



## Screening Methanolic Extracts of *Sutherlandia spp* (Cancer Bush) as Anti-Tumor Agents and their Effects on Anti-Apoptotic Genes

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### Abstract

In South Africa, cervical cancer is the utmost common cancer in women with an annual crude incidence rate of 30.2 per 100,000 women. Many of home are still dependent on traditional medicine. Because of the name “cancer bush” given to *Sutherlandia frutescens* (SF) plant by the traditional healers for its anti-cancer activity, the plant was used lines. MTT and xCELLigence were used to predict the IC50 and evaluate methanolic extract effect on SiHa cell lines. Flow cytometer was used to characterize the cell death type. Caspase 3/7 and ATP assays. MTT and xCELLigence results showed a growth inhibition following treatment with varying concentration of *Sutherlandia frutescens* with IC50 optimal at 50ug/ml. ATP level increased in S.F treated cells. Flow cytometry showed cells dying by apoptosis and with many cells trapped in S-Phase. Caspase activity was high in methanolic treated cells. The compounds used were: Canavanine, GABA and Pinitol. These compounds induced cell death at the highest concentration used. *Sutherlandia frutescens* has shown potential to disrupt DNA replication thereby damaging DNA and arresting cell at S-phase leading to cell death by apoptosis.

**Keywords:** Cervical cancer, *Sutherlandia frutescens*, cell cycle, Pinitol, GABA, apoptosis

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### 1. Introduction

Cervical cancer is one of the most common malignancy worldwide next to breast cancer in women (Liu et al., 2009). According to WHO about 13.240 new cases will be diagnosed in 2017 in the United States of America, and 4,210 women are estimated to die in the same year as a result of cervical cancer. Without action, the cervical cancer burden is estimated to reach 21 million new diagnoses and 13

million deaths in 2030 (IARC, 2012). Worldwide, 90% of cervical cancers, occur in low and middle-income countries. Cervical cancer is diagnosed in females as early as 15 years old. The diagnosis is confined mainly to the ages of 30-69 years old (Van Schalwyk et al., 2008). Cervical cancer has the largest rate in South Africa as compared to other cancers affecting women. It affects an estimated 30.2 in 100,000 women and the highest rates are found in women between the ages of 66-69 years (Mqoqi et al., 2004). Approximately 6800 women in South Africa face a new case of cervical cancer, which may account for the 3700 cancer deaths annually (Moqoqi et al., 2004). Because of unequal access to health facilities, socio-economic differences, HPV and HIV infection, the rate of cervical cancer in black women is higher (42.1%) compared to the lower rate in white women (14.5%) (Mqoqi, 2004). South African women undergo cervical excision of the premalignant condition and experience premature delivery as a result of HPV infection (Talip et al., 2010). *Sutherlandia frutescens* (S.F) is an indigenous plant to South Africa belonging to the family of Galegeae (Van Wyk and Albrecht. 2008). The Xhosas, Zulu, Sotho and Cape Dutch used the plant for various illnesses such as stomach ailments, fever, internal cancer, diabetes and wounds (Van Wyk and Gericke, 2000). Moshe et al. (1998) established that the decoction consumed as blood tonic was used also for cough, uterine cancer, and eye infection. The plant is closely related to *Lessertia D.C* (van Wyk and Albrecht, 2008) and is found in the southern part of Namibia, southern eastern

parts of Botswana, western, central and eastern parts of South Africa and most parts of Lesotho (Van Wyk and Albrecht. 2008). The other name of the plant is cancer bush or “kankerbos” owing to its anti-cancer activity. We evaluated *Sutherlandia frutescens* for its cellular impact on cancer cells.

## 2. Materials and Methods

### 2.1. Cervical Cancer Cell Lines

SiHa is a cell line of human squamous carcinoma of the cervix. The cell line was purchased from Japanese Health Science Resource.

### 2.2. Plant Extraction

Plant material (leaves) was dried for 3 days at 50°C, powdered and autoclaved. The plant was then identified and verified at the University of the Witwatersrand botany department. The powdered plant was extracted by dissolving 10mg in 10ml of methanol at room temperature for 24 hours with shaking. The supernatant was removed and filtered through a 0.2 µm filter (Chinkwo, 2005), Methanol was then evaporated. The extracted material was suspended in 5% DMSO (dimethyl sulfur oxide) to make an initial concentration of 5.0 mg/ml of stock solution. The concentration of DMSO was diluted to 0.05% at treatment.

### 2.3. Cell Culture

The cervical cancer cell (*SiHa* cell line) was cultured in RPMI 1640 medium supplement with 10% Foetal Bovine Serum

(FBS), 1 % of 2 mM L-glutamine (Keawpradub *et al.*, 1999), 1 % penicillin/streptomycin/fungizone cocktail and 0.5 % gentamycin. The culture was grown at 37°C in a humidified atmosphere of 5 % (v/v) CO<sub>2</sub> in air. When confluent the cells were either stored by freezing with 10% DMSO or plated in either 96 or 6 well plates for experimental purpose.

