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# Cytotoxicity of South-African medicinal plants towards sensitive and multidrug-resistant cancer cells

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ABSTRACT

*Ethnopharmacological relevance:* Traditional medicine plays a major role for primary health care worldwide. Cancer belongs to the leading disease burden in industrialized and developing countries. Successful cancer therapy is hampered by the development of resistance towards established anticancer drugs.

*Aim:* In the present study, we investigated the cytotoxicity of 29 extracts from 26 medicinal plants of South-Africa against leukemia cell lines, most of which are used traditionally to treat cancer and related symptoms.

*Material and methods:* We have investigated the plant extracts for their cytotoxic activity towards drug-sensitive parental CCRF-CEM leukemia cells and their multidrug-resistant P-glycoprotein-overexpressing subline, CEM/ADR5000 by means of the resazurin assay. A panel of 60 NCI tumor cell lines have been investigated for correlations between selected phytochemicals from medicinal plants and the expression of resistance-conferring genes (ABC-transporters, oncogenes, tumor suppressor genes).

*Results:* Seven extracts inhibited both cell lines (*Acokanthera oppositifolia, Hypoestes aristata, Laurus nobilis, Leonotis leonurus, Plectranthus barbatus, Plectranthus ciliates, Salvia apiana).* CEM/ADR5000 cells exhibited a low degree of cross-resistance (3.35-fold) towards the L. *leonurus* extract, while no cross-resistance was observed to other plant extracts, although CEM/ADR5000 cells were highly resistant to clinically established drugs. The $\log_{10}IC_{50}$ values for two out of 14 selected phytochemicals from these plants (acovenoside A and ouabain) of 60 tumor cell lines were correlated to the expression of ABC-transporters (*ABCB1*, *ABCB5*, *ABCC1*, *ABCG2*), oncogenes (*EGFR*, *RAS*) and tumor suppressors (*TP53*). Sensitivity or resistance of the cell lines were not statistically associated with the expression of these genes, indicating that multidrug-resistant, refractory tumors expressing these genes may still respond to acovenoside A and ouabain.

*Conclusion:* The bioactivity of South African medicinal plants may represent a basis for the development of strategies to treat multidrug-resistant tumors either by phytotherapeutic approaches with whole plant preparations or by classical drug development with isolated compounds such as acovenoside A or ouabain.



## 1. Introduction

One major obstacle in cancer management with established anticancer agents is drug resistance (Mukamoto et al., 2013). The phenomenon of multidrug resistance (MDR) became a point of interest in cancer research after the discovery of P-glycoprotein, which exhibits not only resistance to one but to many drugs (Juliano and Ling, 1976). This phenomenon is defined as cross-resistance of cancer cells to the cytostatic and cytotoxic actions of many anticancer drugs, which are structurally or functionally unrelated and which have different molecular targets (Nakamura et al., 2013). Overexpression of ATP-binding cassette (ABC) transporters such as P-glycoprotein (P-gp) has been shown to be responsible for MDR in clinical tumors (Efferth and Osieka, 1993).

Interestingly, between the years 1981 and 2006 about a hundred anticancer agents have been developed, and the majority are either pure natural products, natural product derivatives or synthetic

*Abbreviations:* ABC, ATP-binding cassette; $IC_{50}$, 50% inhibition concentration; MDR, multidrug resistance; NCI, National Cancer Institute; (P-gp), P-glycoprotein

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compounds with pharmacophores mimicking natural products. Thus, natural products can be considered as a promising source of novel anticancer drugs to combat MDR (Newman and Cragg, 2007). South Africa has a rich flora, including a plethora of medicinal plants, which have not been investigated in detail (Hutchings et al., 1996; Van Wyk et al., 1997; Watt and Breyer-Brandwijk, 1962), although some South-African medicinal plants are known to exhibit cytotoxic activity against breast cancer and leukemia cell lines (Chang et al., 2001; Steenkamp and Gouws, 2006).

Here, we performed a study on 26 South-African medicinal plants, which have a rich tradition in the practice of healers for a wide range of diseases and symptoms (Cordell et al., 1991). We tested extracts of these plants for their cytotoxicity towards sensitive and multidrug-resistant, P-gp-overexpressing leukemia cell lines. Interestingly, extracts from seven herbs revealed high cytotoxicity and these seven plants have a documented use in traditional medicine for cancer or cancer-related symptoms. Cancer is frequently not exactly defined in traditional African medicine. Therefore, symptoms and activities related to anticancer and cytotoxic activity should be taken into account to describe the ethnopharmacological use in an appropriate manner.

*Leonotis leonurus* has many traditional applications (Mazimba, 2015). The hottentots used leaf decoctions as purgative and emmenagogue. Colonial settlers employed it against leprosy.

Other applications include among many others, influenza, bronchitis, wound healing, asthma tuberculosis, jaundice, muscular cramps, high blood pressure, diabetes, viral hepatitis, dysentery, diarrhea, gynecological disorders, cardiovascular disorders, and epilepsy (Bienvenu et al., 2002; Kuchta et al., 2012; Mazimba, 2015; Scott et al., 2004). Tea made from the whole plant is used against cancer, arthritis, piles, bladder and kidney disorder, obesity, and rheumatism (Thring and Weitz, 2006). Generally the plant is a general tonic, having reputed dermatological, hypertension, anti-inflammatory, pain and wound healing properties.

