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Ratsimbazafy AM
Department of Biology, Laurentian University, Sudbury, Ontario, Canada

P Michael
Biomolecular Sciences Program, and Laurentian University, Sudbury, Ontario, Canada

SD Rakotoarivelo
Department of Biology, Laurentian University, Sudbury, Ontario, Canada

A Ratiarson
Department of Biology, Laurentian University, Sudbury, Ontario, Canada

CB Lariviere
1) Biomolecular Sciences Program, and Laurentian University, Sudbury, Ontario, Canada
2) School of Human Kinetics, Laurentian University, Sudbury, Ontario, Canada

KK Nkongolo
1) Department of Biology, Laurentian University, Sudbury, Ontario, Canada
2) Biomolecular Sciences Program, and Laurentian University, Sudbury, Ontario, Canada

Correspondence
KK Nkongolo
1) Department of Biology, Laurentian University, Sudbury, Ontario, Canada
2) Biomolecular Sciences Program, and Laurentian University, Sudbury, Ontario, Canada

Cytotoxicity activities against cervical and skin cancer cell lines of *Smilax kraussiana* and *Anthocleista rhizophoroides*, two endemic species widely used in traditional medicine in Madagascar

Ratsimbazafy AM, P Michael, SD Rakotoarivelo, A Ratiarson, CB Lariviere and KK Nkongolo

Abstract
Smilax kraussiana and *Anthocleista rhizophoroides* are endemic plant species widely distributed in Madagascar and are used as herbal remedies. But their cytotoxicity has never been investigated. We evaluated the cytoxicity of extracts from these species on normal, cervical and skin cancer cell lines. The cell panel analyzed consisted of cervical cancer cell lines (HeLa cells containing the HPV18, SiHa cells carrying the HPV16), Human fibroblast cell (CCL-110), and mouse normal fibroblast cell (NIH3T3). Transformed NIH3T3 carrying oncogenes E6 and E7 were developed. Taxol was used as reference drug. Effects of plant extracts at different dosages and Taxol were analyzed using microscope observations, try pan blue test, and conventional MTT assay. The cytotoxicity activity was inferred by the presence of cell membrane blebbingin the cells treated with plant extracts compared to controls. These characteristics were different from observations made for Taxol that generated trypsinized-like rounded cells. The extracts from *S. kraussiana* (1.1 mg/kg) and *A. rhizophoroides* (1.2 mg/kg) exhibited higher cytotoxicity properties on both normal and cancer cell lines from fibroblast and cervix. Their effect was comparable to that of the reference drug Taxol used at low dose. Insertion of E6 and E7 oncogenes in normal fibroblast cells increases their resistance to low dose of plant extracts. All the assays showed dose-dependent responses for the cell lines tested. The extracts in the present study like many others currently used in traditional medicine show general toxicity due to a lack of specificity for cancer cells. This prevents their consideration for clinical trials.

Keywords: Cytotoxicity; *Smilax kraussiana, Anthocleista rhizophoroides*; cervical cancer and fibroblast cell lines

1. Introduction
Cervical cancer is the second most common cancer in women worldwide, with about 500 000 new cases and 250 000 deaths each year [1]. In Madagascar, since the early 90s, it has become a major health issue and one of the principal causes of death among women between 15 and 44 years old representing the population at risk [2]. The main causes and risk factors for cervical cancer have been well characterized. They include the human papillomavirus (HPV) infection, having many sexual partners, smoking, taking birth control pills, and engaging in early sexual contact [1, 3, 4]. HPV infection may also cause cervical dysplasia. In general, cervical cancer in its early or precancerous stages does not cause any symptoms at all and it is often undetected until it has reached advanced stages. These cancerous cells could be completely removed or cured if detected at early stage of development [5, 6].
Typical treatment includes targeting the tumor with ionizing radiation, surgical removal of tumor tissue, and chemotherapy [5]. These methods are expensive and cause severe systemic side effects [5]. For this reason, recent research has focused on the search for alternative drugs extracted from plant-based sources. The use of alternative medicines, especially when combined with conventional cancer treatments, can minimize many of the side effects [7–9]. Since these drugs are primarily extracts of naturally occurring flora, their bioavailability can strengthen the immune system and sometimes enhances the uptake of conventional drugs [10].
Medicinal plants have been of paramount importance in the treatment of many diseases in different parts of the world, owing to the challenges confronting the appropriate delivery of official health care to millions of people in remote and rural communities [11–13]. The World Health Organization (WHO) has called for the identification, sensible exploitation, scientific development and appropriate use of herbal medicines which provide safe and effective remedies in therapies.