#### 2.4. MTT Assay

The MTT {3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide} assay measures the cell's ability to survive in unfavorable conditions. For this study, the assay was used to check the survival of the cells when subjected to methanolic plant extract of *Sutherlandia* spp. Five thousand cells were plated into 96 well culture plates and incubated overnight. The cells were then treated with varying concentrations (50 µg/ml - 200 µg/ml) of the herbal extract and isolated compounds. Cells were treated for 24 hours, followed by the addition of MTT and further incubated for 4 hours at 37°C in the dark. The formazan crystals formed were dissolved by DMSO and the plate was read using a Bio-Rad Microplate reader at an absorbance of 570 nm. Percentage Cell Viability was calculated as follows:

$$\% \text{ Cell viability} = \frac{\text{Absorbance of treated cells} - \text{Absorbance of blank}}{\text{Absorbance of untreated cells} - \text{Absorbance of blank}} \times 100$$

#### 2.5. Flow Cytometry

Flow cytometry was employed following the MTT and xCELLigence assays to investigate the type of cell death linked to treatment of the cells with the plant extract. Annexin V and propidium iodide measured the number of cells that underwent apoptosis, necrosis as well as viable cells. Annexin V has a high calcium-dependent affinity to phosphatidylserine residues; these residues are normally embedded in the cytoplasmic plasma membrane in healthy cells but move to the surface of the cells during apoptosis. Therefore, Annexin V bound to the residues acting as a probe for flow cytometric analysis of cells that were undergoing apoptosis. Propidium iodide, a reagent that is used in tandem with Annexin V, is a non-cell permeable DNA dye that will stain cells undergoing necrosis. Cells were treated with plant extract for 24 hours and they were stained with Annexin V-FITC Apoptosis Detection Kit according to the manufacturer's specifications. Other cells were stained with PI for cell cycle analysis. The cells were then analyzed by flow cytometry.

#### 2.6. Caspase 3/7 Assay

To further distinguish between apoptosis and necrosis, Caspase 3/7 assay was used to determine if the type of cell death induced was caspase-dependent which then suggests that apoptosis might be involved. The cells were seeded in the 96-well plate and incubated overnight to allow the cell to attach on the bottom of the plate. Cells were then treated with extracts for 24hours. CaspaseGlo

substrate was added on to wells and incubated for an hour at 37°C in the dark. The luciferase activity was then read using the Glomax Fluorescence®-96 microplate luminometer.

### 2.7. ATP Assay

Adenosine Triphosphate is used to measure the biological activity of the cells and the amount of ATP is directly proportional to the number of live cells. In this study, we used ATP activity to analyse further how the plant extract affected cell activity or its mitochondrial activity since ATP is mainly synthesized in the mitochondria. The depletion of ATP over time suggested mitochondrial depletion and apoptosis activation. So cells were seeded in 96 well plates and then treated with plant extract for 24 hours followed by addition of CellTiter substrate in the buffer and incubated for an hour at 37°C in the dark. The ATP level was quantified using GLOMAX Fluorescence (Promega, USA).

### 2.8. Real Time PCR

Having observed cell cycle arrest in S-phase and also caspase activity associated with plant extract treatment, we elected to check using qPCR the impact of the plant extract on genes associated with such activities. Luminaris Colour HI Green qPCR Master Mix (Inqaba Biotech) dye was used to quantify the amount of gene expression on cDNA synthesized using total RNA that was isolated from cultured cells. Briefly, cells were treated with IC50 of methanolic extract for 48 hours. Total RNA was isolated using the Zymoresearch Quick RNATM Miniprep and it

was quantified with NanoDrop. Normalized amounts of total RNA were reverse transcribed using the ImProm-II Reverse Transcription System (Promega) according to the manufacturer's specifications. The real-time qPCR was performed in a 20µl reaction mixture containing 2mg cDNA, SYBR Green, and specific primers of our desired genes. The data were analyzed using REST 2009 software.

### 2.9 Statistical Analysis

Two-tailed student t-test was carried out in order to compare the significance of the difference between treated and untreated cancer cells. The probability value ( $p > 0.05$ ) of less than 0.05 indicated a significance.

