxicity Assay. The cytotoxicity assay performed using resazurin reduction assay was applied to the crude extract (DCB), compounds I-4, and doxorubicin [18, 20, 21] with similar experimental conditions as those reported earlier [13, 19, 22, 23]. The Infinite M2000 Pro™ plate reader (Tecan, Crailsheim, Germany) with excitation wavelength of 544 nm and an emission wavelength of 590 nm was used to read the fluorescence after 72 h incubation. IC₅₀ values earlier defined [13] were calculated from a calibration curve by linear regression using Microsoft Excel [24]. The degree of resistance (D.R.) was determined as the IC₅₀ value of the resistant cell line versus that of its sensitive congeners; meanwhile, the selectivity index (S.I.) was the IC₅₀ value in normal AML12 hepatocytes versus that in HepG2 hepatocarcinoma.

2.6. Cell Cycle Analysis and Detection of Apoptotic Cells by Flow Cytometry and Annexin V/PI Staining. Aliquots of 1×10⁶ CCRF-CEM cells were treated with the studied samples (DCB and compound I), the reference drug (doxorubicin), or the solvent control (DMSO) at various concentrations. The distribution of CCRF-CEM cycle was analyzed as described earlier in similar experimental conditions (24 h incubation; humidified 5% CO₂ atmosphere; 37°C) [13, 22, 23]. The BD Accuri C6 Flow Cytometer (BD Biosciences, Heidelberg, Germany) was used to measure the propidium iodide (PI) fluorescence of individual nuclei. Assays were repeated at least three times and in triplicate.
To perform the annexin V/PI staining, DCB, betulinic acid (1), and doxorubicin were used to treat an amount of 1x10⁶ per 1 ml CCRF-CEM cells. The experimental conditions were similar to those earlier reported (24 h incubation; humidified 5% CO₂ atmosphere; 37°C) [13]. The BD Accuri C6 Flow Cytometer was then used to analyze apoptosis using fluorescein isothiocyanate (FITC)-conjugated annexin V/PI assay kit (eBioscience™ Annexin V; Invitrogen, San Diego, USA) similarly as reported earlier [13, 22, 23]; early apoptosis for cells stained with only annexin V; late apoptosis or in a necrotic stage for cells stained with both annexin V and propidium iodide [13, 25, 26].

2.7. Assessment of Caspases Activation Using the Caspase-Glo Assay. After 6 h treatment of CCRF-CEM cells with DCB and triterpenoid 1 for 6 h, caspases activities were evaluated with Caspase-Glo 3/7, 8, and 9 assay kits (Promega, Mannheim, Germany) similarly as previously reported [13, 18, 27].

2.8. Assessment of the Integrity of the Mitochondrial Membrane. The mitochondrial membrane potential (MMP) of CCRF-CEM cells was analyzed after 24 h treatment with DCB, compound 1, or valinomycin (mitochondrial gradient dissipation substance or positive control). The 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolyl carbocyanine iodide (JC-1; Biomol, Hamburg, Germany) staining was used to measure the MMP similarly as previously reported [13, 18, 22, 23].

2.9. Evaluation of the Production of Reactive Oxygen Species (ROS). The measurement of ROS production using 2',7'-dichlorodihydrofluorescein diacetate (H₂DCFH-DA) (Sigma-Aldrich) was done in CCRF-CEM cells were treated with DCB, compound 1, a solvent control (DMSO), or a positive control, hydrogen peroxide (H₂O₂) for 24 h, in similar experimental conditions as documented earlier [13, 18, 28, 29].

3. Results

3.1. Phytochemistry. Physical and NMR data with direct comparison with literature was used to elucidate the chemical structures of phytochemicals isolated from the bark of Dichrostachys cinerea. They were betulinic acid, C₃₀H₅₀O₁ (m.p. 216°C; m/z 426) [30], glyceryl-1-hexacosanoate, C₂₉H₅₂O₄ (2; m.p. 91-95°C; m/z 470) [31], 7-hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one, C₁₅H₁₀O₃ (3; m.p. 315°C; m/z 254) [32], and 6-hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one, C₁₅H₁₀O₄ (4; m.p. 325°C; m/z 254) [33] (Figure 1).

