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Immune Modulating and Antiproliferative Potential of *Withania somnifera* Crude and Pre-purified Fractions on Selected Cancerous and Normal Cells

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Authors' contributions

This work was carried out in collaboration between all authors. Authors PGM, NCM, LK, JG, FK, PK and SW wrote the protocol. Authors PGM and NCM designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Author PGM managed the analyses of the study. Authors PGM and NCM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Cancer is a major public health problem globally. The burden of cancer is greater in developing countries where the cost of treatment and management is way beyond the reach of many resource challenged individuals. Plants are a good source for anticancer agents. *Withania*

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somnifera has been widely used for its great pharmacological value. Previous studies have shown that *W. somnifera* has potential as an immune modulating agent by up-regulating the Interleukin-7 cytokine gene expression. Interleukin-7 cytokine (IL-7) is required for the development of the immune system. Studies have shown that exogenous IL-7 increases T cell and B cell numbers. Activation of $CD4^+$ and $CD8^+$T cells may be effective agents for the treatment of malignant tumors. The objective of this study was to confirm that Kenyan *W. somnifera* plant parts extracts have antiproliferative activity and ability to raise IL-7 levels.

Methodology: The MTT assay was used to evaluate the anti-proliferative potential of the extracts. The conventional drug 5-Flourouracil was used as the positive control. For IL-7 analysis, the plant extract with the best antiproliferative activity was used. The cells were recultured in T25 flask then treated with the extract at 50µg/ml for 48hrs. After which the RNA was extracted using Pure Link RNA mini kit (Thermoscientific USA). The extracted RNA was quantified and its quality assessed using a NanoDrop ND-2000 spectrophotometer. Reverse transcription and cDNA amplification was done by a single step reaction technique using Invitrogen Super Script IV Reverse Transcriptase and Thermo scientific real time SYBR green kits according to the manufacturer's instructions. Quantitative real-time PCR was performed in a reaction volume of 25 *µ*L under different condition and time. A total of 50 cycles using RT- PCR Quant studio V system were performed. The primers were the designed using Expasy and Jusbio after which the ct cycle was used to determine the expression levels of IL-7.

Results: The stem methanol-dichloromethane extracts on HCC (IC_{50}-25.23±1.23), roots water extract on DU 145 (IC_{50}-8.04±1.55), leaves methanol-dichloromethane extracts on 22RVI (IC_{50}-2.21±0.577) had the lowest IC_{50} values signifying high activity. The methanolic-dichloromethane extract stem bark gave the highest selectivity index on HCC (breast cancer, SI-98.403) compared to the other extracts. The most inhibitive methanol-dichloromethane extracts against cancerous cells did not inhibit the growth of IEC6 (normal cells). However after 48hrs of exposure of the plant's extract on IEC6 cells expression of IL-7 gene was upregulated 2 times for crude *W. somnifera* leaves organic extract and up to 5.37 times for the prepurified fraction. Our findings indicate that Kenyan *W. somnifera* extracts especially in the pre-purified form have potential to treat and manage cancer. Of great significance is the potency of the plants extracts more so the prepurified fraction to up-regulate IL-7 which is an anticancer cytokine important in proliferation and maturation of immune system cells. This cytokine has been implicated in improved cancer prognosis. The potency of the *W. somnifera* extracts and pre-purified fraction to induce up-regulation of this could infer its probable immune modulation pathway as a possible mechanism of action of the plant's extracts.

Keywords: Antiproliferative; anticancer; Withania somnifera; immune modulation.

1. INTRODUCTION

Cancer is a generic term for a large group of diseases that can affect any part of the body. It encompasses malignant tumors and neoplasms. Cancer is the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs. This process is referred to as metastasis. Metastases are the major cause of death from cancer [1,2]. Cancer is also characterized by malfunctions in immunity. Malignant cells manage to escape recognition and elimination by the immune system [3]. Anyone can develop cancer; however, the risk of being diagnosed with cancer increases with age. About 77% of all cancers are diagnosed in people 55 years of age and older [1, 2].