This is based on the inherent value of herbal remedies to primary care and the fact that over three quarters of the world's population rely on plants for medical care [12, 14–16]. Hence, therapeutic evaluations are critical in drug development [17].

Toxicity of herbal plants has been reported in many studies [18–25]. But studies comparing effects of natural products on normal and cancer cells are limited. *Smilax kraussiana* and *Anthocleista rhizophoroides* are endemic plant species widely distributed in Madagascar. An ethnobotanical surveythat we conducted among traditional healers in Madagascar revealed that these two species are traditionally used as herbal remedies to treat cancer and venereal diseases.

The main objectives of the present study is to determine the cytoxicity of *Smilax kraussiana* and *Anthocleista rhizophoroides* extracts on normal, cervical and skincancer cell lines.

2. Materials and methods:

2.1. Preparation of ethanolic extracts from *Smilax kraussiana* and *Anthocleista rhizophoroides*

Smilax kraussiana leaves and *Anthocleista rhizophoroides* roots were collected from Antsirabeand Manjakandrianaareas (Madagascar), respectively. The plants were dried at room temperature in a ventilated area protected from light for better preservation. Ethanol extracts of each plant sample were prepared as follows: 100 g of dried plants were soaked in 250 ml of 70 % ethanol for 3 days at room temperature. The mixtures were then filtered through Whatman No. 1 filter paper to remove particulate matters. The filtrates were evaporated under reduced pressure at 24°C using a rotary evaporator that produced gummy concentrates of ethanol extracts.

Dried extracts were dissolved in DMSO to prepare a stock solution with a concentration of100mg of dry matter/ml of DMSO. They were diluted directly in cell culture media and tested on the cancer cell lines at concentration ranging from 0.7 to 1.2 mg/mL. DMSO without plant extracts was used as negative control. Taxol or paclitaxel, one of the most conventional products widely prescribed for cancer treatment was used as a reference compound in measuring the efficacy of the plant extracts.

2.2. Cell Lines

Two human cervical cancer cell lines (HeLa and SiHa), Human fibroblast cell (CCL-110), and mouse normal fibroblast cell (NIH3T3) were used for this study. These cell lines were purchased from ATCC (Rockville, MD, USA). Transformed cell lines obtained by transfection of NIH3T3 and CCL-110 were also included in this study. The cells were cultured and grown at 37°C and 5% CO_2 in sterile DMEM medium with 10% fetal bovine serum, after the addition of 1% of penicillin-streptomycin. Cells were grown in standard tissue culture flasks and were passaged with a solution of 0.25% trypsin-EDTA upon reaching 80% confluence.

2.3. Construction of recombinant DNA and transfection

The goal was to construct a recombinant eukaryotic expression plasmid pCDNA3 containing the E6 and E7 viral oncogenes that can be expressed in mouse fibroblast NIH3T3 cells and human skin fibroblast CCL-110 cells. Therefore DNA extraction was performed from Hela and SiHa cells by using the QIAamp kit provided by Qiagen. A dynamic template PCR technique was used to amplify and to synthesize the sequence of interest. The PCR program included pre-denaturation at 95°C for 5 minutes, 30 amplification cycles each consisting of denaturation at 94°C for 30 seconds, annealing at 55°C for 60 seconds, and extension at 72°C for 50 seconds; followed by further extension at 72°C for 5 minutes [26]. A Kozak sequence GCCACC was inserted at the 5' end of E6 and E7 before the ATG start codon [27]. A BamH1 restriction site was set up just before the Kozak sequence of E6and an EcoR1 restriction site was inserted after the E7 stop codon. The plasmid vector pCDNA3 was digested by EcoR1 and BamH1. The digested products were checked on a 1.5% agarose gel electrophoresis and ligated with the previous nucleotide to form the recombinant DNA. The recombinant plasmid vector pCDNA3 was amplified in *Escherichia coli*HB101 and DH5 alpha competent cells and then extracted by a DNA purification system (Promega) according to the manufacturer's instructions. NIH3T3 and CCL-110 cells at 60-80% confluency were transfected with pCDNA3 recombinant using phosphate-calcium transfection technique [28]. After 6-hours exposure, the normal culture medium, Dulbecco's Modified Eagle Media (DMEM), supplemented with 10% fetal bovine serum (Gibco, USA) was added into cells. Forty eight hours later, cells were exposed to 450 and 500 µg/mL G418 (Gibco, USA) for three days for each concentration. After 18 days of selection, G418-resistant clones were randomly picked up, and cultured in new flasks. Thus, only transfected cells from single clones that were stable were selected for the toxicity assays [29]. For this step, NIH3T3 cells and CCL-110 cells that were transfected with pCDNA3.0 were used as controls. The human fibroblast transformed CCL-110 cells were not included in future toxicity assays because of the lack of cell resistance (high mortality rate) after completion of transfection process.