*Plectranthus barbatus* has been traditionally used for a wide range of complaints, *e.g.* stomach ache and as purgative drug (Abdel-Mogib et al., 2002), against hypertension, congestive heart failure, eczema, colic, respiratory disorders, painful urination, insomnia, convulsions, and cancer prevention (Mwitari et al., 2013). In traditional medicine, several species of this genus are used to treat ailments of the digestive tract, respiratory system, nervous system, inflammation, infections, pain and different types of cancers (Lukhoba et al., 2006). The cytotoxic activity of *P. barbatus* and *P. ciliates* may be also supported by the contraceptive properties (Yashaswini and Vasundhara, 2011).

*Hypoestes aristata* has been used against eye sores (Hulme, 1954) breast diseases, respiratory infections and malaria (Iwu, 1993; Kokwaro, 1976). According to healers of the Xhosa tribe, the plant is also used to treat complicated and major diseases like cancer, arthritis, tuberculosis, bone fractures, etc. (Bhat, 2014).

*Acokanthera oppositifolia* (Vhavenda name: *mutsilili*; English name: Common poison bush) is traditionally used as highly effective arrow poison in South-Africa (Steyn, 1934; Van Wyk et al., 2002), which indicates his cytotoxic properties. It is also used as anthelminthic against snake-bites and gynecological disorders (Adedapo et al., 2008; Bisi-Johnson et al., 2010; Fouche et al., 2008; Steenkamp, 2003). Applied as powdered snuff, it has been traditionally used to treat headaches and as infusions for abdominal pains and convulsions and septicemia (Bhat and Jacobs, 1995; Dold and Cocks, 2001; Watt and Breyer-Brandwijk, 1962).

*Salvia apiana* has been traditionally used for respiratory tract infections and acute vaginitis caused by *Candida* infection (Foster and Hobbs, 2002; Moore, 1993). It is recommended by herbalists to treat prostate hyperplasia (http://oneearthherbs.squarespace.com/diseases/benign-prostatic-hyperplasia-bph.html).

*Laurus nobilis* is used among other applications against skin diseases and cancer in the Middle East (Said et al., 2002). This plant is also used in South-Africa.

We exemplarily selected two compounds from one of the cytotoxic plants, acovenoside A and ouabain and correlated their 50% inhibition concentrations ($IC_{50}$) values in a panel of 60 tumor cell lines of the National Cancer Institute (NCI, USA) with the expression of genes that are known to confer MDR, *i.e.* the ABC-transporters *ABCB1/MDR1, ABCB5, ABCC1/MRP, ABCG2/BRCP*, the oncogene *EGFR*, and the tumor suppressor gene *TP53*. Furthermore, we correlated the response of acovenoside A and ouabain with 85 standard anticancer drugs to identify cross-resistance profiles of these two natural compounds with established drugs. The present study may serve as a starting point to further characterize phytochemicals from these plants with the potential to kill MDR cells.

## 2. Material and methods

### 2.1. Plant material

Twenty-six plant species were collected from Pretoria (South-Africa) during September 2011 and identified at the H.G.W.J. Schweickerdt Herbarium (PRU) at the University of Pretoria. Traditional uses of the plants against cancer and their voucher numbers are shown in Tables 1 and 2 respectively. The plant name has been verified with www.theplantlist.org. The phytochemicals of these cytotoxic plants identified in the literature are given in Table 3.

### 2.2. Extract preparation

Twenty-nine extracts have been prepared from 26 plant species in this study. Plant material was subjected to the following extraction protocol separately; ~50 g fresh leaves (unless otherwise stated) were homogenized with 150 mL methanol (Merck, Germany). After filtration, the plant material was washed twice with fresh solvent, the combined extract was concentrated under reduced pressure using a vacuum rotary evaporator (Buchi Labortechnik, Flawil, Switzerland). The solvent free extracts were kept at 4 °C for further biological evaluation. In case of resin samples, ~2.0 g have been extracted.

### 2.3. Cell lines

Human drug sensitive CCRF-CEM and multidrug-resistant CEM/ADR5000 leukemia cells have been generated as described (Kimmig et al., 1990). The panel of 60 human tumor cell lines of the Developmental Therapeutics Program of the National Cancer Institute (NCI, USA) consisted of leukemia, melanoma, non-small cell lung cancer, colon cancer, renal cancer, ovarian cancer, breast cancer, and prostate carcinoma cells as well as tumor cells of the central nervous system (Alley et al., 1988).

### 2.4. Cytotoxicity assay

The resazurin reduction assay is based on reduction of the indicator dye, resazurin, to the highly fluorescent resorufin by viable cells (O'Brien et al., 2000). The protocol used in the present investigation has been previously reported (Kuete et al., 2012). Cells of the National Cancer Institute (NCI, USA) cell line panel were assayed by means of a sulforhodamine B assay (Rubinstein et al., 1990).

### 2.5. Statistical analysis

mRNA microarray data and $\log_{10}IC_{50}$ values of the NCI tumor cell line panel are available (Scherf et al., 2000; Staunton et al.,