Statistics show that cancer death cases were in excess of 8.2 million and was ranked as one of the leading causes of death in 2012 [3]. Cancer cases are expected to rise from 14 million in 2012 to an overwhelming 22 million in the next two decades [4]. The National Institutes of Health estimates overall costs of cancer in 2010 at $263.8 billion, $102.8 billion for direct medical costs (total of all health expenditures); $20.9 billion for indirect morbidity costs (cost of lost productivity due to illness); and $140.1 billion for indirect mortality costs (cost of lost productivity due to premature death) [1].

The most fatal forms of cancer include lung, liver, stomach, prostate, colorectal and breast cancers [1]. About a third of all cancer cases can be prevented through improving one's diet, exercise,

lifestyle change viz: reducing tobacco, alcohol and sugar use.

Physical carcinogens, such as ultraviolet and ionizing radiation; chemical carcinogens, such as asbestos, components of tobacco smoke, aflatoxin, arsenic and, biological carcinogens, such as infections from certain viruses, bacteria or parasites interact with an individual's genetic factors to cause an alteration in a normal cell which leads to cancerous cell development. Ageing is another fundamental factor for the development of cancer. Tobacco use, alcohol use, unhealthy diet, and chronic infections from hepatitis B virus (HBV), hepatitis C virus (HCV) and some types of Human Papilloma Virus (HPV) are leading risk factors for cancer in low- and middle-income countries. Cervical cancer, which is caused by HPV, is a leading cause of cancer death among women in low-income countries [1,5,4].

Cancer can be reduced and controlled by implementing evidence based strategies for cancer prevention, early detection of cancer and management of patients with cancer. Treatment includes psychosocial support, surgery, radiotherapy, chemotherapy all aimed at curbing the disease or prolonging and improving quality of life considerably [1].

The efficacy of surgery, chemotherapy and radiation is limited as these treatments do not eliminate all cancerous cells, do not address the immunosuppressive nature of the disease and can further impair the patient's immune response weakening patient's resistance to cancer and other opportunistic infections [2].

It is important to address the health challenge due to cancer by identifying possible solutions to reducing and treating cancer. Medicinal Plants and their components have continued to provide lead compounds in drug development and should be exploited to the benefit of mankind.

Since time immemorial, mankind has used traditional medicines for human health care with terrestrialplants occupying a significant therapeutic role [6]. Over 80% of the world's inhabitants still depend ontraditional medicines for primary health care [7]. At least 120 important drugs are obtained from plants. Many of thenow clinically useful anticancer drugs are either natural plant products or derivatives of naturalproducts [6].

W. somnifera (L.) Dunal is an important plant in the traditional medicine of Africa and Asia. *W. somnifera* contains more than 80 chemical compounds, mainly alkaloids and steroids (withanolides). Numerous studies have been published on the activities of these compounds, mostly obtained from the leaves and roots. These studies have demonstrated antibiotic, anti-inflammatory, cytotoxic, anti-tumor, cholesterol-lowering activities and immune modulating potential [8,9,10]. The chemistry of *W. somnifera* has been extensively studied and over 35 chemical constituents have been identified, extracted, and isolated. The biologically active chemical constituents are alkaloids (isopelletierine, anaferine), steroidal lactones (withanolides, withaferins), saponins containing an additional acyl group (sitoindoside VII and VIII), and withanolides with glucose at carbon 27 (sitoindoside IX and X). *W. somnifera* is also rich in iron [10,11]. The cytotoxicity associated with *W. somnifera* extract is relatively low [8].

Immunologists have proposed and tested a variety of novel strategies for generating cell-based tumor vaccines, focusing on the stimulation of $CD8^+$ CTLs because these effector cells are capable of specifically and directly destroying malignant tumor cells [12].Various cytokine genes and/or surface molecules have been transfected into tumors, and the modified tumor cells have been used as cell-based vaccines to enhance antitumor immune responses [12]. Cell-based vaccines targeting the activation of $CD4^+$ and $CD8^+$ T cells may be effective agents for the treatment of malignancies, such as breast cancer, where the primary tumor is curable by conventional methods, but metastatic lesions remain refractile to current treatment modalities [12].