2.4. Measuring cytotoxicity

Each of the cell lines (HeLa, SiHa, NIH3T3, CCL-110, transfected NIH3T3) underwent different treatments including DMSO, Taxol, *Smilax kraussiana* extracts at doses of 1.1 mg / ml, 1 mg / ml and 0.7 mg / ml, *Anthocleista rhizophoroides* extracts at doses of 1.2 mg / ml, 1.1 mg / ml and 0.8 mg / ml. Three different methods were used to measure the effects of extracts on cell lines in culture. They include morphological analysis, cell counts, and MTT test.

2.4.1. Morphological analysis

Cytotoxicity test was conducted to check the extracts ability to kill cancerous cells. From the different cell lines, the normal media was removed and then 100uL of the medium containing the extract was added. After 24 hours of incubation, the culture was observed under microscope to identify any morphological change. Photographs were taken at 100x magnification.

2.4.2. Trypan blue test/ Dye exclusion

A cell suspension (approximatively 10,000 cells) was prepared for the trypan blue test. Clean hemocytometer slides and cover slips were used; 100 µl /well of cell suspensions were seeded in 96 well micro titer plates and incubated at 37˚C. The medium wasthen removed from each well. Extract mixed with media was added to each well and allowed to incubate for 24 hours. The well content was then emptied and 50 µlof trypan blue was added. This dye was then completely removed from the well after 30 sec and the stained well content was observed under microscope. The number of stained cells and the total number of cells were counted. Viable cells exclude trypan blue, while dead cells will uptake the dye and will stain blue. The experiment was performed in

triplicate. The following formula was used to calculate the concentration of viable cells per well: Cell concentration per ml = NT x 2500 x dilution factor (NT = total number of cells counted in the four outer perimeters).

2.4.3. Cytotoxicity Analysis with MTT Assay

The analysis was performed as described in Chan *et al*. [10] Cytotoxicity of the plant extracts on the cell lines was determined using the MTT Proliferation Assay kits from Invitrogen. Cells were seeded in 96-well flat bottom tissue culture plates at a density of approximately 5,000-10,000 cells/well and allowed to attach for 24 hours at 37°C. They were then incubated with 100 µL of extracts at 100 µg/mL for 24 hours to 120 hours. Control cultures received 100 µL of medium, and blank wells without cells contained 100 µL of medium. After treatment with plant extracts, the cells were grown for additional 24 hours in extract-free fresh medium. A volume of 10 µL of the MTT reagent was then added to each well, and the plate was incubated for 4 h at 37°C. The MTT crystals were then solubilized overnight with 100 µL of the MTT detergent reagent. Absorbance measurements were made at 570 nm using a Spectrophotometer. Cytotoxicity was expressed as the percentage of cells surviving relative to untreated cultures. The percentage of cell viability was calculated using the following formula: Percentage cell viability = (OD of the experiment samples/OD of the control) × 100 [30]. All MTT experiments were performed in triplicate and repeated twice.

2.5. Statistical Analysis

Cell survival data were analyzed using SPSS 20 for Windows, with all data being transformed using a $\log_{10}$ transformation to achieve a normal distribution. ANOVA, followed by Tukey's HSD multiple comparison analysis, were performed to determine significant differences among treatments ($P \leq 0.05$).