## 3. Results and Discussion

### 3.1. Discussion

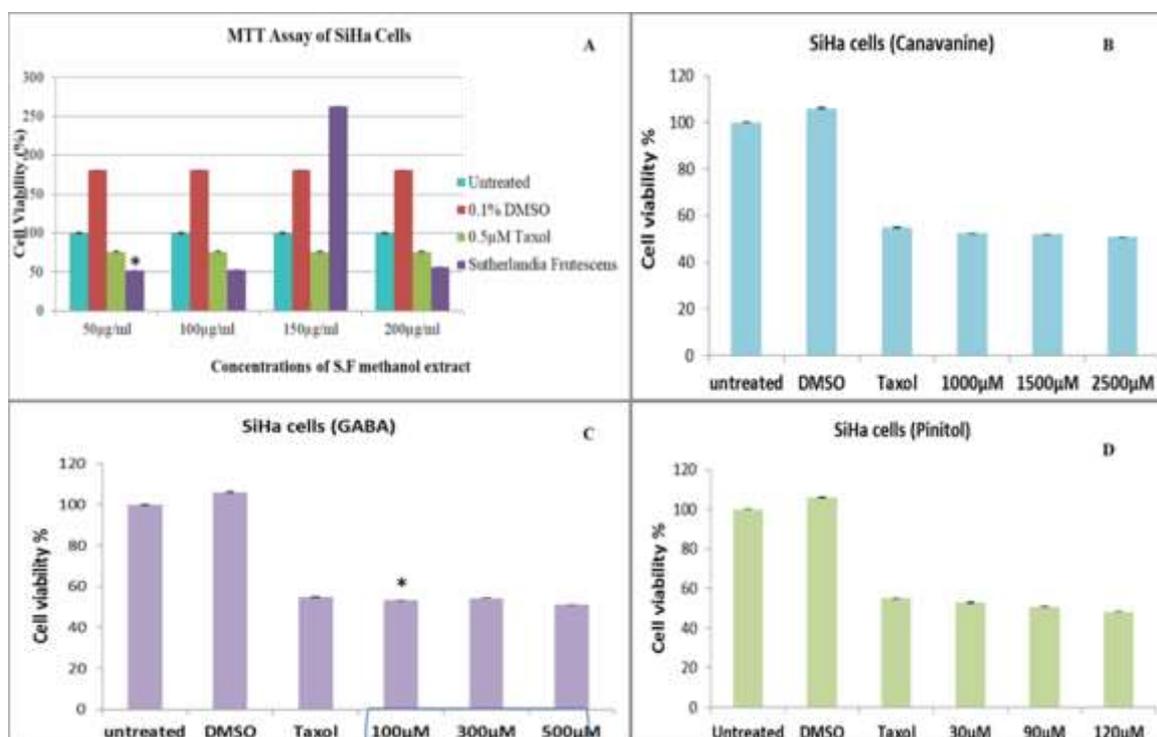
*Sutherlandia frutescens* (S.F) has been used for years by Traditional Healers in most of South Africa as anticancer remedy hence the name Cancer Bush. However, like many more plants used as a treatment for other diseases, there is little to no scientific evidence to suggest that the plant is effective against cancer or has any scientific properties that support the claim it is effective against cancer. In this section, we evaluate the effect of SF on cervical cancer cell line SiHa and examine also its immediate impact on molecular biology. The section of results shows our observed impact of methanolic extract of *Sutherlandia* spp on cell viability and gene expression analysis. It further identifies the

type of compounds present in the plant extract and their impact on cellular survival.

### 3.1.1. The Cytotoxic Effect of *Sutherlandia* Extract on SiHa

MTT assay was employed to determine the antiproliferation of *Sutherlandia frutescens*, Pinitol, Canavanine and GABA activity on SiHa and also to determine the IC<sub>50</sub>. Cells were treated with varying concentrations of the methanolic extract and incubated for a period of between 4- 24hours. Untreated cells were used as a 100% cell viability and 0.5 $\mu$ M Taxol as a positive control. From the observed results, cell viability was concentration-dependent rather than time-dependent as the longer we treated there was no significant

change. However, there was little cytotoxicity before 16 hours and above 24 hours which resulted in predicting that any treatment between 16 to 24 hours was enough to induce cell death. From varying concentrations, treatment with 50 $\mu$ g/ml, 100 $\mu$ g/ml and 200 $\mu$ g/ml reduced cell proliferation by 52%, 53%, and 56% respectively while 150 $\mu$ g/ml did not affect at all even after several repeats (Figure 1). From compounds, there was antiproliferation activity in Pinitol of 51% at 30 $\mu$ M and GABA of 52% at 100  $\mu$ M while in Canavanine there was little activity (Figure 1). From the observed results, the plant at the cellular level showed a promising antiproliferation of the cancer cell line. This prompted us to continue with further studies of



**Figure 1.** The cytotoxic effect of methanol extract of *Sutherlandia frutescens* on SiHa cells. Cells treated with varying concentrations and incubated for 24hrs. (a) Represent methanolic total extraction of SF, (b) Canavanine compound of SF, (c) GABA compound of SF and (d) Pinitol compound.

the plant.

