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Antiproliferative effect of hydroethanolic extracts of seeds of *Cola verticillata* and leaves of *Solanum scabrum*

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Abstract

Hydroethanolic extracts of leaves of *Solanum scabrum* and seeds of *Cola verticillata* were analysed for anticancer properties. A MTT assay was done to evaluate their antiproliferative properties after 24 and 48 h treatment with varying concentrations. qRT-PCR was used to evaluate the modulation of seven genes involved in apoptosis and angiogenesis after treating cells with 10 and 50 μg/ml of extracts of *C. verticillata* and *S. scabrum*. IC_{50}s for treatment with *S. scabrum* were 15.00 and 11.30 μg/ml after 24 and 48 h treatment respectively. While for *C. verticillata*, it was 19.83 and 15.30 μg/ml for 24 and 48 h treated cells respectively. Apoptotic genes (*p53*, *BCL-2*, and *TNFα*) were downregulated by both doses of *C. verticillata* except for the 10 μg/ml dose that led to the upregulation of TNFα. *S. scabrum* upregulated *BCL-2* for both doses, p53 was upregulated by the higher dose while the lower dose did not modulate the expression of *p53*. TNFα was downregulated by both doses. All selected angiogenic genes (*ICAM-1*, *PDGF*, and *VEGF*) were downregulated by both extracts and at both doses. The DNA repair associated gene (*ERCC1*) was found to be downregulated by both extracts.

Keywords: *Solanum scabrum*; *Cola verticillata*; antiproliferative; gene modulation.

Introduction

Cancer led to the death of 7.6 million people in 2008 say 21% of deaths caused by noncommunicable diseases (NCD) (WHO, 2011) and could be the first cost of mortality in the coming years (Seffrin, 2008). It is a malignant pathology that implicates three well distinct but connected steps: initiation (normal cell transforms into initiated cell), promotion (initiated cell transforms into pre-neoplastic cell), and progression (pre-neoplastic cell becomes neoplastic) (Thangapazham *et al.*, 2006). It is characterized by the uncontrolled growth and proliferation of abnormal cells. Chemotherapy is a reference in the treatment of cancer but applied alone rarely provides healing (Lake and Robinson, 2005). Its association to other types of treatment like surgery and radiotherapy provide more efficient results (Oba, 2009). Apart from chemotherapy, radiotherapy, and surgery, phytotherapy is highly progressing.

Phytotherapy is well known and available in the whole world be it in developing or developed countries. Due to the high cost of other cancer treatments, in Sub-Saharan Africa, phytotherapy is applied through traditional medicine which implicates the use of medicinal plants in the management of this disease. Among these medicinal plants, some are consumed as food and mostly targeted for their healing potentials; it is the case with certain fruits and vegetables highly consumed in Cameroon. *Cola verticillata* whose seeds are consumed in the name of kola nut and *Solanum scabrum* whose leaves are consumed as vegetable locally called Njama-njama are two of such plants. *C. verticillata* belongs to the family of Sterculiaceae. Most members of this family have been found to have high antioxidant potential (Endrini *et al.*, 2009; Momo *et al.*, 2009) and also to be cytotoxic on some cancer cell lines (Endrini *et al.*, 2009). *S. scabrum* is highly consumed in the north-western region

of Cameroon as a vegetable. It is locally used as analgesic, febrifuge, narcotic, and purgative by local population. A closely related specie, *Solanum nigrum* has been proven to be hepatoprotective (Sultana *et al.*, 1995), neuropharmacological (Perez *et al.*, 1998), and antiproliferative (Son *et al.*, 2003). Because of the growing importance of phytotherapy in the treatment of cancer, phytotherapeutic projects have acquired an important part of the medical market and its progression is noticed in Europe and the United States (Saklani and Kutty, 2008). It is in this continuous search that we proposed to study the possible use of two Cameroonian functional foods in the management of cancer.

Materials and Methods

Preparation of hydroethanolic extracts

Fruits of *C. verticillata* were harvested in Bamena, in the western region of Cameroon in april 2010, while leaves of *S. scabrum* were harvested in Babangui, in the north-west region of Cameroon in march 2010. Both were identified at the National Herbarium in Yaounde, Cameroon. They were then sundried until constant weight was attained. After which they were grinned and the obtained powder was used to prepare hydroethanolic extracts. For this, 500 g of each material was marcerated for 48 h in 2000 ml of 50% ethanol. After maceration, the filtrates were recuperated and concentrated by air drying at 40°C to obtain crude hydroethanolic extracts of *C. verticillata* and *S. scabrum*.

Cell culture

The cell line used was the A2780 human ovarian carcinoma. The A2780 cells used for this study were obtained from the cell bank of the Laboratory of Functional Genomics and Experimental Pathology, The Oncology Institute, Prof. Dr. Ion Chiricuţă, Cluj-Napoca, Romania. They were grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 2 mM L-Glutamine, and 100 IU/ml penicillin + 100 mg/ml streptomycin in 5% CO_2 humidified environment at 37°C. All reagents were purchased from Sigma–Aldrich, Germany.