3. Results and Discussion

The cell panel analyzed consisted of cervical cancer cell lines (HeLa cells containing the HPV18, SiHa cells carrying the HPV16), CCL-110 (Human fibroblast cell), and NIH3T3 (mouse normal fibroblast cell). Previous research has shown that E6 and E7 oncoproteins involved in cervical cancer development are responsible for the deregulation of cellular control system, by suppressing their anti-proliferative response and engaging the cells into an unbridled growth [31, 32]. Thereby the transforming property has been investigated by creating two new cell lines (transformed cells of NIH3T3 mouse normal fibroblast cells and of CCL-110 human normal skin fibroblast cells) with E6 and E7 oncogenes using a Calcium-Phosphate transfection technique. Microscopic characteristics of normal NIH3T3 and transfected NIH3T3 are illustrated in figure 1. Effects on growth rate, morphology and viability of cells were analyzed using a range of approaches that include microscope observation, trypan blue test, and conventional MTT assay.

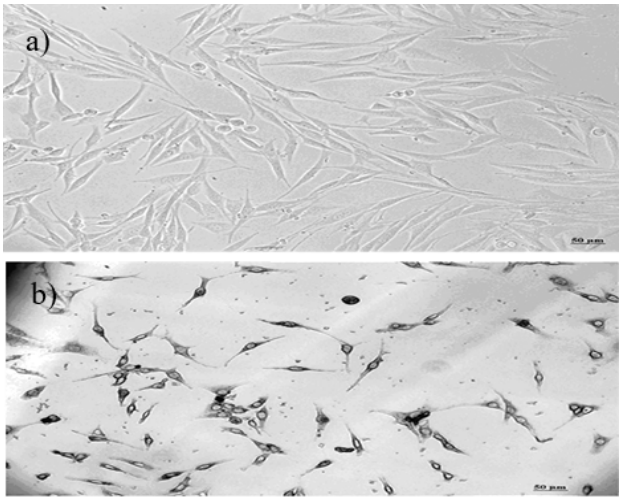

Fig 1: Microscopic illustrations of a) NH3T3 normal cell lines and b) transfected NH3T3 cell lines

3.1. Cell morphology

Microscopic observation of cultures during the treatment period revealed that untreated cells were elongated in shape (Figure 2).

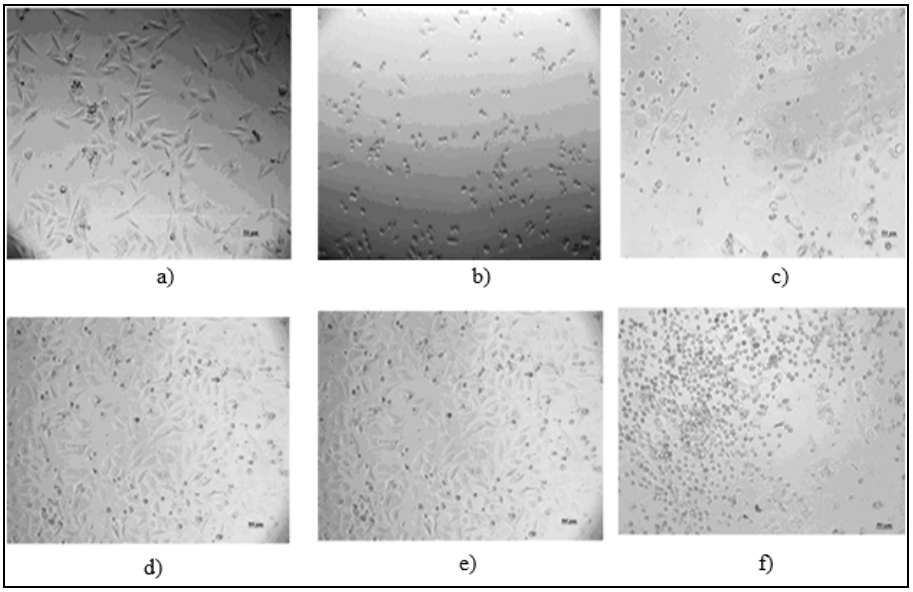

Fig 2: Microscopic illustrations of a) normal Hela cells b) HeLa cells after 120 hours treatment with *Smilax Kraussiana*(1,1mg/ml) after 120 hours; c) HeLa cells after 120 hours treatment with Taxol (10 nM); d) SiHa normal cells; e)SiHa cells after 120 hours treatment with *Anthocleista rhizophoroides* (1,2 mg/ml); f) SiHa cells after 120 hours treatment with Taxol (10 nM).