Natural products with similar stimulating effects on $CD8^+$ CTLs would be extremely useful in addressing tumors. *W. somnifera* has potential to increase expression of IL-7 cytokine mRNA [8]. IL-7 cytokine is associated with the development and maturation of T lymphocytes. IL-7 prolongs survival, decreases tumor burden and enhances tumor T cell infiltration in vaccinated mice [13,14]. Histology of tumors from IL-7–treated mice is heavily infiltrated with both $CD4^+$ and $CD8^+$ T cells [13,14]. The presence of tumor infiltrating lymphocytes (TILs) within the tumor micro-environment is considered to be an indication of the host immune response to tumor antigens [13] and is thought to reflect the dynamic process of "cancer immunoediting".

It would be extremely useful to evaluate various *W. somnifera* plant extracts and pre-purified fractions for antiproliferative activity and potential up regulation on the expression of IL-7 mRNA. *W. somnifera* bioactive components can be targeted for developing into a nutraceutical to boost immunity against cancer.

2. STUDY DESIGN AND METHODS

2.1 Plant Collection and Extraction

2.1.1 Collection and preliminary treatment of leaves, stem and roots of *Withania somnifera*

Withania somnifera was collected from Naivasha sub-county, Nakuru County, Kenya. The collected samples were identified by a specialist botanist and voucher specimens with voucher Mwitari/Naivasha/WS/1/2015 and stored at the University of Nairobi Herbarium. The samples were allowed to dry at room temperature at the Center for Traditional Medicine and Drug Research Botanical laboratory-KEMRI. The dried samples were ground into fine powder using an electrical mill and stored in air tight plastic bags at room temperature until use.

2.2Extraction

Briefly, 200 g of each sample that is the leaves, barks and roots were weighed separately and each put separate in a conical flask, Methanol: Dichloromethane (1:1) 400 ml, was added to cover the plant material completely and left to stand for 24 hours. The extract was filtered using Whatman No. 1 filter paper and residue soaked again for another 24hrs. For the water extracts, the samples were soaked for 90 minutes in double distilled in a water bath at 80ºC. The solvents were removed using a rotor evaporator for organic solvents and water extracts freeze dried. Concentrated extracts were packed in air tight vials and stored at 4ºC until use.

2.3 Cell Line Culturing

ATCC Vero E6 (normal cell line), 22Rv1 (prostate cancer), DU 145 (prostate cancer) and HCC 229 (breast cancer) cell lines were obtained from CTMDR biological unit and used for the study. The cells were cultured in Minimum Essential Media (MEM) (Sigma, USA) supplemented with 10% Fetal Bovine Serum (FBS) and 100 µg/ml streptomycin, then incubated at 5% CO_2 for 72 hours at 37ºC.

2.4 Cells Proliferation Assay

Selected cells (ATCC) viz: HCC, DU 145, 22Rv1 and Vero stored in liquid nitrogen were quickly thawed in a water bath at 37^0C and transferred to MEM media warmed to 37 ^{0}C in 1% DMSO solution. The cells were centrifuged at 800 revolutions per minute for 5 minutes. The supernatant was discarded, and cells re-suspended in complete media; MEM media, 10% Fetal Bovine Serum (FBS), 1% L-Glutamine and 1% antibiotic (PS). The cells were then transferred to a T75 culture bottle and incubated in a high humidity environment at 37^{o}C and 5% CO_2. The cells were passaged after attaining 90% confluence. For anti-proliferation assay, cells were seeded at 50,000 cells per ml concentration in 96 well flat bottomed micro titer plates and incubated in a high humidity environment at 37ºC and 5% CO_2 overnight. A volume of 15 µl of 100mg/ml of the plant's extracts was added to row H, mixed and three-fold serial dilution done up to row B. Row A served as the cell control. The plates were then incubated for a further 48hours. Five-flouro uracil was used as a positive control. Cells at zero concentration of the treatment served as the negative control. Ten µl of MTT dye were added and incubated at 37ºC at 5% CO_2 for 3 hours. Formed formazan was then solubilized using 100µl of DMSO [15], and optical difference (OD) read at 562 nm in a 96-well microtiter plate (MULTISKAN GO, Thermo scientific, USA) OD reader. The test was done in triplicate. Three different independent experiments were set for each test. The effect of the plant extract and 5-flouro uracil on the cells was expressed in IC_{50} values (the extract concentration reducing the absorbance of treated cells by 50% on untreated cells) [8,16].