Cell treatment

Cells were treated for 24 and 48 h at varying concentrations of *C. verticillata* and *S. scabrum* extracts (0.25, 1, 2.5, 5, 10, 50, 100, 250, and 500 μg/ml). Cell treatment was done on 96 well plates where 2×10^4 cells were introduced into each well plate in a volume of 200 μl. Each concentration was done in quadruplet and control wells were present in all plates. Cells were seeded into wells 24 h before treatment. The treatment was done by adding 20 μl extract of well defined concentrations into corresponding wells and they were left for 24 and 48 h after which the MTT viability assay was carried.

Viability test

The viability test was done by the tetrazolium 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye reduction assay (Mosmann, 1983). After treating cells for 24 or 48 h, the culture medium in plates was discarded and wells washed with phosphate buffered saline (PBS). 50 μl of hank's solution containing 1 mg/ml of MTT was pipetted into each well and the plates reincubated for 2 h. The wells were washed once more with PBS then 70 μl of pure DMSO was added to solubilize the formazan crystals formed due to action with MTT. The plates were protected from light and agitated at 300 rpm for 5 min at room temperature after which they were read using a Tecan Sunrise microplate reader at 492 nm.

Cell treatment for RT-PCR

For this, six well plates were used and 4×10^5 cells were seeded into each well 24 h before treatment. Cells were treated for 24 h with concentrations of 10 and 50 μg/ml for both hydroethanolic extracts of *C. verticillata* and *S. scabrum* (the concentrations were chosen based on the calculated Inhibitiory concentration 50 (IC_{50}) after the viability test). Each concentration was done in triplicate for each extract and controls (untreated cells) were present.

Extraction and purification of RNA

Treating cells for 24 h led to RNA extraction using TRI-reagent (Sigma–Aldrich, Germany) by digestion. Seperation of cellular contents was done by adding chloroform to the mixture containing cell debris and TRI-reagent. After centrifugation the top phase containing RNA was pipetted into another tube. 70% ethanol was added to precipitate RNA. Purification was done by repeated washes using 75% ethanol. At the end of the purification process, RNA was solubilized in 30 μl of RNase-free water. Quantity analyses using NanoDrop 1000® spectrometer and quality analyses using Lab-on-a-chip Agilent 2100

Bioanalyzer (Agilent technology) were done to determine if the purified RNA could be used for further analyses. All the RNAs presented a RNA intergrity number (RIN) between 9 and 10 permitting us to continue with our analyses.

cDNA synthesis

For cDNA synthesis in this experiment, the Random Hexamer Priming method was exploited as described by the manufacturer of the Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnosis, Germany). Dilutions were done for each RNA purified sample so as to obtain a final concentration of 1000 ng/11 ul for each sample (starting concentration for cDNA synthesis was the same in each tube).

qRT-PCR (quantitative reverse transcriptase-polymerase chain reaction) assay

The LightCycler® Carousel-Based System (LightCycler® Software 4.0, Roche) in combination with the LightCycler TaqMan Master kit were used for qRT-PCR analyses according to the protocol provided by the manufacturer. Template cDNA was added to TaqMan Universal Master Mix with specific primers and probe for each gene: Beta-actin, left primer CCAACCGCGAAGATGA, right primer CCAGAGGCGTACAGGGATAG; PDGF, left primer TGATCTCCACGCCTGCT, right primer TGCATGTTCAGGTCCAACTCG; p53, left primer AGGCCTTGGAACTCAAGGAT, right primer CCCTTTTTGGACTTCAGGTG; ICAM-1, left primer CCTTCCTCACCGTGTACTGG, right primer AGCGTAGGGTAAGGTTCTTGC; VEGF, left primer CCACTTCGTGATGATTCGC, right primer TACCTCCACCATGCCAAGT; BCL-2, left primer CATGTGTGTGGAGAGCGTCAA, right primer GCCGGTTCAGGTACTCAGTCA; TNFα, left primer CAGCCTCTTCTCCTTCCTGAT, right primer GCCAGAGGGCTGATTAAGAGA; ERCC1, left primer CGGACCTCCTGATGGAGA, right primer TTCACGGTGGTCAGACATTC. The PCR array data analysis software using the ΔΔCT method, which is based on fold-change calculations with normalization for all the genes was used.

Statistical analysis

Analysis were done using Graphpad Prism 5.04 and SPSS 16.0; results were analysed using analysis of variance (ANOVA) and they were expressed in mean ± standard deviation. Where necessary a least significant difference (LSD) test was used to compare means and results were considered statistically different when $p \leq 0.05$.

Results

Antiproliferative assay

Antiproliferative activity of hydroethanolic extracts of *S. scabrum* and *C. verticillata* was evaluated using MTT assay. Cells were treated with extracts for 24 and 48 h after which MTT was carried-out on them.

Figure 1: Percentage control against log of concentration for *S. scabrum* treated cells. Curves showing percentage of control against log of concentration after treating cells for 24 and 48 h with hydroethanolic extract of *S. scabrum*. Both curves follow the normal decay trend with that for 24 h being slightly above that for 48 h. This is in accordance with the calculated IC_{50}s implying that the longer the cells stay exposed to the extract the more their proliferation was inhibited.