3.2. Cytotoxicity. Triterpenoid 1 and flavone 4 had cytotoxic effects towards the 9 tested cancer cell lines with IC₅₀ values below 50 µM (Table 1). Botanical DCB and flavone 3 had selective activities, while no cytotoxic effect (IC₅₀ value above 100 µM) was recorded with fatty acid ester 2. The recorded IC₅₀ values varied from 7.65 µM (towards resistant CEM-ADR5000 leukemia cells) to 44.17 µM (against HepG2 hepatocarcinoma cells) for 1, 18.90 µM (CCRF-CEM leukemia cells) to 88.86 µM (against HCT116p53+/+ colon adenocarcinoma cells) for 4, and 0.02 µM (against CCRF-CEM cells) to 122.96 µM (against CEM/ADR5000 cells) for doxorubicin. The IC₅₀ values in normal AML12 hepatocytes were above 80 µg/mL for DCB and above 100 µM for compounds 2 and 3 (Table 1). Collateral sensitivity (hypersensitivity or D.R. below 1) of all resistant cell lines compared to their sensitive counterparts was observed with triterpenoid 1 (Table 1). Hypersensitivity or normal sensitivity
Table 1: Cytotoxicity of crude extract, compounds isolated from *Dichrostachys cinerea* and doxorubicin in multifactorial drug-sensitive and -resistant cancer cells lines and normal cells following the resazurin assay with 72 h incubation.

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Crude extract</th>
<th>Samples, IC$_{50}$ μM (sub/μM) values and degrees of resistance* or selectivity index** (in bracket)</th>
<th>Doxorubicin μM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DCB</td>
<td>1 2 3 4</td>
<td></td>
</tr>
<tr>
<td>CCRF-CEM</td>
<td>5.69 ± 1.34</td>
<td>8.80 ± 0.35 &gt;100 31.18 ± 0.98 18.90 ± 1.54</td>
<td>0.02±0.00</td>
</tr>
<tr>
<td>CEM/ADR5000</td>
<td>4.13 ± 0.71</td>
<td>7.65 ± 1.46 &gt;100 124.21 ± 20.16 38.82 ± 2.13</td>
<td>122.96±10.94</td>
</tr>
<tr>
<td>Degree of resistance*</td>
<td>(0.73)</td>
<td>(0.87)</td>
<td>(2.05)</td>
</tr>
<tr>
<td>MDA-MB-231-pcDNA</td>
<td>44.72 ± 2.05</td>
<td>38.83 ± 0.94 &gt;100 75.55 ± 5.16 45.75 ± 4.76</td>
<td>0.13±0.01</td>
</tr>
<tr>
<td>MDA-MB-231-BCRP</td>
<td>64.03 ± 2.72</td>
<td>24.91 ± 1.2 &gt;100 80.00 ± 1.61 40.46 ± 3.90</td>
<td>0.79±0.08</td>
</tr>
<tr>
<td>Degree of resistance</td>
<td>(1.43)</td>
<td>(0.64)</td>
<td>(6.68)</td>
</tr>
<tr>
<td>HCT116(p53^{+/+})</td>
<td>&gt;80</td>
<td>31.46 ± 0.49 &gt;100 48.86 ± 5.35 122.96 ± 10.94</td>
<td>0.48±0.06</td>
</tr>
<tr>
<td>HCT116(p53^{−/−})</td>
<td>70.37 ± 9.14</td>
<td>17.07 ± 0.70 &gt;100 48.62 ± 3.82 17.07 ± 0.70</td>
<td>1.78±0.08</td>
</tr>
<tr>
<td>Degree of resistance</td>
<td>(&lt;0.87)</td>
<td>(&lt;0.66)</td>
<td>(3.73)</td>
</tr>
<tr>
<td>U87MG</td>
<td>&gt;80</td>
<td>24.91 ± 0.33 &gt;100 43.86 ± 8.19 31.18 ± 0.98</td>
<td>0.26±0.03</td>
</tr>
<tr>
<td>U87MGΔEGFR</td>
<td>54.65 ± 6.26</td>
<td>13.92 ± 1.06 &gt;100 53.78 ± 6.10 44.57 ± 2.56</td>
<td>0.98±0.07</td>
</tr>
<tr>
<td>Degree of resistance</td>
<td>(&lt;0.68)</td>
<td>(0.56)</td>
<td>(3.79)</td>
</tr>
<tr>
<td>HepG2</td>
<td>&gt;80</td>
<td>44.17 ± 3.15 &gt;100 34.02 ± 2.95 48.58 ± 7.09</td>
<td>4.56±0.48</td>
</tr>
<tr>
<td>AML12</td>
<td>&gt;80</td>
<td>&gt;93.90</td>
<td>52.90±4.09</td>
</tr>
<tr>
<td>Selectivity index**</td>
<td>(&gt;±2.13)</td>
<td>&gt;100</td>
<td></td>
</tr>
</tbody>
</table>