2.5 RNA Extraction and Gene Expression Analyses

The IEC-6 (Intestinal epithelial cells) were used in evaluation of IL-7 gene expression profiles of the most active plant extracts from *W. somnifera*. The cell line used was selected based on antiproliferative effect of the extracts. The cells were cultured in T25 flask in MEM media with 10% Fetal Bovine Serum (FBS), 1% L-Glutamine and 1% antibiotic (PS) at 5% CO_2 and 37^{o}C. The media was supplemented with select plant extracts at 50µg/ml singly for 48 hours. The

media was then decanted and cells washed in PBS. Cell washing was done three times. Trypsinization of the cells was done and RNA extraction was done using Pure Link RNA mini kit (Thermo Scientific, USA). Briefly the cells were lysed using a lysing buffer I for two minutes with frequent shaking and vortexing. The flask contents containing the lysed cells were then transferred into a molecular grade RNase free Eppendorf tube and centrifuged at 12000 rpm and the supernatant discarded. The pellet was re-suspended in washing buffer II containing 1% 2-mecaptoethanol followed by addition of 2 volumes of cold 70% ethanol to precipitate the nucleic acids in the solution. The tube was then incubated for 30 minutes at room temperature and the contents transferred into a spin cartridge. The spin cartridge was then centrifuged to allow the RNA bid to the cartridge RNA binding matrix. This was followed by RNA elution using RNase free water. The extracted RNA was quantified and its quality assessed using a NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA) and concentrations (ng/µl) obtained. The concentra-tion of RNA (ng/µl) was used to calculate the volumes of RNA and water (H_2O) for use in reverse transcription by first dividing 500 by ng/µl of RNA obtained to give volume of RNA suspension in µl. Reverse transcription and cDNA amplification was done by a single step reaction technique using Invitrogen Super Script IV Reverse Transcriptase and Thermo scientific real time SYBR green kits according to the manufacturer's instructions. Quantitative real-time PCR was performed in a reaction volume of 25 *µ*L. Concisely, 12.5 *µ*L of the SYBR Green master mix, 2.5 *µ*L of the gene specific oligonucleotides (10×), and 10 *µ*L of RNA (100 *µ*g) were added into the wells in an optical 96-well plate. The target gene for amplification IL7 at reaction conditions of 50ºC for 30 minutes, 95ºC for 10 minutes at the hold stage, followed by 95ºC for 30 seconds and 60ºC for 2 minutes at the PCR stage, final extension was at 72ºC for 30 minutes). A total of 50 cycles using RT- PCR Quant studio V system (Thermo Scientific, USA) were performed. Three independent replicates were done per sample. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the internal reference gene for normalization of gene expression. The gene-specific oligonucleotide primers (GAPDH-F 5'AGA CAGCCGCATCTCTTG-3', GAPDH-R 5'TGAC TGTGCCGTTGAACTTG-3', IL-7R 5' TTATCC ATCACCAGGAGCCC-3' and IL-7F 5'GCAAC CCTGTCATCTGCAAT-3')were designed using Justbio and Expasywebsites primer design tools [17]. A single narrow peak from each PCR product was obtained by melting curve analysis at specific temperatures. The quantitative RT-PCR data were analyzed by a comparative threshold (Ct) method, and the fold inductions of the genes by extracts compared with the untreated samples. The Ct cycle were used to determine the expression level in control and cells treated with different extracts for 48 hours. The gene-expression levels were calculated as described by Yuan et al. [18]. The data obtained was expressed as the ratio of the reference gene to the target gene using the standard formula: ΔCt = Ct (target gene) − Ct (GAPDH). To determine the relative expression levels, the following formula was used: ΔΔCt = ΔCt (treated) −ΔCt (control). Therefore, the expression levels were presented as *n*-fold differences relative to the calibrator [8,19,20].

2.6 Data Management and Analysis

A Laboratory notebook was used to record all activities related to this project. Raw and processed data was entered into excel data sheets. Concentration required to inhibit growth of 50% of the cells was calculated using Compusyn software. Statistical analysis was done using excel data sheets and statview version 5.0.1. The expression of IL-7 mRNA relative to GAPDH mRNA was calculated, tabulated and bar charts drawn. The differences between the control and the treatments in these experiments were tested for statistical significance by unpaired Student's *t*-test. A value of $p \leq 0.05$ was considered to indicate statistical significance. Values were expressed as mean ±S.E.

