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Cytotoxicity of compounds from *Xylopia aethiopica* towards multi-factorial drug-resistant cancer cells

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ABSTRACT

Introduction: Multidrug resistance (MDR) in cancer represent a major hurdle in chemotherapy. Previously, the methanol extract of the medicinal spice *Xylopia aethiopica* displayed considerable cytotoxicity against multidrug resistant (MDR) cancer cell lines.
Methods: The present study was designed to assess the cytotoxicity of compounds, 16α-hydroxy-*ent*-kauran-19-oic acid (**2**), 3,4′,5-trihydroxy-6″,6″-dimethylpyrano[2,3-g]flavone (**3**), isotetrandrine (**5**) and *trans*-tiliroside (**6**) derived from the methanol crude extract of *Xylopia aethiopica* against 9 drug-sensitive and -resistant cancer cell lines. The resazurin reduction assay was used to evaluate the cytotoxicity of these compounds, whilst caspase-Glo assay was used to detect caspase activation. Cell cycle, mitochondrial membrane potential (MMP) and levels of reactive oxygen species (ROS) were all analyzed *via* flow cytometry.
Results: Flavonoid **3** and alkaloid **5** also displayed IC_{50} values ranging from 2.61 μM (towards leukemia CCRF-CEM cells) to 18.60 μM (towards gliobastoma multiforme U87MG.Δ*EGFR* cells) and from 1.45 μM (towards HepG2 cells) to 7.28 μM (towards MDA-MB-231-*pcDNA* cells), respectively. IC_{50} values ranged from 0.20 μM (against CCRF-CEM cells) to 195.12 μM (against CEM/ADR5000 cells) for doxorubicin. Compound **3** induced apoptosis in leukemia CCRF-CEM cells mediated by the disruption of the MMP, whilst **5** induced apoptosis mediated by ROS production.
Conclusions: Compounds **2** and **5** represent potential cytotoxic phytochemicals that deserve more investigations to develop novel antineoplastic drugs against multifactorial drug-resistant cancers.

Introduction

Multidrug resistance (MDR) in cancer represent a major hurdle in chemotherapy. P-glycoprotein (P-gp), breast cancer resistance protein (BCRP) as well as mutation in p53 tumor suppressor gene and over-expression of the epidermal growth factor receptor (EGFR) play a key role in cancer chemoresistance (Biedler and Spengler, 1994; el-Deiry, 1997; Efferth et al., 2003a, 2003b). Their modulation by dietary botanicals may positively affect the therapeutic efficacy and can be an attractive strategy to combat MDR tumors. There is an urgent clinical need to explore new agents against MDR. Natural products and their derivates act as one of the major sources for anticancer agents (Wu et al., 2015). Several African medicinal plants and derived molecules were successfully screened in the past few years against MDR cancer cell lines (Choumessi et al., 2012a, 2012b; Dzoyem et al., 2012, 2013; Kuete et al., 2011, 2013b, 2013c, 2013d, 2014b, 2014c). Amongst them, the medicinal spice *Xylopia aethiopica* (Dunal) A.Rich. (Annonaceae), traditional used to treat wounds and skin infections, fever, tapeworm, stomach ache, dysentery, stomach ulcer (Irvine, 1961; Thomas, 1965) and cancers (Kuete et al., 2011, 2013c). The plant is commercially known as Guinea pepper, spice tree or negro pepper (Irvine, 1961) The fruits of *X. aethiopica* are the parts most commonly used for commercial applications (Irvine, 1961; Thomas, 1965). The seeds methanol extract of this displayed profound cytotoxicity in a previous study towards a panel of cancer cell lines, including MDR phenotypes (Kuete et al., 2011, 2013c). Within our ongoing search of antiproliferative drugs from edible plants, the present work was designed to identify the cytotoxic constituents of *Xylopia aethiopica*. More accurately, the work was undertaken to evaluate the cytotoxicity of compounds from the methanol extract of this

