Selective Cytotoxicity of Ethiopian Plants

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Few studies have reported the in vitro anticancer activities of medicinal plants that are collected from different agro-ecological zones of Ethiopia. Hence, the main aim of this study was to screen the cytotoxic activities of 80% methanol extracts of 22 plants against human peripheral blood mononuclear cells (PBMCs), as well as human breast (MCF-7), lung (A427), bladder (RT-4), and cervical (SiSo) cancer cell lines. Active extracts were further screened against human large cell lung carcinoma (LCLC-103H), pancreatic cancer (DAN-G), ovarian cancer (A2780), and squamous cell carcinoma of the esophagus (KYSE-70) by using the crystal violet cell proliferation assay, while the vitality of the acute myeloid leukemia (HL-60) and histiocytic lymphoma (U-937) cell lines was monitored in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) microtiter assay. Euphorbia schimperiana, Acokanthera schimperi, Kniphofia foliosa, and Kalanchoe petitiana exhibited potent antiproliferative activity against A427, RT-4, MCF-7, and SiSo cell lines, with IC50 values ranging from 1.85 ± 0.44 to 17.8 ± 2.31µg/mL. Hence, further studies focusing on bio-assay-guided isolation and structural elucidation of active cytotoxic compounds from these plants are warranted.

Ethiopia medicinal plants cytotoxicity extractions cancer

1. Introduction

Medicinal plants have been traditionally used in Ethiopia for the treatment of various diseases, including cancer[1][2][3]. However, the cytotoxic activities of plants that are traditionally used to treat cancer in Ethiopia have not been reported for samples collected in Ethiopia. Only a few plants collected from Ethiopian geographic locations have been investigated so far for their antiproliferative/cytotoxic activities [4][5][6]. Therefore, in continuation with our previous studies in which we reported ethnobotanical evidence of Ethiopian anticancer plants, as well as the cytotoxic activities of 21 plants against MV4-11 (human myeloid leukemia) cell line, we have further screened the cytotoxic activities of these and one additional plant (22 plants) against MCF-7, A427, RT-4, SiSo, LCLC-103H, DAN-G, A2780, KYSE-70, HL-60, and U-937 human cancer cell lines. The phytoconstituents, including anticancer compounds, previously reported from these active plants have also been discussed in this paper.

2. Results and Discussion

From 73 plants that were reported for their traditional anticancer use in our ethnobotanical survey [1], 22 were selected based on their ethnobotanical and chemotaxonomic data. The majority of selected plants belong to Lamiaceae (18.2%), Asteraceae (13.6%), and Euphorbiaceae (9.1%) families. The 80% methanolic extracts of different parts of these plants were tested for their cytotoxic activity against A427, MCF-7, RT-4, and SiSo human

cancer cell lines and peripheral blood mononuclear cells (PBMCs) by using the crystal violet cell antiproliferation and MTT cell viability assays, respectively. The extracts were tested in primary screening at a concentration of 50 µg/mL. Four plant extracts—*A. schimperi*, *E. schimperiana*, *K. foliosa*, and *K. petitiana*—showed negative T/Ccorr. values, indicating relevant cytotoxic activity at 50 µg/mL.

Based on the primary cytotoxic data, these four plants were selected for secondary screening at a range of concentrations against A427, MCF-7, RT-4, SiSo, and four additional adherents (LCLC-103H, DAN-G, KYSE-70, and A2780), and two suspensions (HL-60 and U-937) cell lines. PBMCs were included to compare the results obtained by the HL-60 and U-937 to primary noncancer cells. This should allow an initial assessment of the selectivity of the extracts. Accordingly, concentration–response curves of the extracts against 10 cell lines were generated and IC50 values were calculated (Table 1).

Selectivity is a desired property of active lead anticancer agents [7]. Different studies used PBMC as a model to check the cytotoxic effect of agents on normal human cells [8][9]. In the current study, tested extracts exhibited a much higher cytotoxic effect toward HL-60 and U-937 cell lines than PBMC. The cytotoxicity of all extracts against PBMC was greater than the highest concentration tested (IC50 ˃ 50 µg/mL).

Table 1. IC50 values (µg/mL) for the activities of crude extracts against 10 human cancer MCF-7, A427, RT-4, SiSo, LCLC-103H, DAN-G, A2780, KYSE-70, HL-60, U-937 cell lines, and PBMC.