3. RESULTS

Tables 1, 2 and 3 presents the IC_{50} values of different extracts *W. somnifera*as mean ±SE from three different experiments. Antiproliferative activities of the extracts were categorized based on median inhibitory concentration (IC_{50}) into four groups: ≤ 20 µg/ml, active >20-100 µg/ml, moderately active, >100-1000 µg/ml, weakly active and >1000 µg/ml, inactive [21,22]. The activity of the extracts was also expressed in selectivity index (SI) values (Tables 1-4). The SI demonstrates the differential activity of the plants on normal cells compared to cancerous cells. High SI value depicts high selectivity. Selectivity index less than 2 indicates general toxicity and greater than 3 depicts high selectivity [23].

All the extracts inhibited the growth of 22Rv1 (prostate cancer). The plant's stem methanolic-dichloromethane extract was more selective in the inhibition of 22 Rv1 compared to Vero cell line (Table 1). The leaves water extract was significantly toxic to normal Vero cells compared to 22Rv1 cells and the roots were not toxic to 22Rv1 cells. Stem's methanol-dichloromethane extract had the highest selectivity index on HCC cell line (Breast cancer) compared to normal cells (Fig. 1). The leaves water extract was more toxic to normal cells and roots water extract was not toxic to HCC cells. The roots water extract was more selective on DU 145 (SI = 44.401) compared to the other extracts tested. The leaves water extract was more toxic to Vero compared to DU 145 (Table 3).

Table 1. The IC_{50} values of *W. somnifera* extracts on 22Rv1 cells (human prostate cancer cell line) and verocells (normal)

Plant	Part used	Type of extract	22Rv1(Prostate cancer cell line)	Vero (normal cell line)	Selectivity index
W. somnifera	Leaves	M/D	2.21±0.577[a]	30.29±5.77	13.706
W. somnifera	Roots	M/D	98.65±9.64[a]	313.14±5.77	3.174
W. somnifera	Stem	M/D	11.27±2.89[a]	412.53±61.73	42.520
W. somnifera	Leaves	W	122.23±6.35[b]	83.64±5.77	0.684
W. somnifera	Roots	W	N/C	356.99±10.77	N/A
W. somnifera	Stem	W	93.79±11.55[a]	420.02±10.09	4.322
5-Flourouracil	N/A	N/A	21.57±16.11[a]	164.63±5.77	7.632

Table 2. The IC_{50} values of *W. somnifera* extracts on DU 145 cells (human prostate cancer cell line) and verocells (normal)

Plant	Part used	Type of extract	DU 145 (Prostrate cancer cell line)	Vero (Normal cell line)	Selectivity index
W. somnifera	Leaves	M/D	20.74±5.77[a]	30.29±5.77	0.648
W. somnifera	Roots	M/D	149.05±15.68[a]	313.14±5.77	2.101
W. somnifera	Stem	M/D	13.33±6.68[a]	412.53±61.73	35.949
W. somnifera	Leaves	W	393.81±10.59[b]	83.64±5.77	0.212
W. somnifera	Roots	W	8.04±1.55[a]	356.99±10.77	44.401
W. somnifera	Stem	W	239.26±20.77[a]	420.02±10.09	1.694
5-Flouro uracil	N/A	N/A	37.68±0.577[a]	164.63±5.77	4.369

Table 3. The IC_{50} values of *W. somnifera* extracts on HCC cells (human breast cancer cell line) and Vero cells (normal)

Plant	Part used	Type of extract	HCC (breast cancer cell line)	Vero (Normal cell line)	Selectivity index
W. somnifera	Leaves	M/D	N/C	30.29±5.77	N/A
W. somnifera	Roots	M/D	25.23±1.23[a]	313.14±5.77	12.688
W. somnifera	Stem	M/D	5.97±1.59[a]	412.53±61.73	89.403
W. somnifera	Leaves	W	392.77±15.57[b]	83.64±5.77	0.213
W. somnifera	Roots	W	N/C	356.99±10.77	N/A
W. somnifera	Stem	W	169.34±12.82[a]	420.02±10.09	2.339
5-Flourouracil	N/A	N/A	10.29±2.95[a]	164.63±5.77	3.701

Key: MD-Methanol-dichloromathane extract (1:1), W- water, N/C- Not cytotoxic within the tested concentrations, N/A – Not applicable. [a]P<0.001 represents high statistical significance for normal (Vero) compared to each cancer cell type (22Rv1, HCC and DU 145). [b]P<0.001 represents lower statistical significance of normal cells (Vero) compared to each cancer cell.