Abbreviations: CC, column chromatography; DCF, dichlorofluorescein; DCM, dichloromethane; ddH_2O, double distilled water; DMSO, dimethylsufoxide; H_2DCF, 2′,7′-dichlorodihydrofluorescein; H_2DCFH-DA, 2′,7′-Dichlorodihydrofluorescein diacetate; H_2O_2, oxygenated water; IC_{50}, inhibitory concentrations 50%; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide; MDR, Multidrug resistant; MMP, mitochondrial membrane potential; mp, Melting points; ROS, reactive oxygen species; TLC, thin layer chromatography; VCC, Vacuum column chromatography; VIN, vinblastine; X. aethiopica, Xylopia aethiopica.

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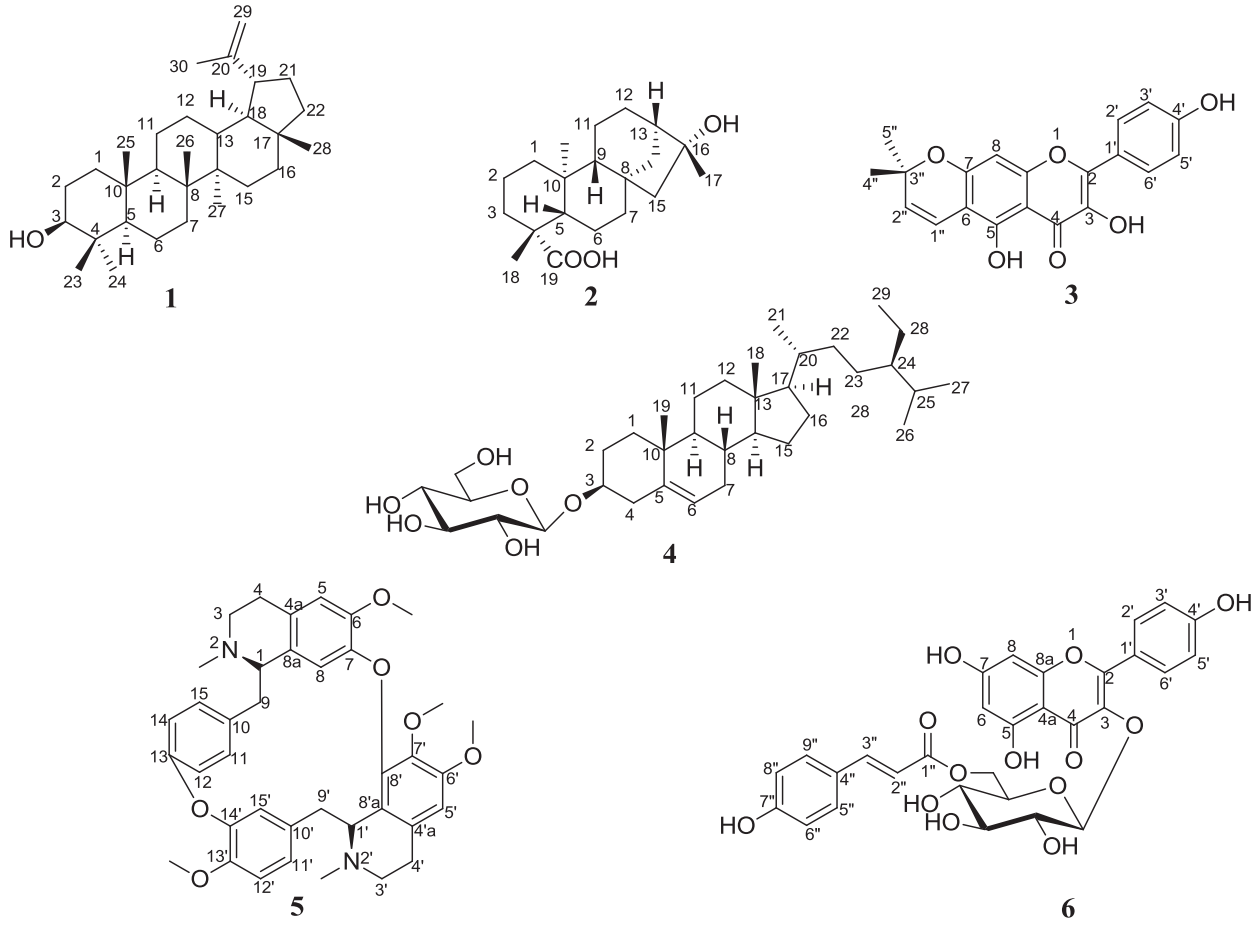