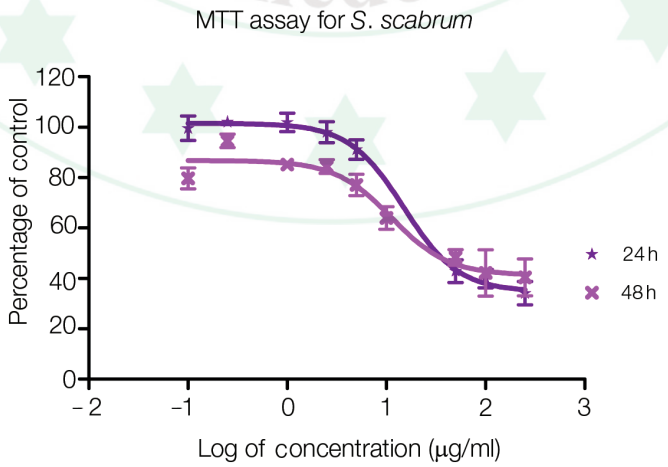
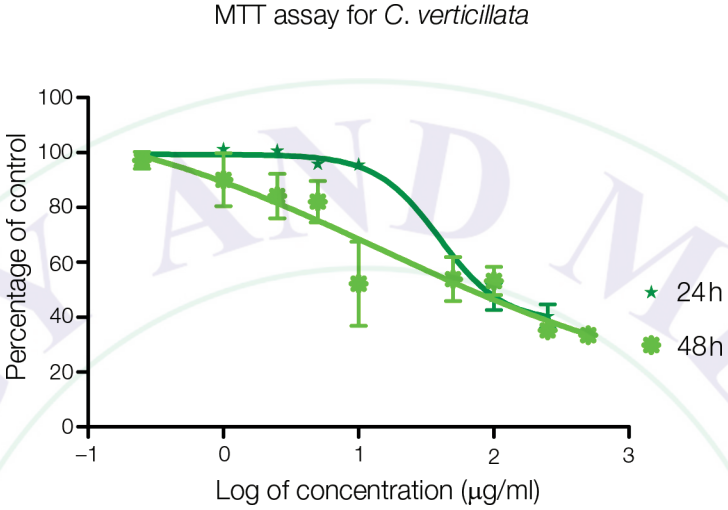


Figure 2: Percentage control against log of concentration for *C. verticillata* treated cells. Curves showing percentage of control against log of concentration after treating cells for 24 and 48 h with hydroethanolic extract of *C. verticillata*. The curve for 24 h treated cells followed the normal decay trend while treatment for 48 h did not. This is in accordance with the calculated IC_{50}s implying that the longer the cells stayed exposed to the extract the more their proliferation was inhibited.



Concerning cells treated with *S. scabrum*, both 24 and 48 h treated gave curves which both followed the normal decay trend (Figure 1). The IC_{50} of cells treated for 24 h (15.00 µg/ml) was higher than for those treated for 48 h (11.30 µg/ml) and these IC_{50}s were found to be statically different ($p \leq 0.01$).

The curve of percentage of control against log of concentration for cells treated with *C. verticillata* for 24 h gave a curve that followed the normal trend while those treated for 48 h did not follow the normal trend (Figure 2). From analysis the IC_{50} for treating cells for 24 h was significantly different from that for 48 h treatment ($p \leq 0.01$); the IC_{50} for 24 h treatment was 19.83 µg/ml while that of cells treated for 48 h was 15.30 µg/ml. Cells treated for a longer period (48 h) showed an IC_{50} that was lower than for cells treated for a shorter period (24 h).

Gene modulation by extracts

For the evaluation of gene modulation by extracts two doses (10 and 50 µg/ml) of each extract (hydroethanolic extracts of *S. scabrum* and *C. verticillata*) were used. The genes evaluated for both extracts included *BCL-2*, *p53*, *PDGF*, *ICAM-1*, *ERCC1*, *TNFα,* and *VEGF*.

Figure 3 below is a graphic representation of gene modulation using *C. verticillata* extracts. Overall, the evaluated genes were downregulated by both doses of the extract except for *TNFα* gene which was upregulated after treatment with 10 µg/ml of *C. verticillata*. *BCL-2* was downregulated and the difference between the two was not significant. This results could imply that apoptosis through a pathway that involves BCL-2 is induced by *C. verticillata*. *p53* was not significantly modulated by the lower dose while it was significant downregulated by the lower dose ($p \leq 0.05$). This could imply that the two doses activated two different apoptotic pathways. The other apoptotic gene evaluated was *TNFα* which was found to be downregulated by the 50 µg/ml dose while it was upregulated by the 10 µg/ml dose (1.84 folds) and the difference between the two was significant. Still these two doses could exploit different pathways for the induction of apoptosis. Apart from these three apoptovtic genes, three angiogenic genes and one DNA repair gene were equally evaluated notably *PDGF*, *ICAM-1*, *ERCC1*, and *VEGF*, which were downregulated even if the folds were different for each gene and dose. The 0.32 and 0.53 fold for the downregulation of the higher and lower doses of PDGF were found to be significantly the same ($p \geq 0.05$). This was also the case with the regulation of *ICAM-1*. As concerns *ERCC1* and *VEGF*, the two doses showed significantly different ($p \leq 0.05$) fold differences with the 50 µg/ml dose being more downregulated than the 10 µg/ml dose. The downregulation of these four angiogenic genes could suppose that hydroethanolic extract of *C. verticillata* fruit is antiangiogenic.