### *3.1.2. The Effects of Sutherlandia frutescens Methanolic Extract on the Cell Growth of SiHa Cell Line.*

Following MTT analysis, cell growth of SiHa cells was monitored over 72 hours to evaluate the impact of plant extracts and compounds on their ability to proliferate. This was done using xCELLigence gold-coated plates that monitor the number of cells attached to the plate surface and present that as a cell index. The more cells attached to the surface the higher the cell index. The effectiveness of the plant extracts or compounds indicated a drop in cell index following treatment. From the observed data, most treatments showed a significant decrease in cell index following 24 hours of treatment with different compounds and plant extracts (Figure 2). In canavanine, there was a brief decline that was followed by a pick in cell index. Both untreated and DMSO treated cells showed a continuous increase in cell index which suggested a little effect on cancer cells.

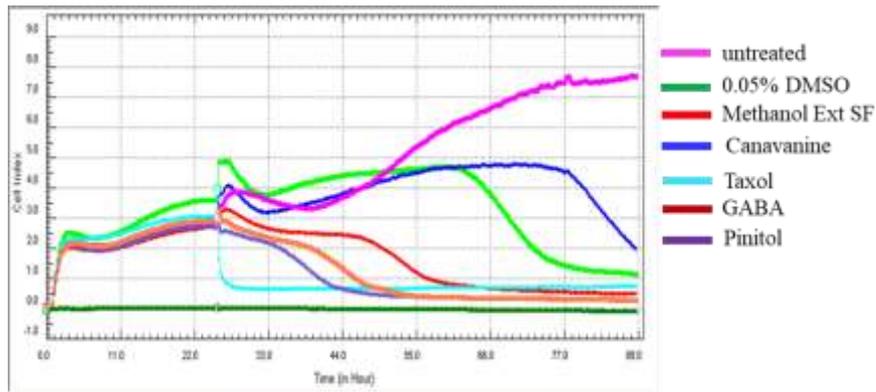
### *3.1.3. The Effect of Methanolic S.F Extract on Cell Cycle and Apoptosis*

The IC<sub>50</sub> observed through MTT assay and xCELLigence was then used to verify if the antiproliferation effects of the plant extracts on cancer cells were either due to Necrosis or programmed cell death. Annexin V was used to bind to the phosphatidylserine serine to measure the early apoptosis. In early apoptosis, membrane asymmetry is lost and phosphatidylserine translocate to the leaflet.

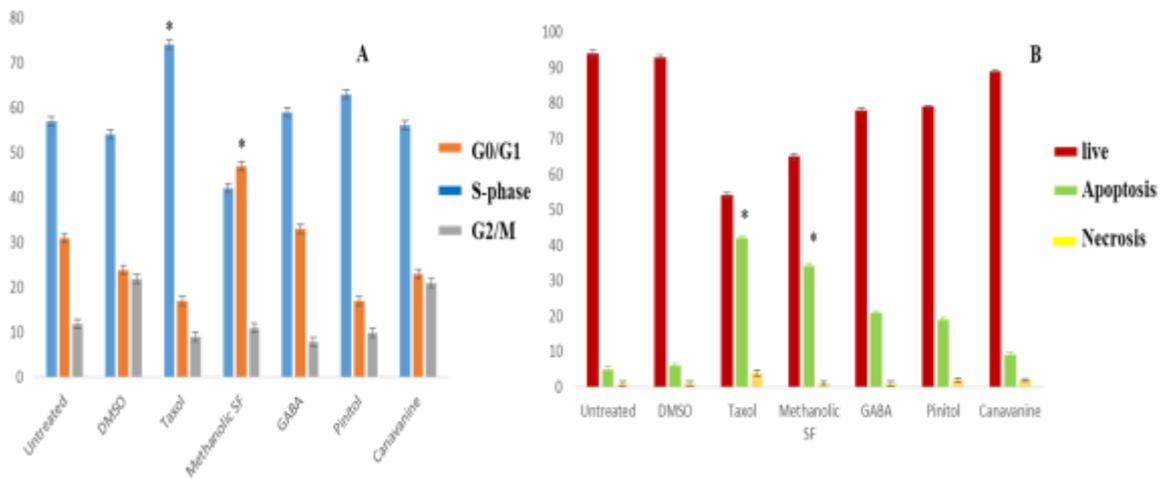
Following treatment with plant extract and compound, there was a significant increase in apoptosis in cells treated with Taxol and Methanolic extract to about 41% and 37% respectively. While other compounds presented an increase, it was however not significant. As expected, necrosis was not significantly increased, which ranged between 1-4% in all treatment and untreated cells. Many compounds may result in apoptosis whether through direct activation of apoptosis or cell cycle arrest. To verify if cells were directly induced through apoptosis to undergo cell death or they were first arrested to repair the damaged cell we conducted cell cycle arrest thereby staining cells with PI following 24 hours treatment with plant extracts. From our study, we observed increased accumulation of cells treated with methanolic extract in the S-Phase (46%) while in other treatment many cells were arrested in the G<sub>0</sub>/G<sub>1</sub> (Taxol 78%, GABA 59% and pinitol 63%) phase (Figure 3).