(*): 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 U87MGΔEGFR were used as the corresponding resistant counterparts for CCRF-CEM, MDA-MB-231-pcDNA, HCT116(p53^{−/−}), and U87MG, respectively. (**): the selectivity index was determined as the ratio of IC$_{50}$ value in the normal AML12 hepatocytes divided by the IC$_{50}$ in HepG2 hepatocarcinoma cells. In bold: significant cytotoxic effect [4, 11, 12]; (μ): values in μg/mL; (μM): values in μM; (nd): not determined: 1: betulinic acid; 2: glyceryl-1-hexacosanoate; 3: 7-hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one; and 4: 6-hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one.
of at least one resistant cell line to botanical DCB as well as compounds 3 and 4 was also recorded (Table 1). Selectivity indexes above 2 were also observed with compound 1 (S.I.: >2.13) and doxorubicin (S.I.: 11.59) in HepG2 as compared with normal AML12 hepatocytes (Table 1).

3.3. Cell Cycle Distribution and Apoptosis. Upon treatment of CCRF-CEM cells with botanical DCB, triterpenoid 1, and the reference compound doxorubicin, the cell cycle phases were modified in concentration-dependent manner (Figure 2). Increase of cells in sub-G0/G1 phase was observed with all samples, and DCB induced cell cycle arrest in G0/G1 phase, while triterpenoid 1 caused cycle arrest in G2/M; doxorubicin induced arrest of cell cycle between S and G2/M. The percentage of CCRF-CEM cells in sub-G0/G1 phase in nontreated cells only was 1.78%; meanwhile, it varied upon treatment from 4.00% (1/4 nontreated cells only was 1.78%; meanwhile, it varied upon doxorubicin-induced arrest of cell cycle between S and G2/M. The percentage of CCRF-CEM cells in sub-G0/G1 phase in nontreated cells only was 1.78%; meanwhile, it varied upon treatment from 4.00% (1/4 × IC50) to 32.18% (2 × IC50) for DCB, 15.30% (1/4 × IC50) to 48.40% (2 × IC50) for compound 1, and 4.81% (1/4 × IC50) to 10.35% (2 × IC50) for doxorubicin (Figure 2). These data suggested that DCB, compound 1, and doxorubicin induced apoptosis in CCRF-CEM cells. In the annexin V/PI staining, the induction of apoptosis was further investigated. The results depicted in Figure 3 showed a dose-dependent induction with DCB, triterpenoid 1, and doxorubicin. When cells were treated with 2 × IC50, for example, DCB induced apoptosis with 39.8% early apoptotic V (+)/PI (-) cells, 8.8% late apoptotic V (+)/PI (+) cells as well as necrosis with 12.8% annexin V (-)/PI (+) cells; triterpenoid 1 induced 51.0% early apoptotic cells and 5.1% necrotic cells, while doxorubicin induced 11.8% late apoptotic cells.

3.4. Activation of Caspases, Integrity of MMP, and ROS Production. Treatment of CCRF-CEM cells with DCB did not activate the activity of caspases 3/7, 8, and 9 contrary to triterpenoid 1 (Figure 4). In effect, a dose-dependent activation of caspases upon treatment with 1 was observed, with optimal effects at 8.8 μM; up to 3.19-fold, 2.91-fold, and 2.37-fold increases in the activity of caspases 3/7, 8, and 9, respectively, were recorded. The effects of DCB, betulinic acid (1), and valinomycin on integrity of MMP in CCRF-CEM are depicted in Figure 5. Both DCB and compound 1 considerably modified the MMP with up to 90.3% and 57.5% (at 2 × IC50), respectively; valinomycin at 10 μM induced 45.9% alteration.