Figure 3: Gene expression level against evaluated gene after treatment with *C. verticillata*. Graph shows relative gene expression level (fold) for each evaluated gene (*BCL-2*, *p53*, *PDGF*, *ICAM-1*, *ERCC1*, *TNF*α, and *VEGF*) for cells treated with 10–50 μg/ml of hydroethanolic extract of *C. verticillata*. The evaluated genes were all down for both doses but for TNFα that was upregulated by the 10 μg/ml dose.

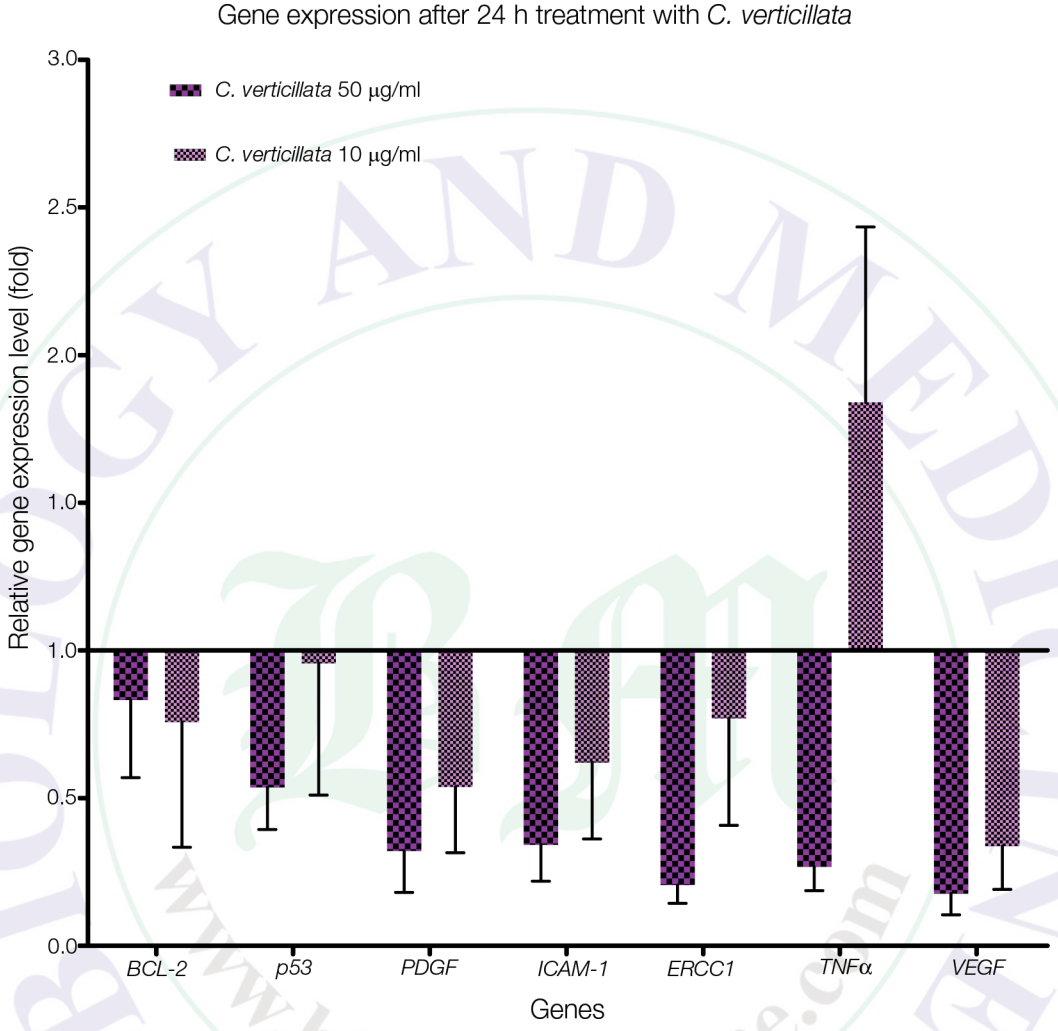
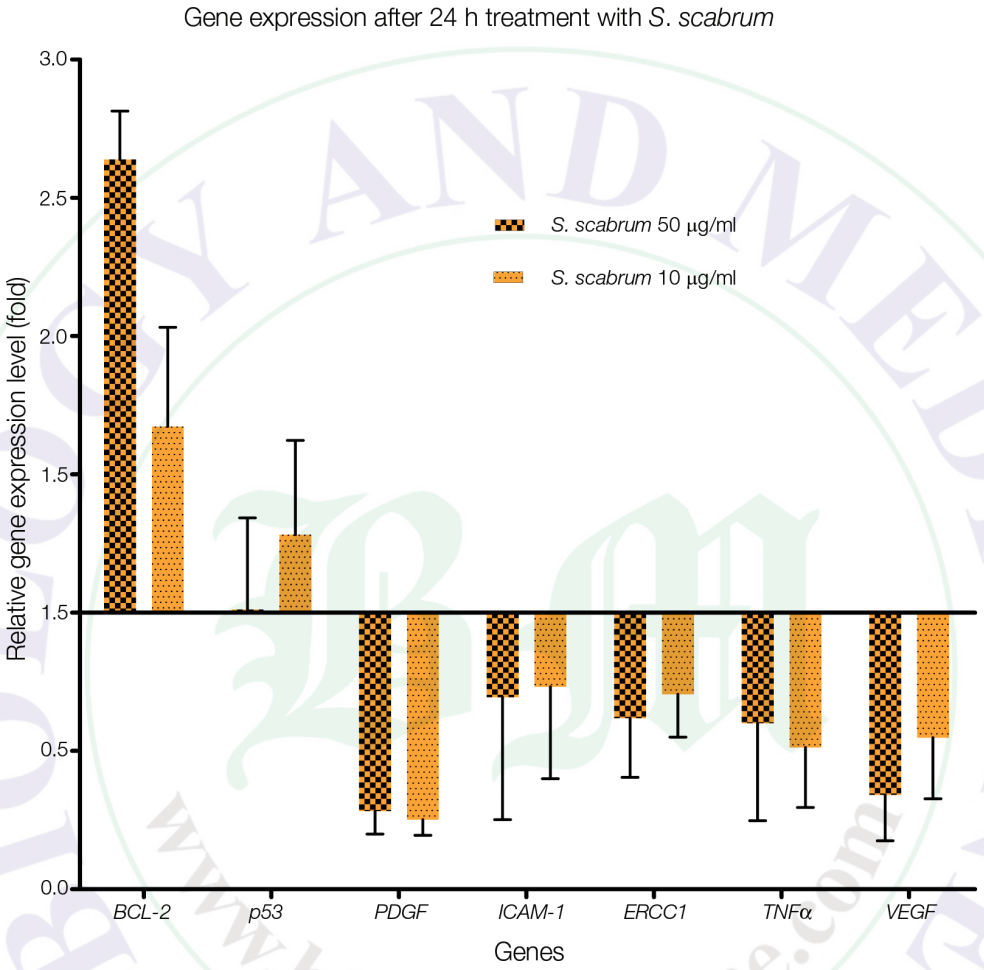