Cell Lines	Mean ± Standard Error of Mean (µg/mL)			
	A. schimperi	*E. schimperiana*	*K. petitiana*	*K. foliosa*
A427	2.17 ± 0.41	1.85 ± 0.44	2.09 ± 0.43	14.54 ± 4.14
MCF-7	10.31 ± 3.45	Nd	10.41 ± 5.59	14.89 ± 2.38
RT-4	5.18 ± 0.69	2.13 ± 3.78	6.83 ± 0.79	17.3 ± 5.44
SiSo	2.86 ± 0.29	3.28 ± 1.2	3.79 ± 0.49	17.8 ± 2.31
LCLC-103H	3.06 ± 0.3	0.086	7.33 ± 2.7	24.16 ± 0.4
DAN-G	5.23 ± 1.7	Nd	9.6 ± 1.6	27.06 ± 10.8
KYSE-70	2.87 ± 0.3	30.37	3.45 ± 1.6	22.03 ± 3.4
A2780	1.87 ± 0.4	26.54 ± 18.5	2.35 ± 0.9	16.77 ± 4.6
HL-60	4.08 ± 1.4	Nd	8.0 ± 1.7	24.2 ± 0.3
U-937	9.76 ± 6.8	47.68	8.58 ± 3.5	16.9
PBMC	˃50	˃50	˃50	˃50

Testing was conducted with the crystal violet assay except for the HL-60 and U937 cell lines, which were tested with the MTT assay. All values are averages with a standard error of mean of three independent experiments; Nd (not determined).

3. Materials and Methods

3.1. Plant Material

Different parts of 22 plant species were collected from 9 districts, namely, Bale Robe, Bale Goba, Bahirdar Zuria, Abay Gorge, Gewane, Wondo Genet, Doyo Gena, North Bench, and Mizan Aman, of Ethiopia. These specimens were identified by a botanist and a voucher specimen of each plant was deposited at the National Herbarium, Addis Ababa University, Addis Ababa. Each plant materials were shade dried and ground into powder.

3.2. Preparation of Crude Extract

The dried powder (200 g each) was macerated in 1 L (80% methanol) and shaken for 48 h. The macerated plant material was then filtered through Whatman No.1 filter paper by using a Buchner funnel. The crude methanol extracts were concentrated with a rotary evaporator (Büchi Rotavapor®®, R-200and R-210, Duisburg, Germany) with heating (Büchi heating bath®®, B-490 and B-491) at 37–40 °C, followed by freeze-drying (VaCo5, Zirbus Technology, Bad Grund, Germany) the aqueous concentrate.

3.3. Cell Culture

The cancer cell lines in this study were routinely maintained in 75cm2 culture flasks (Sarstedt, Nümbrecht, Germany), in a humid atmosphere of 5% CO2 at 37 °C [10]. Cells were grown in 90% RPMI-1640 media containing, 10% (v/v) heat-inactivated fetal bovine serum (Sigma-Aldrich, Munich, Germany) and supplemented with 30 mg/L penicillin and 40mg/L streptomycin. Cells were incubated in a 5% CO2 humidified incubator (Heracell, Thermo Fisher Scientific, Waltham, MA, USA), at 37 °C, and passaged weekly. Peripheral blood from healthy humans was provided by the blood bank of the University Medicine Greifswald.

3.4. Crystal Violet Cell Proliferation and MTT Assay

Crystal violet and MTT assays were used for both primary and secondary screening of extracts, as described previously in [11] and [12], respectively.

3.5. Statistical Analysis

All tests were independently performed in triplicate. IC50 values were calculated with the software GraphPad Prism 7.0a by determining the inflection point of the simulated sigmoidal curves. The results are presented as means ± standard error of mean where appropriate.

4. Conclusions

The results of this study indicate that crude extracts of 4 out of the 22 plant species have good cytotoxic activity against human cancer cell lines. Among these, four plants, *A. schimperi*, *E. schimperiana*, *K. petitiana* and *K. foliosa*, showed cytotoxic activity against all ten cell lines. Moreover, these extracts possessed selective cytotoxicity toward suspension cell lines (HL-60 and U-937) when compared to their effect on PBMC, consistent with their traditional use in anticancer therapies. In addition to these four plants, *C. abyssinica* and *G. involucrata* also showed selective cytotoxic/antiproliferative activities against some of the human cancer cell lines used in this study. These encouraging results have motivated us to begin isolating and identifying the active components of these four plant extracts, which may contain novel lead compounds for the treatment of cancer.

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