Antiproliferative effect of hydroethanolic extracts of seeds of *Cola verticillata* and leaves of *Solanum scabrum*
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 48h 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 50µg/ml of extracts of C. verticillata and S. scabrum. IC₅₀s for treatment with S. scabrum were 15.00 and 11.30µg/ml after 24 and 48h treatment respectively. While for C. verticillata, it was 19.83 and 15.30µg/ml for 24 and 48h treated cells respectively. Apoptotic genes (p53, BCL-2, and TNFa) were downregulated by both doses of C. verticillata except for the 10µg/ml dose that led to the upregulation of TNFa. 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. TNFa 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 by 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, phytherapeutic 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 macerated 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), 2mM L-Glutamine, and 100IU/ml penicillin + 100mg/ml streptomycin in 5% CO₂ 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 500µg/ml). Cell treatment was done on 96 well plates where 2 × 10^4 cells were introduced into each well plate in a volume of 200µl. 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 20µl 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). 50µl of Hank’s solution containing 1mg/ml of MTT was pipetted into each well and the plates reincubated for 2 h. The wells were washed once more with PBS then 70µl 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 300rpm 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 × 10^6 cells were seeded into each well 24 h before treatment. Cells were treated for 24 h with concentrations of 10 and 50µg/ml for both hydroethanolic extracts of C. verticillata and S. scabrum (the concentrations were chosen based on the calculated Inhibitory concentration 50 (IC₅₀) 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. Separation 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 30µl 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 integrity 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 TGATCTCCACGCCCTGCT, right primer TGACCTCTACACCTGCTGTCG; p53, left primer AGGCCCTTGAAGATCATG, right primer CCCTTTTTGGACTTCAGGTG; ICAM-1, left primer CTTCTTCACCGTGTACTGG, right primer AGCGTAGGGTAGGTCTCTGT; VEGF, left primer CCACCTCGTGAAGCAAGCC, right primer TACCTCCACCCATGCAAACT; BCL-2, left primer CATTGTCGAGCGCCAG; p53, left primer CCACCTCTTCACCCTGACT; TNF, left primer CAGGCTCTTCTTTCCCTCT, right primer GCCAGAGGGCTGATTAAGAG; ERCC1, left primer CCGACCTCTCCTGATTTGGATG, right primer TTCACCAGTTGTCAGCACATC. 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 ≤ 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.
Concerning cells treated with S. scabrum, both 24 and 48 h treated gave curves which both followed the normal decay trend (Figure 1). The IC₅₀ 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 IC₅₀s were found to be statically different (p ≤ 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 IC₅₀ for treating cells for 24 h was significantly different from that for 48 h treatment (p ≤ 0.01); the IC₅₀ 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 IC₅₀ 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 ≤ 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 apoptotic 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 ≥ 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 ≤ 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 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 treatment with 50 (2.63 folds) and 10 µg/ml (1.67 folds) and the increased expression was different for the two doses (p ≤ 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 *TNFα* and it was found to be underexpressed in cells after treatment with *S. scabrum* with no significant difference between the two doses (p ≥ 0.05). This could imply that induction of apoptosis does not exploit the *TNFα* 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 ≤ 0.05).
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 ≤ 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. These two were significantly different (p ≤ 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 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, TNFα, and VEGF) were downregulated.

### 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.

<table>
<thead>
<tr>
<th>Hydroethanolic extract</th>
<th>24h treatment</th>
<th>48h treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. verticillata</td>
<td>19.83</td>
<td>15.30</td>
</tr>
<tr>
<td>S. scabrum</td>
<td>15.00</td>
<td>11.30</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Hydroethanolic extract of C. verticillata</th>
<th>Hydroethanolic extract of S. scabrum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 µg/ml</td>
<td>10 µg/ml</td>
</tr>
<tr>
<td>BCL-2</td>
<td>0.83 ± 0.385</td>
<td>0.75 ± 0.43</td>
</tr>
<tr>
<td>p53</td>
<td>0.53 ± 0.195</td>
<td>0.95 ± 0.84</td>
</tr>
<tr>
<td>PDGF</td>
<td>0.32 ± 0.247</td>
<td>0.53 ± 0.37</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>0.34 ± 0.193</td>
<td>0.62 ± 0.44</td>
</tr>
<tr>
<td>ERCC1</td>
<td>0.20 ± 0.09</td>
<td>0.77 ± 0.82</td>
</tr>
<tr>
<td>TNFα</td>
<td>0.26 ± 0.117</td>
<td>1.84 ± 0.59</td>
</tr>
<tr>
<td>VEGF</td>
<td>0.17 ± 0.122</td>
<td>0.33 ± 0.26</td>
</tr>
</tbody>
</table>

there was a significant difference between the 50 µg/ml dose of C. verticillata compared with both doses of S. scabrum (p ≤ 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 ≤ 0.05). TNFα showed significantly different results between the lower dose of C. verticillata and the higher dose of S. scabrum (p ≤ 0.05) but significantly the same with the lower dose of the same extract (p ≤ 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 ≤ 0.05) but no difference with the 50 µg/ml dose of S. scabrum (p ≥ 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 IC50s 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; Frédéric et al., 2002). The National cancer institute estimates that a plants extract that has shown an antiproliferation inhibitory activity of IC50 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 IC50 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 suppress 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 mitochondrial stimuli (Burz et al., 2009). Nevertheless, both pathways are linked with other signaling proteins, such as p53, MDM2, and Nkx-B and converge at the level of caspasess (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 caspasess (Tsujimoto and Shimizu, 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, 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), pancratistatin (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, TNF-α, and TRAIL. TNF-α 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 receptor. 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 angiogenesis 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 cisplatin in cells that had been proven to be resistant to cisplatin 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 Cameroonian. 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.

**References**