### *3.1.4. the Effect of Sutherlandia frutescens Methanolic Extract on the Caspase and Mitochondrial Activity of SiHa Cell Lines*

Caspases 3 activation plays a role in cellular events during the apoptosis process. Caspases have been identified as a major contributor to the execution of apoptosis. Both caspases 3 and 7 are responsible for the downstream cleavage of the substrate such as PARP. The caspase activity is measured by the Luminescent which is dependent on the luciferase formulated to generate the glow type luminescent. The results depicted in Fig. 4A



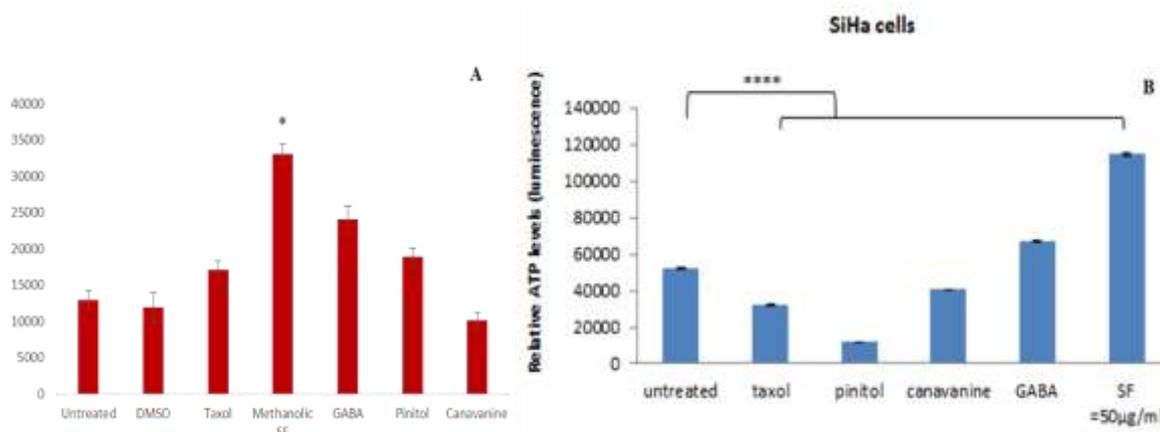
**Figure 2.** xCELLigence analysis of the effect of methanol extract on the cell growth on SiHa in real time. Cells were seeded in 96 well plates and incubated for 24 hours. The SiHa cells were then treated with the increasing concentration 50 µg/ml and various compounds extracted from the plant based on the MTT results.



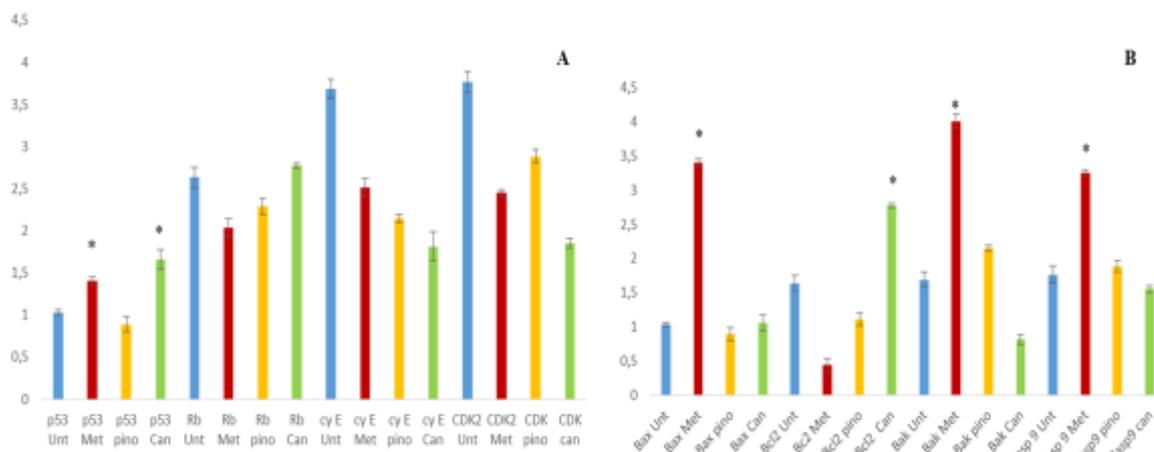
**Figure 3.** Flow Cytometry analysis of SiHa cell subjected to plant extract and Taxol. (a) Cell cycle analysis showing cells accumulating in the S-phase following treatment. (b) Apoptosis analysis showing significant increase in apoptosis in methanolic treatment.