Fig. 1. Chemical structures of the compounds isolated from *Xylopia aethiopica*. Lupeol (**1**), 16α-hydroxy-ent-kauran-19-oic acid (**2**), 3,4′,5-trihydroxy-6″,6″-dimethylpyrano[2,3-g]flavone (**3**), 3-*O*-β-sitosterol β-*D*-glucopyranoside (**4**), isotetrandrine (**5**), *trans*-tiliroside (**6**).

plants, and the mode of action of its most active constituents namely 3,4′,5-trihydroxy-6″,6″-dimethylpyrano[2,3-g]flavone (**3**) and isotetrandrine (**5**). To the best of our knowledge, the cytotoxicity of flavonoid **3** and alkaloid **5** is being herein reported for the first time against MDR cancer cell lines.

Materials and methods

Chemicals

The physical and spectroscopic data of the compounds **1, 2, 3, 4, 5** and **6** are in full agreement with those reported in the literature: **1, 3** and **4** (Kuete et al., 2015), **2** (Hsieh et al., 2004), **5** (Bick et al., 1986) and **6** (Backhouse et al., 2002). The isolation procedure of compounds **1**–**6** is provided as supplementary information. Doxorubicin 98.0% and vinblastine ≥96% from Sigma-Aldrich (Munich, Germany) were provided by the University Pharmacy of the Johannes Gutenberg University (Mainz, Germany) and dissolved in PBS (Invitrogen, Eggenstein, Germany) at a concentration of 10 mM. Geneticin > 98% (72.18 mM; Sigma-Aldrich, Munich, Germany).

Cell cultures

The cell lines used in the present work, their origins and their treatments were previously reported. They include drug-sensitive CCRF-CEM and multidrug-resistant P-glycoprotein-over-expressing CEM/ADR5000 leukemia cells (Kimmig et al., 1990; Efferth et al., 2003b; Gillet et al., 2004), MDA-MB-231-pcDNA3 breast cancer cells and its resistant subline MDA-MB-231-*BCRP* clone 23 (Doyle et al., 1998), HCT116 ($p53^{+/+}$) colon cancer cells and its knockout clone HCT116 ($p53^{-/-}$), U87MG glioblastoma cells and its resistant subline U87MG.Δ*EGFR* (Kuete et al., 2013b, 2013c, 2013d).

Resazurin reduction assay

The cytotoxicity of the tested samples was performed by resazurin reduction assay as previously described (O'Brien et al., 2000; Kuete et al., 2013a, 2014c).

2.7. Flow cytometry for cell cycle analysis and detection of apoptotic cells

All reagents, experimental conditions and apparatus were identical to those previously reported (Kuete et al., 2013a, 2014c).

Caspase-Glo 3/7, caspase-Glo 8 and caspase-Glo 9 assay

Caspase activity in CCRF-CEM leukemia cells was detected using Caspase-Glo 3/7, Caspase-Glo 8 and Caspase-Glo 9 Assay kits (Promega, Mannheim, Germany) as previously reported (Kuete et al., 2013a, 2014c).

Analysis of mitochondrial membrane potential (MMP)

The MMP was analyzed in CCRF-CEM cells by 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide) (JC-1; Biomol, Hamburg, Germany) staining as previously reported (Kuete et al., 2013a, 2014c).