The cytotoxicity activity was inferred by the presence of cell membrane blebbing (transient detachment of the cell) in the cells treated with plant extracts compared to controls. Cells treated with extracts of plants at high dose shrunk in size and lost their cytoplasm. They were distorted by the mechanism of apoptosis or natural cell death and their overall morphology resembled cellular debris. Irregularly shaped, these cells were floating on the surface of culture media. These cell characteristics are different from observations made for Taxol that generated trypsinized rounded cells (Figure 2).

Toxicity against cells can result in either apoptosis that characterizes a cellular program induced by phytochemicals that cause specific or nonspecific killing of cells[30]. Thus, quantitative measurements of proteins associated with this phenomenon as Bax, Bcl-2, Caspase-3 and PARP-1 may be carried out by immunoblot analysis.

3.2. Cell counts

The number of viable cells for a given cell line varies with different treatments. Tables 1 to 5show that the presence of DMSO in the culture medium does not significantly modify cell growth and proliferation 5 days after the treatment compared to the untreated cells.

Table 1: Viable transfected HeLa cells per µl of suspension after extract treatments.

				Smilax kraussiana			***Anthocleista rhizophoroides***		
Days	**Non treated**	**DMSO**	**Taxol**	**1.1 mg/ml**	**1 ml/ml**	**0.7mg/ml**	**1.2mg/ml**	**1 mg/ml**	**0.8mg/ml**
0	1035^{a}	1013^{a}	998^{a}	1026^{a}	1020^{a}	1021^{a}	1033^{a}	1029^{a}	1014^{a}
1	1224^{a}	1203^{a}	723^{b}	755^{b}	888^{b}	1232^{a}	782^{b}	912^{ab}	1295^{a}
2	1426^{a}	1417^{a}	452^{b}	389^{b}	724^{b}	1475^{a}	418^{b}	771^{b}	1430^{a}
3	1701^{a}	1692^{a}	201^{b}	183^{b}	586^{b}	1624^{ac}	223^{b}	524^{b}	1583^{a}
4	1954^{a}	1923^{a}	54^{b}	64^{b}	465^{b}	1873^{a}	101^{b}	448^{b}	1725^{a}
5	2112^{a}	2129^{a}	5^{b}	2^{b}	364^{c}	1980^{ad}	84^{b}	387^{c}	1757^{d}

Mean values with the same letter are not significantly different based on Tukey's HSD multiple comparison test ($P \geq 0.05$).

Table 2: Viable SiHa cells per µl of suspension after extract treatments.

				Smilax kraussiana			***Antholeistica rhizophoroides***		
Days	**No treatment**	**DMSO**	**Taxol**	**1.1 mg/ml**	**1 mg/ml**	**0.7 mg/ml**	**1.2 mg/ml**	**1 mg/ml**	**0.8 mg/ml**
0	910^{a}	895^{a}	873^{a}	915^{a}	884^{a}	896^{a}	868^{a}	875^{a}	905^{a}
1	1245^{a}	1222^{a}	557^{b}	641^{bc}	702^{c}	1014^{c}	614^{b}	732^{c}	1286^{a}
2	1564^{a}	1546^{a}	340^{b}	422^{eb}	563^{c}	1272^{d}	548^{ec}	575^{c}	1492^{a}
3	1875^{a}	1725^{b}	115^{c}	186^{c}	402^{d}	1417^{e}	319^{d}	402^{d}	1723^{b}
4	2153^{a}	1998^{ab}	47^{c}	48^{c}	257^{d}	1692^{e}	124^{dc}	365^{d}	1886^{b}
5	2355^{a}	2334^{a}	13^{b}	14^{b}	184^{c}	1800^{d}	92^{bc}	215^{c}	2038^{e}

Mean values with the same letter are not significantly different based on Tukey's HSD multiple comparison test ($P \geq 0.05$).

Table 3: Viable normal NIH3T3 cells per µl of suspension after extract treatments.