shows that there was a significant double-fold increase (33000 RLU) in caspase3/7 activity following treatment with total methanolic extract of the plant in comparison to Taxol and untreated cells. While in other compounds GABA showed a second-largest increase in caspase activity (25000RLU) as compared to Pinitol at 19000 RLU and Canavanine at about 10000 RLU. Mitochondria is the powerhouse of the cell and its main function is to produce

the ATP that will be used during cell function. Apoptosis is an active process which means it requires ATP for it to be executed. This will be indicated by the increased activity of the mitochondrial. From our analysis of mitochondrial activity, we have observed a double-fold increase in mitochondrial activity from 6000 RLU to 120000RLU within 8 hours which is a suggestion that indeed ATP was highly synthesized immediately after treatment



**Figure 4.** The Caspase and mitochondrial activity graph depicting the results after the cells treated with 0.5µM Taxol and IC<sub>50</sub> from MTT. (a) Caspase 3/7 showed doubling following treatment with methanolic extract. (b) Mitochondrial activity was high within the first 8 hours of treatment in GABA and methanolic extract.



**Figure 5.** qPCR analysis of cell cycle gene expression. Note blue represent untreated cells, Red methanolic extract treated cells, yellow Pinitol treated cells and green Canavanine treated cells.

with plant extract (Figure 4B). The results were similar to that of Taxol and GABA while Pinitol was lower as compared to its caspase activity assay.

### 3.1.5. the Effect of *Sutherlandia frutescens* Methanolic Extract on Cell Cycle and Apoptosis Associated Genes

A cell cycle is a molecular event associated with the production of various proteins that are required to execute cell cycle arrest and if the

repair fails then programmed cell death. One of the main proteins in apoptosis activation is p53 that is negatively regulated by MDM2 during normal cell growth. Following treatment with S.F methanolic extract and observed an increase in cells in S-phase, we analyzed genes associated with the cell cycle. From the results of qPCR p53 was significantly increased by about 1.5 fold in methanolic extract and by 2 fold in canavanine treated cells. Rb was also increased by 3 fold

in canavanine treated cells while other genes such as cyclin E and CDK2 remained unchanged but higher in all treatments (Figure 5A) these results suggest that the cell cycle might not be a reliable reason for the observed cell death. When looking at the apoptosis gene, there was a significant increase in Bax, Bak and Caspase 9 from 0.9 to 3.4 folds, 1.6 to 4.2 folds and 1.7 to 3.2 folds respectively in methanolic plant extract (Figure 5B). Interestingly Bcl2, was significantly decreased in canavanine from 1.8 to 0.8 fold similarly in Methanolic extract from 1.67 to 0.75 fold.

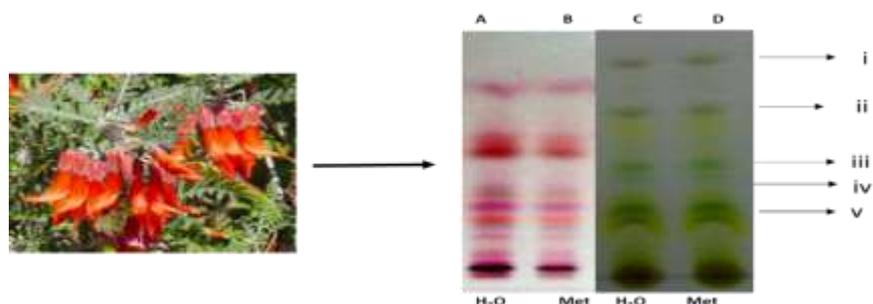
### 3.1.6. Phytochemical Compounds found from Methanol and Aqueous Extracts of *Sutherlandia frutescens* after Mass Spectrophotometry

Fig. 6 shows the TLC profile of *Sutherlandia frutescens* methanolic extract detected with vanillin (A) and fractions of methanolic extract showing over 5 different fragments of compounds present in the plant-based of the Rf values. Further analysis and separation of the compound were identified as shown in table 1. Many of the compounds

present were shown to induce apoptosis in cancer cells such as GABA and Canavanine (Table 1).