Figure 4 is a representation of gene modulation using *S. scabrum* extracts. The two doses tested were 10 and 50 μg/ml same as with *C. verticillata*. Of all evaluated genes, two of them were upregulated while the remaining five were downregulated. *BCL-2* was upregulated by 24 h treatement with 50 (2.63 folds) and 10 μg/ml (1.67 folds) and the increased expression was different for the two doses ($p \leq 0.05$). The apoptotic mechanism involving *BCL-2* is possibly not that exploited by the hydroethanolic extract of *S. scabrum*. As concerns *p53*, treatment with the 50 μg/ml dose did not modulate this gene (1.00 folds), while treatment with the 10 μg/ml dose significantly increased the expression of the *p53* gene (1.28 folds). This result suggested that apoptosis induced by this extract was *p53* dependent. The other apoptotic gene evaluated was *TNF*α and it was found to be underexpressed in cells after treatment with *S. scabrum* with no significant difference between the two doses ($p \geq 0.05$). This could imply that induction of apoptosis does not exploit the *TNF*α path. Like in treatment with *C. verticillata*, all angiogenic genes as well as the DNA repair gene were underexpressed in cells treated with this extract and this was independent of the dose. Contrarily to the other three genes which showed no significant difference between the 50 and 10 μg/ml dose, *VEGF* showed a difference in the expression levels between the lower and the higher dose ($p \leq 0.05$).

Figure 4: Gene expression level against evaluated gene after treatment with *S. scabrum*.
Graph shows relative gene expression level (fold) for each evaluated gene (*BCL-2*, *p53*, *PDGF*, *ICAM-1*, *ERCC1*, *TNF*α, and *VEGF*) for cells treated with 10–50 μg/ml of hydroethanolic extract of *S. scabrum*. The evaluated genes were modulated differently independent of the dose administered. Both doses of *S. scabrum* led to the upregulation of *BCL-2* and *p53* genes for both doses while the other genes (*PDGF*, *ICAM-1*, *ERCC1*, *TNF*α, and *VEGF*) were downregulated.



S. scabrum and *C. verticillata* affected the evaluated genes differently for some and same for others (Table 1). *BCL-2* was downregulated by *C. verticillata* but upregulated by *S. scabrum* ($p \leq 0.01$). *p53* was downregulated by *C. verticillata* and for *S. scabrum* the higher dose did not alter regulation while the lower dose upregulated *p53* and these two were significantly different ($p \leq 0.05$) from the downregulation of the two doses of *C. verticillata*. Regulation of *PDGF* was significantly the same for both extracts independent of the doses ($p \geq 0.05$). For *ICAM-1*,

Table 1: IC_{50} after treating cells with *C. verticillata* and *S. scabrum*.
IC_{50} of hydroethanolic extracts of *C. verticillata* and *S. scabrum* after 24 and 48 h treatment of A2780 ovarian cancer cells. The lowest IC_{50} was obtained with cells treated for 48 h with *S. scabrum* extracts (11.30 μg/ml) followed by 24 h treatment with the same extract (15.00 μg/ml) then 48 h treatment with *C. verticillata* (15.30 μg/ml) and then 24 h treatment of *C. verticillata* (19.83 μg/ml). *S. scabrum* was found to inhibit the proliferation of A2780 cells more than *C. verticillata*.