The conventional drug (5-Flourouracil) selectively inhibited the growth of all cancer cells compared to the normal cells. However, the selectivity indexes of the roots water (SI=44.401) and stem methanol-dichloromethane extracts (SI=35.949) Selectivity Index's were significantly higher compared to those of the 5-Flouro uracil on DU 145 ($p<0.001$). The extracts of the stem and root methanol-dichloromethane were significantly more selective compared to 5-Flourouracil drug on HCC cells. Leaves and stem's methanol-dichloromethane extracts selectivity indexes on 22Rv1 were significantly high compared to 5-Flouro uracil the positive control drug.

Table 4. Fold change in expression of mRNA of interleukin 7 gene

Sample	IL7 gene expression
W. somnifera MD	2.934±0.314
W. somnifera pre-purified fraction	4.873±0.965

***Withania somnifera* extracts up-regulation of interleukin 7 gene**

In order to evaluate the immunomodulatory effect of *W. somnifera* extracts, the up regulation and down regulation of interleukin 7 gene (IL-7) was analyzed. *W. somnifera* mediated expression was performed by treating IEC-6 cells with the plants extracts and pre-purified fractions. Cells treated with *W. somnifera* leaves methanol-dichloromethane (1:1) extract showed a two times up-regulation of the IL-7 gene. The up regulation of IL-7 was found to be higher where IEC-6 cells were subjected to the pre-purified fraction to over 5 times compared to the house keeping gene, GAPDH.

4. DISCUSSION

The study sought to determine the probable anticancer activity of Kenyan *W. somnifera* extracts and pre-purified fraction. In 2012 more than 32.6 million people worldwide had cancer [24]. Radiotherapy, chemotherapy and surgery have been the main stay techniques in cancer treatment and management for centuries. In 1996 Dana Leach proposed for the first time immune approaches in treatment and management of cancer. This proposal accelerated the search for new immune-modulatory agents which led to approval of the first immune checkpoint inhibitor by FDA in 2011 [25]. As progress continues in immunotherapy it is evident that the discovery of this new therapeutic approach was a landmark innovation [26]. Immunomodulation used to control cancer development through cytokine induction has been discovered to be one search novel approach. *W. somnifera* has been known to be a potential anticancer agent in studies done in various parts of the world. Given the fact that phytochemical composition could differ with geographical location of the plant [27].