The effects of DCB and compound 1 on the production of ROS in CCRF-CEM cells are given in Figure 6. The two samples dose-dependently enhanced the production of ROS in CCRF-CEM cells. The ROS level in nontreated cells was 0.2%, whilst at 2 × IC50, DCB caused increased ROS production by up to 61.1% and triterpenoid 1 by 53.30%. H2O2 induced ROS production by 98.8% at 50 μM.
Figure 3: Evaluation of apoptosis induced by the crude extract, betulinic acid (1), and doxorubicin on CCRF-CEM leukemia cells after 24 h as determined by annexin V/PI assay. Apoptosis was assessed by flow cytometry after annexin V-PI double staining. IC50 values were 5.69 µg/mL for the crude extract, 8.80 µM for 1, and 0.02 µM for doxorubicin. Necrotic cells lose membrane integrity, allowing PI entry. Q9-LL: viable cells exhibit annexin V (-)/PI (-); Q9-LR: early apoptotic cells exhibit annexin (+)/PI (-); and Q9-UR and Q9-UL: late apoptotic cells or necrotic cells exhibit annexin V (+)/PI (+) or annexin V (-)/PI (+).

4. Discussion

Phytochemicals isolated from the bark of *Dichrostachys cinerea* were one triterpenoid 1, one ester of fatty acid 2, and two flavone-type flavonoids 3 and 4. Previous phytochemical investigation of the bark of *Dichrostachys cinerea* led to the isolation of meroterpene derivatives, dichrostaches A-R [10] which were not isolated in this study, probably due to the isolation procedure used or the fact that the plant was harvested in different geographic locations.

Drug resistance of malignant cells seriously hampers the chemotherapy of cancer. In the search for cytotoxic compounds, scientists should take into consideration the ability of these cells to rapidly develop drug resistance. This is possible when investigations also consider resistant phenotypes of malignant cells. In the present study, we have used several models of MDR cancer cell lines including ATP-binding cassette (ABC)-transporter-overexpressing MDR-mediating P-glycoprotein (P-gp; ABCB1/MDR1) or breast cancer resistance protein (ABCG2/BCRP), a p53 knockout
Figure 4: Effects of 6 h treatment of CCRF-CEM cells with crude extract and betulinic acid (I) on caspases activity. Samples were tested at their 1/2 × IC₅₀, IC₅₀ and 2 × IC₅₀; IC₅₀ values were 5.69 μg/mL for the crude extract and 8.80 μM for I. Caspase activity is expressed as percentage (%) compared to untreated cells. Shown are mean±SD of three independent experiments.

Figure 5: Effect of the crude extract, betulinic acid (I) and valinomycin for 24 h on the MMP of CCRF-CEM cells. IC₅₀ values were 5.69 μg/mL for the crude extract, 8.80 μM for I, and 0.02 μM for doxorubicin. Intact cells (Q1), loss of MMP (Q2), and ruptured cell membrane (Q3 and Q4).
Shown are mean ± SD of three independent experiments. Figure 6: ROS production in CCRF-CEM cells treated for 24 h with the crude extract, betulinic acid (1), and hydrogen peroxide (H$_2$O$_2$). Samples were tested at their 1/4 × IC$_{50}$, 1/2 × IC$_{50}$, IC$_{50}$, and 2 × IC$_{50}$; IC$_{50}$ values were 5.69 μg/mL for the crude extract and 8.80 μM for 1. Shown are mean±SD of three independent experiments.