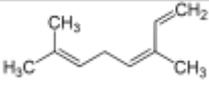
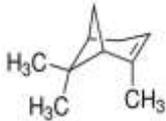
### 3.2. Discussion

Plants have been utilized in the treatment of diabetes, blood pressure and stress, as well as anticancer, antiviral, antifungal and antibacterial agents among others [8]. Up until 2010, 170 anticancer agents were clinically approved, approximately 25% of these constituted by compounds directly from or derived from higher plants [9]. Six clinically utilized groups of chemotherapeutic agents are derived from plants, including Taxol/Paclitaxel from the bark of the Pacific Yew, *Taxus brevifolia* Nutt. (Taxaceae). This compound disrupts the cell cycle through crosslinking microtubules, reducing cell proliferation and inducing apoptosis, and is currently utilized in the treatment of ovarian cancer, advanced breast cancer, as well as small and non-small cell lung cancer [10]. Another chemotherapeutic utilized in the treatment of ovarian cancer is Topotecan, a derivative of the compound camptothecin



**Figure 6.** TLC analysis of *Sutherlandia frutescens*. (a) Vanillin stained plate showing similar fragments to the one unstrained. (b) Methanolic extract fragments of *Sutherlandia frutescens*.

**Table 1.** The table shows the other (not presented on a literature) phytochemical constituents of the methanol and aqueous extract of *Sutherlandia frutescens* using the time of flight mass spectrophotometer as well as the research done on each.

Compound	Structure	Solvent and Cancer type for anti-proliferative effect	Reference
(+)-3-carene C <sub>10</sub> H <sub>16</sub>		DMSO Solvent, Breast, colon, lung and glioma cancer	Mahahpara and Washat
(E)-β-ocimene C <sub>10</sub> H <sub>16</sub>		Colon, glioblastoma, adenocarcinoma and carcinoma	human hepatocellular carcinoma De Oliveira <i>et al.</i> , 2015
(+)-β-pinene C <sub>10</sub> H <sub>16</sub>		Ovarian and Hepatocellular carcinoma, Lung, glioma, breast and colon cancer	DMSO Wang <i>et al.</i> , 2012 Wajahaka Shah 2014
(+)-α-pinene C <sub>10</sub> H <sub>16</sub>		Ovarian and Hepatocellular carcinoma, Lung, glioma, breast and colon cancer	DMSO Wang <i>et al.</i> , 2012 and Chen <i>et al.</i> , 2015, Wajahaka Shah, 2014
15-cis-phytoene C <sub>15</sub> H <sub>22</sub> O <sub>2</sub>		Reduce risk of lung and epithelial cancers on human	Pharmacodynamic basis of herbal medicine, second edition by Manuchair Ebadi

isolated from the bark of the *Camptotheca accuminata* tree, which acts to inhibit cell cycle progression and induce apoptosis through preventing the function of DNA Topoisomerase I [11].

In developing and undeveloped countries medicinal plants remain the main source of health care. However, many plants are able to naturally synthesis toxic or poisonous chemicals for their defense. These chemicals might be dangerous for human consumption and might even lead to fatality. One such plant that many in South Africa depend on is *Sutherlandia Frutescens* also known as cancer

bush for its use or perceived success in the treatment of cancers. The phytochemistry of *Sutherlandia Frutescens* has been studied but their role in cancer treatment still not been clarified [1]. In this study, we looked at the role of *Sutherlandia Frutescens* methanolic extract and its single extracted compounds Pinitol, Canavanine and GABA in anticancer activity. The results obtained using SiHa cells indicate that methanolic extracts of *Sutherlandia Frutescens* had some antiproliferative activity as compared to the 3 compounds (Figure 1). These results suggest that perhaps not individual compounds that

might have anticancer activity but the cocktail of all compounds present in the extract. The results were further confirmed by xCELLigence in which similar results were seen in which cell index was significantly affected following treatment with *Sutherlandia Frutescens* extracts than individual compounds. Of note, GABA has shown some cell growth promotion than inhibition. Canavanine was one of the compounds that showed some positive cell index inhibition. These results are similar to those previously reported by other researchers whereby they have shown that total extracts of the plant were more effective than the individual compounds [12-14].

The only way to successful cancer therapy is the ability of new targets whether plant extracts of small molecule targets to restore the cell's ability to self-regulate meaning restoring the apoptotic and cell cycle machinery. From our analysis of the effect of *Sutherlandia Frutescens* methanolic extracts and the 3 compounds on apoptosis and cell cycle arrest, we observed contradictory results with the 3 compounds failing to induce apoptosis as compared to the earlier MTT assay analysis that showed about 52% cell proliferation inhibition. This was further supported by the mitochondrial activity that showed little to none in the activity following treatment with the 3 compounds. However, GABA showed some apoptotic activity as indicated by the shrinking of the cancer cell that stained positive for Annexin V (Figure 3). The results were similar to that of Moela et al in which camptothecin was able to induce cell

morphological changes that resemble that of apoptosis in MCF-7 breast cancer cell line.