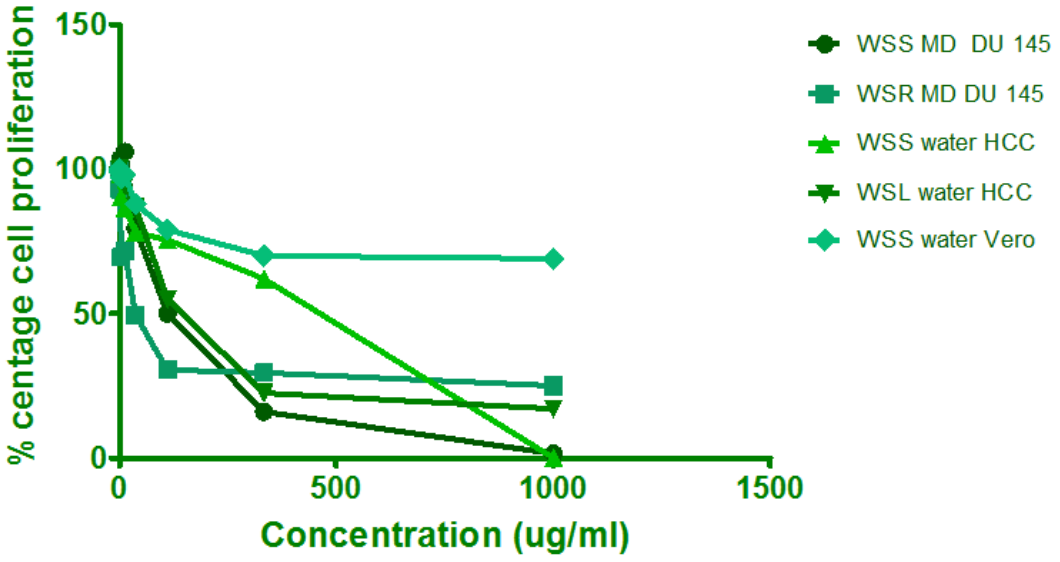

Fig. 1. Effect of the plant extracts on prostate and breast cancer cells

This study evaluated and demonstrated the antiproliferative activity of the plant extracts against breast and prostate cancer cells. The selectivity indices demonstrating both toxic and selective potential of the different plant part extracts. This could be attributed to the different phytochemical composition and concentration in the leaves, stem and roots. The results obtained are in conformation with previous studies in which various extracts from the plant were found to be active against various cancer cell lines [28,29,30]. However, the study is different in that we report a comparison between methanol-dichloromethane and water extracts potential use in breast and prostate cancer treatment and management. Previous studies had indicated that the roots are the most potent [31]. However, the data obtained in this study shows varied inhibition of the cancer cells by extracts from different parts of the plant. The disparity may be due to phytochemical composition in different parts of the plant, environmental pressure and geographical location.

The effect of the plant's extracts on IL-7 gene expression was also evaluated. From this study we were able to see the up regulation of IL-7 gene by the plant extracts. Several studies have demonstrated the importance of cytokines, they provide support, growth and differentiation signals to the cells of the immune system and are of interest for therapeutic development [32]. Of these, the cytokines that signal through the gamma common (*γc*) chain, such as IL-2, IL-4, IL-7, IL-9 or IL-15, are among the most essential [33].

Intestinal epithelial cells (IEC) have been implicated in IL-7 synthesis [8,34]. IL-7 plays an important role in immune processes in our bodies. Several studies have indicated that IEC may play an important role in mucosal immune responses by helping to regulate intestinal intraepithelial lymphocytes (IEL) [35].Importance and usefulness of Cytokine IL 7 as a tool in immunologic activities has been demonstrated before [36]. IL-7 has a potential for adoptive immunotherapy [37,38]. IL-7 is required for the development of the immune system, and studies have shown that exogenous IL-7 increases T cell and B cell numbers in mice [39].

Plant extracts that enhance or stimulate the production of IL-7 cytokine therefore provide potential candidates in cancer treatment as immune boosters. IEC and IL-7 are viable research tools in evaluation of potential plant medicines and their mode of action. Other studies have reported that the plant extracts inhibit expression of IL-8 and Cyclooxgenase -2 (COX-2) in prostate cancer cells. Interlukin-8 and cyclooxygenase -2 over expression is followed by chronic inflammation, increased proliferation, angiogenesis, apoptosis inhibition and metastasis [40]. The dimethyl sulfoxide extract is cytostatic and cytotoxic to leukemia cells. The plant's extract also induces immunogenic cell death [41].

W. somnifera is widely used traditionally in treatment of various diseases including cancer. *W. somnifera* is among the GRAS (Generally Regarded as Safe) family which is a group of plants that have been found to be useful in treatment of various diseases [42].

Strong scientific justification on the use of plants as nutraceuticals or leads will go a long way in treatment and management of cancer among the poor [43].

This study shows that *W. somnifera* could be a potent agent in cancer treatment and management.

5. CONCLUSION

W. somnifera might have an important role among other plants as a source of anticancer agents. Different parts of the plant extracted and tested in this study gave varied activity on different cancer cells. The solvent used in extraction might have varied activity on different cancer cells due to chemical composition of the extracts. Of more importance to note is the ability of the plant's extracts to upregulate the expression of IL 7 gene, a major immunomodulator in cancer: immunoediting. Isolation of pure compounds is being considered to identify the bioactive compounds. Formulation of *W. somnifera* extract as a nutraceutical to aid cancer management and treatment remains a novel idea. Studies to deduce other probable mechanisms of action are underway to establish the potential of the plant in cancer treatment and management.

CONSENT

Consent to publish this manuscript sought from Director, KEMRI through the KEMRI publications committee.

ETHICAL APPROVAL

Ethical approval was sought from Kenya Medical Research Institute (KEMRI); Scientific and Ethics Review Unit (SERU) before conducting the study (Approval number SSC 2902.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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