To the best of our knowledge, this is the first intensive study on cytotoxicity of Dichrostachys cinerea and its constituents 3 and 4 against MDR cancer cell lines. However, preliminary antiproliferative effects of this plant were reported towards DU145 and 22Rv1 prostate cancer cells and HeLa cervical cancer cells, with the lowest IC$_{50}$ values of 8.04 μg/mL recorded in 22Rv1 cells [7]. Also, betulinic acid is a well-known cytotoxic compound [34]. Its effects have been reported towards several cancer cell lines including sensitive and resistant phenotypes such as CCRF-CEM cells and CEM/ADR5000 leukemia cells, MDA-MB-231-pcDNA and MDA-MB-231/BCRP breast adenocarcinoma cells, HEK293 and HEK293/ABCBC5 embryonic kidney cells, and U87.MG and U87.MGΔEGFR glioblastoma cells with IC$_{50}$ values.
ranging from 15.1 μM (against HEK293 cells) to 29.4 μM (towards CCRF-CEM cells) [34, 36].

In this study, the crude extract DCB and triterpenoid 1 had the best cytotoxic effects on the two leukemia cells with IC_{50} values below 10 μM. They were consequently selected for further cellular mechanistic studies towards CCRF-CEM cells, such as induction of apoptosis, caspases activation, and alteration of MMP as well as the production of ROS [37]. DCB and compound 1 induced apoptosis in CCRF-CEM cells (Figures 2 and 3). Induction of apoptosis by DCB was mediated by MMP alteration and increased ROS production, while that induced by triterpenoid 1 was mediated by caspases activation (Figure 4), MMP alteration (Figure 5), and increased ROS production (Figure 6). Previous studies on the molecular mechanism of the cytotoxic action of compound 1 showed that it inhibited P-gp, BCRP, and ABCB5 and mutation activated EGFR overexpressing cells. Besides, various genes significantly correlated to its activity on cell cycle regulation, microtubule formation, signal transduction, transcriptional regulation, chromatin remodeling, cell adhesion, tumor suppression, ubiquitination, and proteasome degradation [34].

5. Conclusions

The present study indicated that *Dichrostachys cinerea* is a potential cytotoxic plant and should be further explored to develop new antineoplastic agents to fight recalcitrant cancers. The crude extract DCB induced apoptosis in CCRF-CEM cells mostly mediated by MMP alteration and enhanced ROS production; compound 1 induced apoptosis through caspases activation and MMP alteration and increased ROS production.

### Abbreviations

1: Betulinic acid  
2: Glyceryl-1-hexacosanoate  
3: 7-Hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one  
4: 6-Hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one  
ABC: ATP-binding cassette  
BCRP: Breast cancer resistance protein  
CC: Column chromatography  
CH_2Cl_2: Dichloromethane  
DCB: Crude extract from the bark of *Dichrostachys cinerea*  
DMSO: Dimethylsulfoxide  
D.R.: Degree of resistance  
EGFR: Epidermal growth factor receptor  
ESI-MS: Electrospray ionization mass spectrometry  
EtOAc: Ethyl acetate  
FITC: Fluorescein isothiocyanate  
H_2O_2: Hydrogen peroxide  
H2DCFH-DA: 5',6',7',8'-Tetrachloro-1',3',3'-tetraethylbenzimidazolylcarbocyanine iodide  
IC_{50}: 50% inhibitory concentration  
MDR: Multidrug resistance  
MeOH: Methanol  
MMP: Mitochondrial membrane potential  
NMR: Nuclear magnetic resonance  
P-gp: P-glycoprotein  
PI: Propidium iodide  
ROS: Reactive oxygen species  
Sub-frs: Subfractions  
TLC: Thin layer chromatography  
TMS: Tetramethylsilane.

### Data Availability

The data used to support the findings of this study are included within the article.

### Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

### Authors’ Contributions

Armelle T. Mbaveng and Francois Damen carried out the experiments. Victor Kuete, Pierre Tane, and Thomas Efferth designed the study. Maurice D. Awouafack performed NMR experiments. Francois Damen, Maurice D. Awouafack, and James D. Simo Mpetga contributed to structural elucidation. Armelle T. Mbaveng and Victor Kuete wrote the manuscript. Thomas Efferth supervised the work, corrected the manuscript, and provided the facilities for the study. All authors read and approved the final manuscript.

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### Supplementary Materials

*Supplementary file.docx.* RMN 1H, 13C and major chemical shifts of studied compounds, betulinic acid (1), glyceryl-1-hexacosanoate (2), 7-hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one (3), and 6-hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one (4). *(Supplementary Materials)*
References