				Smilax kraussiana			***Antholeistica rhizophoroides***		
Days	**No treatment**	**DMSO**	**Taxol**	**1,1 mg/ml**	**1 mg/ml**	**0,7 mg/ml**	**1,2 mg/ml**	**1 mg/ml**	**0,8 mg/ml**
0	752^{a}	760^{a}	810^{a}	795^{a}	780^{a}	804^{a}	820^{a}	807^{a}	824^{a}
1	1086^{a}	1187^{b}	502^{c}	441^{c}	645^{df}	737^{e}	659^{f}	719^{ef}	916^{g}
2	1402^{a}	1492^{a}	326^{b}	318^{b}	583^{c}	721^{d}	386^{b}	572^{c}	734^{d}
3	1763^{a}	1738^{a}	154^{b}	128^{b}	357^{c}	462^{d}	143^{b}	311^{c}	769^{e}
4	1994^{a}	1989^{a}	38^{b}	12^{b}	141^{b}	506^{c}	85^{b}	146^{b}	541^{c}
5	2127^{a}	2120^{a}	0^{b}	0^{b}	32^{b}	555^{c}	0^{b}	94^{b}	632^{c}

Mean values with the same letter are not significantly different based on Tukey's HSD multiple comparison test ($P \geq 0.05$).

Table 4: Viable transfected CCL-10cells per µl of suspension after extract treatments.

.				***Smilax kraussiana***			***Antholeistica rhizophoroides***		
Days	**No treatment**	**DMSO**	**Taxol**	**1.1 mg/ml**	**1 mg/ml**	**0.7 mg/ml**	**1.2 mg/ml**	**1 mg/ml**	**0.8 mg/ml**
0	1020^{a}	1008^{a}	980^{a}	994^{a}	1001^{a}	983^{a}	991^{a}	1013^{a}	982^{a}
1	1247^{a}	1256^{a}	687^{bc}	623^{b}	776^{c}	1045^{d}	654^{b}	839^{c}	1117^{d}
2	1518^{a}	1572^{a}	416^{b}	484^{bcd}	539^{d}	1259^{e}	467^{bd}	553^{cd}	1375^{f}
3	1721^{a}	1783^{a}	201^{b}	312^{c}	497^{d}	1584^{e}	354^{c}	487^{d}	1505^{f}
4	1866^{a}	1878^{a}	45^{b}	123^{c}	245^{d}	1668^{e}	181^{f}	326^{g}	1726^{h}
5	1995^{a}	1996^{a}	1^{b}	46^{bc}	187^{d}	1752^{e}	62^{c}	204^{d}	1884^{f}

Mean values with the same letter are not significantly different based on Tukey's HSD multiple comparison test ($P \geq 0.05$).

Table 5: Viable transfected NIH3T3 cells per µl of suspension after extract treatments.

				Smilax kraussiana			***Antholeistica rhizophoroides***		
Days	**No treatment**	**DMSO**	**Taxol**	**1.1 mg/ml**	**1 mg/ml**	**0.7 mg/ml**	**1.2 mg/ml**	**1 mg/ml**	**0.8 mg/ml**
0	529^{a}	550^{a}	558^{a}	534^{a}	532^{a}	546^{a}	553^{a}	528^{a}	557^{a}
1	745^{a}	837^{bf}	386^{c}	365^{c}	427^{cd}	763^{a}	396^{ce}	441^{de}	790^{abf}
2	996^{a}	1018^{a}	259^{b}	242^{b}	389^{c}	932^{ad}	275^{b}	382^{c}	884^{d}
3	1279^{a}	1322^{a}	163^{b}	157^{b}	253^{c}	1276^{a}	164^{b}	269^{c}	1036^{d}
4	1342^{a}	1649^{a}	47^{b}	28^{b}	125^{b}	1431^{a}	79^{b}	183^{b}	1352^{a}
5	1763^{a}	1884^{b}	0^{c}	0^{c}	74^{d}	1587^{e}	25^{c}	92^{d}	1613^{e}

Mean values with the same letter are not significantly different based on Tukey's HSD multiple comparison test ($P \geq 0.05$).

The effect of Taxol resembles that of 1.1 mg / ml of *S. kraussiana* leaf extracts and to some extend to 1.2 mg / ml of *A. rhizophoroides* root extracts after 5 days of treatment of the HeLa, SiHa, CC1-110, NIH3T3 fibroblast and transfected cells. There were no significant differences among the lowest dose of *S. kraussiana* and *A. rhizophoroides* plant extracts and the negative control (untreated and DMSO) cells from the first day (day 0) to 5 days after treatments.