Hydroethanolic extract	IC_{50} (μg/ml)	
	24 h treatment	48 h treatment
C. verticillata	19.83	15.30
S. scabrum	15.00	11.30

Table 2: Gene modulation after treatment with different doses of *C. verticillata* and *S. scabrum*. Cells were treated for 24 h with hydroethanolic extracts of *C. verticillata* and *S. scabrum* using two doses (10–50 μg/ml) for each extract. The extract of *C. verticillata* led to the downregulation of all genes (*BCL-2*, *p53*, *PDGF*, *ICAM-1*, *ERCC1*, *TNFα*, and *VEGF*) for both doses except for 10 μg/ml dose that led to the upregulation of *TNFα*. *S. scabrum* led to the upregulation of *BCL-2* and *p53* genes for both doses while the other genes (*PDGF*, *ICAM-1*, *ERCC1*, *TNFα*, *VEGF*) were downregulated.

Genes	Hydroethanolic extract of *C. verticillata*		Hydroethanolic extract of *S. scabrum*	
	50 μg/ml	10 μg/ml	50 μg/ml	10 μg/ml
BCL-2	0.83 ± 0.385	0.75 ± 0.43	2.63 ± 0.17	1.67 ± 0.35
p53	0.53 ± 0.195	0.95 ± 0.84	1.01 ± 0.33	1.28 ± 0.34
PDGF	0.32 ± 0.247	0.53 ± 0.37	0.28 ± 0.11	0.25 ± 0.07
ICAM-1	0.34 ± 0.193	0.62 ± 0.44	0.69 ± 1.21	0.73 ± 0.60
ERCC1	0.20 ± 0.09	0.77 ± 0.82	0.61 ± 0.32	0.70 ± 0.19
TNFα	0.26 ± 0.117	1.84 ± 0.59	0.60 ± 0.84	0.51 ± 0.37
VEGF	0.17 ± 0.122	0.33 ± 0.26	0.34 ± 0.32	0.54 ± 0.36

there was a significant difference between the 50 μg/ml dose of *C. verticillata* compared with both doses of *S. scabrum* ($p \leq 0.05$). As concerns *ERCC1*, there was also a significant difference between the higher dose of *C. verticillata* and both doses of *S. scabrum* ($p \leq 0.05$). *TNFα* showed significantly different results between the lower dose of *C. verticillata* and the higher dose of *S. scabrum* ($p \leq 0.05$) but significantly the same with the lower dose of the same extract ($p \geq 0.05$). There was a significant difference in the modulation of *VEGF* between the treatment with the 10 μg/ml dose of *S. scabrum* and both doses of *C. verticillata* ($p \leq 0.05$) but no difference with the 50 μg/ml dose of *S. scabrum* ($p \geq 0.05$). Table 2 shows the fold changes for each gene for every corresponding dose of each extract.

Discussion

Consumption of fruits and vegetables has been associated with reduced risk of chronic diseases such as cardiovascular diseases and cancer.

In this study, *C. verticillata* and *S. scabrum* which are two functional foods highly consumed in Cameroon were tested for antiproliferative potential using the MTT assay. Their IC_{50}s were determined and it was found that hydroethanolic extracts of both plants altered cell viability and this at concentrations that were within the micromolecular range; the best concentration obtained with *S. scabrum* (11 μg/ml). The potential use of plant derived molecules as active substances in the management of bacterial infections, malaria, and cancer is well documented (Philippe and Angenot, 2005; Frédérich *et al.*, 2002). The National cancer institute estimates that a plants extract that has shown an antiproliferation inhibitory activity of IC_{50} lower than 30 μg/ml during the preliminary cytotoxic test could be considered a potential source of cytotoxic molecules (Jokhadze *et al.*, 2007). *C. verticillata* and *S. scabrum* extracts that were tested gave IC_{50} that were lower than 20 μg/ml. Prior to these, hydroethanolic extracts of seeds of *C. verticillata* and leaves of *S. scabrum* could be considered to have a potential anticancerous activity and could be further studied in view of obtaining new bioactive molecules destined for the treatment of cancer.

In the last decade, basic cancer research has produced remarkable advances in our understanding of cancer biology and cancer genetics. Among these the realization that, apoptosis and the genes that control it have a profound effect on the malignant phenotype. For example, it is now clear that some oncogenic mutations disrupt apoptosis, leading to tumor initiation, progression or metastasis. Conversely, compelling evidence indicates that other oncogenic changes promote apoptosis, thereby producing selective pressure to supress apoptosis during multistage carcinogenesis. The life span of both normal and cancer cells within a living system is regarded to be substantially affected by rate of apoptosis.

p53 is a gene that codes for a protein p53 that regulates cell cycle and hence functions as a tumor suppressor gene. It is a key factor in the initiation of apoptosis as it holds an important

role in cell cycle arrest at the G1 phase. Its positive or negative regulation is dependent on the expression of many proto-oncogenes (Favaudon, 2000). *C. verticillata* extract used in our study was found to downregulate *p53*. Downregulation of *p53* here does not correspond to the antiproliferative property that this extract has. It can be proposed here that apoptosis induced by this extract was not *p53* dependent. In *p53* deficient cells, intrinsic apoptosis can be induced by the tumor protein p73. Apparently, p73 shares numerous regulatory principles and effector pathways with *p53* (Bitomsky and Hofmann, 2009). p73 dependent apoptosis seems to be primarily regulated by its ability to transcriptionally activate pro-apoptotic *p53* target genes (Pietsch *et al.*, 2008). Also it has been proposed that apoptosis induced by DNA damage is not necessarily p53 dependent and that apoptosis due to DNA damage is p53 independent and is correlated to G2/M cell cycle arrest (Arita *et al.*, 1997). Contrarily to *C. verticillata* extracts, A2780 ovarian cancer cells treated with *S. scabrum*, did not downregulated *p53*; the higher dose (50 μg/ml) did not modify *p53* expression while the lower dose (10 μg/ml) upregulated its expression. As a matter of fact, the *p53* gene is a well-known tumor suppressor gene with a significant role in the regulation of cell death and angiogenesis but its molecular mechanisms are far from completely understood. However, *p53* can downregulate some pro-angiogenic proteins like VEGF or upregulate antiangiogenic proteins. The apoptotic process can be induced by two molecular pathways, an extrinsic pathway that operates via death receptors on the cell surface and an intrinsic pathway triggered via mitochondria stimuli (Burz *et al.*, 2009). Nevertheless, both pathways are linked with other signaling proteins, such as p53, MDM2, and NK-κB and converge at the level of caspases (effector proteolytic enzymes). Resveratrol is an anticancer agent that induces apoptosis through the activation of *p53* tumor suppression gene (Laux *et al.*, 2004).

The common mechanism of apoptosis is negatively regulated by several sets of genes, of which the best characterized is the still growing BCL-2 family (Adams and Cory, 1998; Tsujimoto, 1998). The BCL-2 family of proteins that consists of antiapoptotic and pro-apoptotic members determine life-or-death of a cell by controlling the release of mitochondrial apoptogenic factors, cytochrome c, and apoptosis-inducing factor (AIF), that activate downstream executional phases, including the activation of death proteases called caspases (Tsujimoto and Shimzu, 2000). The BCL-2 proteins are a family of proteins involved in the response to apoptosis. Some of these proteins (such as BCL-2 and BCL-XL) are antiapoptotic, while others (such as Bad, Bax, Bak or Bid) are pro-apoptotic (Adams and Cory, 1998; Tsujimoto, 1998). The sensitivity of cells to apoptotic stimuli can depend on the balance of pro- and antiapoptotic BCL-2 proteins. BCL-2 family proteins influence the mitochondrial pathway of apoptosis (Athar *et al.*, 2009). When there is an excess of pro-apoptotic proteins the cells are more sensitive to apoptosis, when there is an excess of antiapoptotic proteins the cells will tend to be more resistant. *BCL-2* of the BCL-2 family prevents all mitochondrial changes including cytochrome c release (Jürgensmeier *et al.*, 1998; Narita *et al.*, 1998). It thus protects cells from undergoing apoptosis through the *p53* pathway but it does not alter the trans-activation of Bax and p21 by *p53*. It is thought that *BCL-2* has an additional ability to prevent caspase activation by sequestrating *Apaf-1* (Hu *et al.*, 1998; Pan *et al.*, 1998). *S. scabrum* used in treating cells led to the upregulation of the *BCL-2* gene. Knowing that antiproliferation was observed anyway in cells treated with *S. scabrum*, it could be supposed that cell death did not depend on a pathway that directly implicated BCL-2. Cytotoxicity due to *S. scabrum* could be linked to ATP depletion in treated cells. Reduction of ATP levels induces a disorganisation of the actine cytoskeleton in treated cancer cells. Quite a number of bioactive molecules have been determined to be cytotoxic by causing ATP depletion. It is the case with cardiotonic glycosides (Van Quaguebeke *et al.*, 2005), pancratistatine (Kekre *et al.*, 2005), and tetrandrine (Yan *et al.*, 2006). Most often, ATP reduction is associated to increase in ROS (Yan *et al.*, 2006) which is often thought to be a factor capable of activating apoptosis (Kekre *et al.*, 2005). Cells treated with 50 and 10 μg/ml of *C. verticillata* for 24 h had their *BCL-2* downregulated. BCL-2 is an antiapoptotic protein that protects cells from programmed cell death by preventing the activation of pro-apoptotic caspase proteins like Bax, Bak, and Bok. Its underexpression would thus not block apoptosis but favor it.

Death receptors are cell surface receptors that transmit apoptotic signals initiated by specific ligands such as Fas, TNFα, and TRAIL. TNFα is a ligand that binds to a death receptor

on the surface of the cell even if its role in inflammation is far more important than its role in apoptosis. In our experiment, both doses of *S. scabrum* led to the downregulation of *TNF* gene. The 50 µg/ml dose of *C. verticillata* equally led to downregulation of this gene. At this level it could be suggested that the extrinsic apoptotic pathway is not stimulated at least concerning the TNF-R1 recptor. Fas was not evaluated and so it cannot be assumed that this pathway is not activated by these extracts. This withstanding, the lower dose (10 µg/ml) of *C. verticillata* led to the upregulation of *TNF*. It can be suggested that, upregulation of TNF is very sensitive to dose and so far as TNF is concerned, very low doses of hydroethanolic extracts of *C. verticillata* are necessary to lead to its over expression.