3.3. MTT assays

The dose-dependent responses for all the cells tested are illustrated in figure 3. As expected, treatments with DMSO resulted in the highest level of viability for all the cell lines. The cell viability for the lowest dose of plant extracts varies between 80% and 100% with the exception of normal NIH3T3 cell line that showed a sensitivity to a low dose of plant extracts with a survival rate of less than 40% after 5 days of treatments. Transfected NIH3T3 cells were resistant to low doses of plants extracts with a cell survival rate similar to that of HeLa, SiHa, and CCL-110 cells. Taxol produced the same high toxicity response as the 1.1 mg / ml of *S. kraussiana* and 1.2 mg / ml of *A. rhizophoroides* after 5 days of treatment for all the cells lines with survival rates of < 1%. The other doses (D2 and d2) showed also significant cytotoxicity and affected cell growth and survival at different degrees (Figure 3).

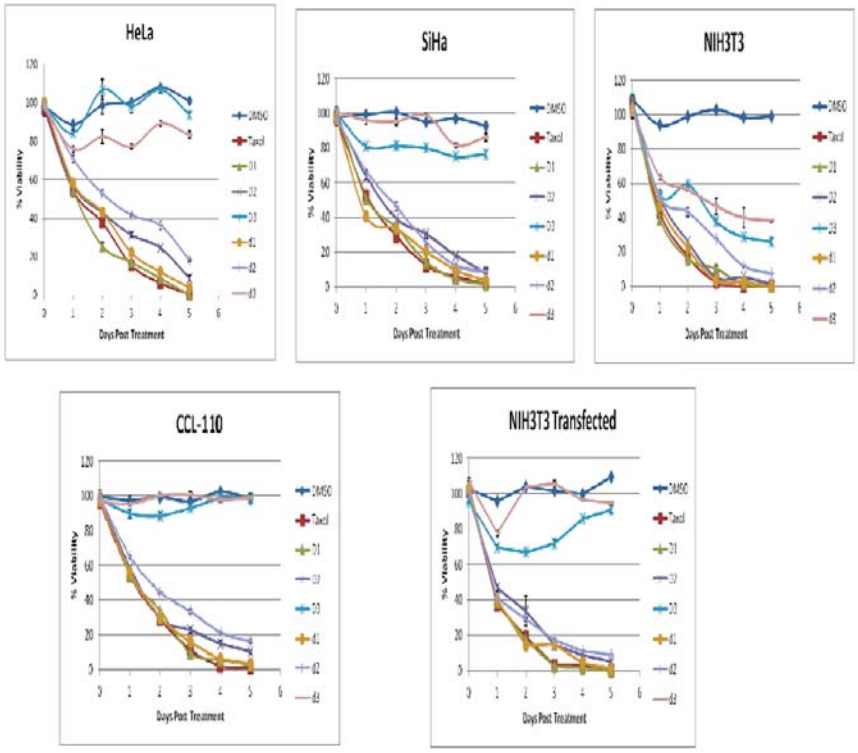

Fig 3: Cell survival rates after five days of treatments in a MTT assay using extracts from *Smilax kraussiana*(D1, 1.1 mg/ml) (D2, 1 mg/ml)(D3, 0.7 mg/ml) and *Anthocleista rhizophoroides*(d1, 1.2 mg/ml) (d2, 1 mg/ml) (d3, 0.8mg/ml) on different cell lines; HeLa cells (A), SiHa cells (B), NIH3T3 cells (C), CCl-110 cells (D), Transfected NIH3T3 cells (E). Taxol-treated cells were used as positive control groups and DMSO-treated cells as negative control groups.

Transfection of CCL-110 Human fibroblast cells with E6 and E7 resulted in severe cell toxicity and inhibition of cell proliferation. Hence, the transformed CCL-110 cell line was not included in the toxicity assays.