The formation of new blood vessels through a process called angiogensis is a necessary one in living organisms. It becomes a nuisance when it allows for tumors to grow by offering them opportunities to feed their cells. These new blood vessels most frequently have thin walls causing them to be permeable, these allowing tumors cells to leave their primary sites more readily. Thus, new vessels embedded in a tumor provide a channel for tumor cells to enter circulation and to metastasize to other sites. If contrarily tumors cannot form these vessels, they will not be able to grow in size and their ability to metastasize will be lower.

There has been evidence that VEGF plays a central role during angiogenesis. Higher levels of VEGF have been found in cancer cells when compared to normal cells (Loncaster *et al.*, 2000). This higher level has been correlated with limited overall survival of cancer patients (Cheng *et al.*, 2000). VEGF-A is a pluripotent vasoactive cytokine that stimulates endothelial cell proliferation and chemotaxis, and increases endothelial solute permeability (Kevil *et al*., 1998). In our study 24 h treatment of A2780 ovarian cancer cells with hydroethanolic extracts of *C. verticillata* and *S. scabrum* led to the downregulation of *VEGF* gene for both doses that were tested. It could be concluded that downregulation of *VEGF* leads to an inhibition of the production of VEGF.

An important family of growth factors implicated in cell proliferation and migration is the PDGF family. PDGF regulates a diverse array of cellular processes including cell proliferation, migration, transformation, and apoptosis. PDGF in its paracrine role is indirectly implicated in angiogenesis induced by stimulation of pericytes and mural cells whose secretion is stimulated by the pro-inflammatory agent VEGF (McCarty *et al*., 2007). Its autocrine role, is its activation of the PI3K/Akt and MAPK (Kitadai *et al*., 2006) pathway thus inducing the survival of tumor cells. In our study all tested extracts, this independent of the dose were found to downregulate the expression of *PDGF* gene. These results suggest that underexpression of PDGF together with underexpression of other pro-angiogenic genes in treated A2780 ovarian cancer cells could be responsible for the antiproliferation these cells.

ICAM-1 (Intercellular adhesion molecule 1) also known as CD54 is a protein that in humans is coded for by *ICAM-1* gene and is a member of the immunoglobuline superfamily. Studies have proven that ICAM plays an important role in modulating angiogenic activity (Goebel *et al*., 2006). Little is known about the mechanism through which it influences the angiogenesis. It is at least known that stimulation of VEGF-A of endothelial cells increases ICAM-1 expression (Goebel *et al*., 2006). ICAM-1 thus facilitates VEGF-A mediated angiogenesis. A novel mechanism whereby ICAM-1 expression regulates VEGF-A mediated eNOS activity and angiogenesis through regulation of endothelial glutathione levels has been proposed (Langston *et al*., 2007). In our study both hydroethanolic extracts of *C. verticillata* and *S. scabrum* downregulated ICAM-1 for both the 10 and 50 µg/ml doses. Reduction of the expression of ICAM-1 by extracts is important as this is associated with increase in GSH levels which upregulates phosphatase and tensin homolog (PTEN). *PTEN* is a well known tumor suppressor gene (Cantley and Neel, 1999) whose modulation could be exploited in the quest for antitumoral cancer agents.

Excision repair cross complementing gene 1 (ERCC1) is a highly conserved enzyme which is specific to the nucleotide excision repair (NER) 1 pathway and its absence is incompatible with life. It is involved in the repair of damaged DNA (McHugh *et al*., 2001). In normal tissue, a disorder leading to the suppression of *ERCC1* is associated with DNA repair deficiency and this can be highly deleterious to the organism as may lead to irreparable genetic disorders and even carcinogenesis. In cancer cells, ERCC1 levels are high but this is not an asset; high levels of ERCC1 have led to resistance to certain cancer drugs (Lord *et al*., 2002). In our study, both tested extracts were found to reduce the expression of ERCC1 protein as *ERCC1* gene was found to be

downregulated by these two extracts. In a study carried out by Tsai and collaborators, administration of curcumin significantly increased the cytotoxicity due to cisplatine in cells that had been proven to be resistant to cisplatine treatment (Tsai *et al*., 2011). These two extracts could thus act as curcumin by reducing ERCC1 levels leading to increased sensitivity to cancer treatments.

Practical Applications

Leaves of *S. scabrum* and seeds of *C. verticillata* are highly consumed in Cameroonian diets especially in the grassfield region. Like most fruits and vegetables they have been proven to possess high antioxidant potentials. Consumption of such foods has been associated with decrease risk of degenerative diseases like cancer.

In our study we found that hydroethanolic extracts *S. scabrum* and *C. verticillata* possessed antiproliferative capacity and modulated the expression of certain gene that have been proven to be affected in cancer patients. From the obtained results, these two plants were found to possess anticancer properties.

The results of this pilot study indicate that these two foods could be highly considered for anticancer studies. Knowing that the prevalence of cancer is fastly increasing in developing countries like Cameroon, the consumption of these foods could be advised to Cameroonians. This study would thus have served for the valorization of these foods.

Conflict of Interests

None declared.

Authors' Contributions

All authors contributed equally to this study.

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