The effect of extracts of transfected NIH3T3 cells is different from the normal NIH3T3 cells. The presence of oncogenes E6 and E7 conferred resistance to this cell line. In fact the transformed and normal NIH3T3 were equally affected by the extracts at higher doses. But the lowest dose at each respective extract (0.7 mg / ml and 0.8 mg / ml) was ineffective against the rapid proliferation of transfected NIH3T3. In contrast, normal NIH3T3 cells remained sensitive even at the lowest dose. This demonstrates once again that these cells have acquired specific characteristics of cancer cells. It is interesting to note that the normal human fibroblast cells CCl-110 have a relatively high resistance compared to NIH3T3 cells. Indeed, the cell viability at 0.7 mg/ml for *S. kraussiana* and at 0.8 mg/ml for *A. rhizophoroides* was 100% for CCL-110 and < 40% for NIH3T3. The induction of resistance to low dose of plant extracts observed in toxicity assays involving NIH3T3 transfected with oncogenes E6 and E7 could be related to a phenomenon similar to that of cancer drug resistance. The most common reason for acquisition of resistance to anticancer drugs is expression of one or more energy-dependent transporters that detect and eject anticancer drugs from cells. Other known mechanisms of resistance that probably play an important role in acquired anticancer drug resistance include insensitivity to drug-induced apoptosis [33]. This is the most likely mechanism of resistance to the extracts at low doses. The activation of drug transporters is much less valid in this situation given the known effects of these viral oncogenes.

The difference between cytotoxicity levels of the two plant extracts based on different essays performed may suggest that they contain different chemicals causing cell toxicity. Phytochemical analysis of *S. kraussiana* revealed the presence of glycosides, flavonoids, and anthraquinones [12]. Analysis of rhizome extracts from *Smilax domingensis* a, a relative of *S. kraussiana*, detected flavonoids, saponins, sequiterpene lactones, coumarins, and tannins [34]. Further, Petrica *et al*., [35] reported that quercetin-3-O-α-Lramnopyranoside (1-6)-O-β-D-glucopyranoside, and quercetin- 3-O-β-D-

galactopyranoside are the main flavoides in *Smilax fluminensis* leaves. Phytochemical study of *Anthocleista djalonensis*, a close relative of *A. rhizophoroides* revealed that these plant leaf extracts contains alkaloids, tannins, flavonoids, cardiac glycoside, and saponin [12, 36]. Several lab screening tests illustrated antibacterial and antifungal properties of these plant extracts [12, 36]. The present study showed for the first time the cytotoxicity effects of *S. kraussiana* (1.1 mg/mg) and *A. rhizophoroides* (1.2 mg /ml) on human normal and cancer cell lines.

Taxol at concentration of 10 nMused in this study was as toxic as the high concentrations of *S. kraussiana* (1.1 mg/mg) and *A. rhizophoroides* (1.2 mg /ml). Cancerous and normal cells were equally sensitive to these three compounds five days after treatments. Taxol is a mitotic spindle poison extracted from the bark of *Taxus brevifolia* [37, 38]. It is widely used in the treatments of ovarian, breast, and lung cancer [38]. Taxol at low concentrations ranging from 10 to 50nM showed a cytotoxic effect against neoplastic cells but not normal fibroblasts cells [39]. Taxol decreases the critical concentration of microtubule protein required for microtubule assembly [40]. At low concentration, the principal effect of Taxol is suppression of microtubule dynamics without altering the polymer mass [40, 41]. Analysis of several studies revealed that the mitotic effects of Taxol is dose – dependent [40, 42]. At lower concentration of Taxol (5 – 10 nM) in the treatment of human cancer, cells exhibit aberrant mitosis such as aneuploidy while at high concentrations of Taxol (>20 nM), the cell cycled is blocked at metaphase by spindle checkpoint activity. Surprisingly, in the present study, all the cells were killed at low concentration of Taxol (10 nM) used suggesting that response to this drug might be tissue (cell) specific. The results suggest that the high concentrations of the plant extracts have the same effect as the low concentration of Taxol.

Although a number of plant extracts have shown much promise as potential drugs in cancer phyto therapies based on cytotoxicity studies, most of them are not approved by any governing agency. This is because the majority of these natural products show general toxicity due to a lack of specificity for cancer cells. This prevents their consideration for clinical trials.

4. Conclusion

The two extracts from *Smilax kraussiana*and *Anthocleista rhizophoroides* exhibited higher cytotoxicity properties on normal skin and cervical cells. In fact, the activity is comparable to that of the reference drug Taxol at low dose. Insertion of E6 and E7 oncogenes in normal cells increases their resistance to low dose of plant extracts used. Further studies are required to isolate and characterize bioactive secondary metabolites with cytotoxic properties in the two targeted plant species.

Conflict of Interest

Authors have no conflict of interest to declare

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