*Sutherlandia Frutescens* extracts induction of both cell cycle and apoptosis was similar to the work by [15] in which they reported cell cycle arrest at G1/S phase in Cannabis in particular cannabidiol treated melanoma cells with the studies indicating cell death via apoptosis as the main effector mechanism of treatments [16]. The fact that Taxol and methanolic extract had presented similar trends in cell cycle arrest both at G0/G1 and G2/M phase is an indication that perhaps there extract might be possessing similar properties to that of Taxol and can act on DNA replication to arrest the cell cycle. This was evident in all the results of SiHa cell treated with GABA, Pinitol, Canavanine and methanolic extracts of *Sutherlandia Frutescens* which showed an increased accumulation of cells in the S-phase suggesting that these cells were able to enter DNA replication but like camptothecin treated cells, topoisomerase I or other DNA replication proteins were interfered with resulting in the disruption of replication (Figure 3; [17-19]). These results suggest that somehow the plant might contain compounds able to inactivate certain enzyme responsible for DNA replication and thereby suggesting that *Sutherlandia Frutescens* might contain properties of anticancer agents.

Like many apoptotic cells, cell morphologically shrink and develop blebs and other characteristics. Similarly, in this study we have observed cell shrinkage following treatment with Canavanine and methanolic extract which further supported our view that

through several other compounds in the plant, *Sutherlandia Frutescens* does induce apoptosis in SiHa cancer cells and that was supported by the fact that Canavanine was able to show similar results to that of *Sutherlandia Frutescens* (Figure 3). These results, therefore, correlate well with apoptosis induction and enhanced caspase-3 activity noted in response to cannabidiol treatment in numerous cancer cell lines [19]. In this study, we further report a significant double-fold increase in caspase3/7 activity (Figure 4). The increase in caspases can only implicate one process in cell biology that is apoptosis because these molecules are known to be the executor's of the cell through apoptosis machinery [20].

Important to cell biology is a mitochondrial activity that is indicative of ATP production which is required for apoptosis induction. In this study, we also observed an increase in mitochondrial activity within 8 hours of treatment with methanolic extract followed by a drop immediately after which suggested disintegration of mitochondria and loss of cell function (Figure 4A). Contrary to these results, none of the 3 compounds were able to induce mitochondrial activity; further supporting the earlier hypothesis that it is the total extract of *Sutherlandia Frutescens* rather than individual compounds that act as anticancer agents. These results are similar to those by [21] in which they have shown that treating lung cancer cell lines with a cocktail of the concentration range of vitamin K from 7.5 to 75  $\mu$ M they were able to induce apoptosis. Previous studies further demonstrated that VK2 inhibits proliferation of various cancer

cells, such as leukemia and hepatocellular carcinoma which further support our observations [22; 23]. This further suggests that several compounds in the plant might be engaging in an organized hierarchical manner that leads to cell death.

Throughout this study, we have shown that methanolic extract of *Sutherlandia Frutescens* is a potent inhibitor of cell proliferation and induce apoptosis possibly through its ability to significantly increase caspases and mitochondrial activity. On a molecular level, there was little difference between treated cells and untreated cells when it comes to cell cycle genes. There was no significant increase or decrease of cyclin and CDKs associated with S-Phase which might suggest that it was not the cyclin that might have been disrupted. However, looking at Bax and Bak there was a significant increase in this gene expression further evidence of apoptosis induction was shown by an increase in caspase 9 in all methanolic extract. With individual compounds, there were no significant changes except that canavanine treatment increases the expression of Bcl2 while decreasing that of BaK. Similar observations were reported by [17] in which they reported no cell cycle or apoptosis induction related to mitochondrial pathway associated with bax in HL-60 cell lines. The results obtained from this study do little to predict any molecular mechanism associated with *Sutherlandia Frutescens* methanolic extracts since there are many pathways upstream or downstream that might be involved and that need to be looked at in the future. Furthermore, the cellular impact of

methanolic extract might also be different from the *in vivo* studies in which many systems will affect the delivery and metabolism of such compounds. However, the cellular model provides a promise and a clear path impact of plant extract on the survival of cancer cells.

#### 4. Conclusion

*Sutherlandia Frutescens* methanolic extract rather than the three extracted compound has a potent anticancer activity which was observed through the activation of apoptosis in a suggested p53 dependent manner and cell cycle arrest, particularly in the S-phase. However, the caspase 3/7 and mitochondrial activity increase highlight the important function of the *Sutherlandia Frutescens* in inducing apoptosis. The molecular mechanism of how apoptosis is induced remains unclear.

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#### Reference

- [1] Liu Y, Cherry JJ, Dineen JV, Androphy EJ, Baleja JD. Determinants of stability for the E6 protein of papillomavirus type 16. *J. Mol. Biol.* (2009) 386 (4): 1123-37.
- [2] Van Schalkwyk SL, Maree JE, Wright SCD. Cervical cancer: the route from signs and symptoms to

treatment in South Africa. *Reprod. Heal. Mat.* (2008) 16 (32): 9–17.

[3] Somdyala NIM, Bradshaw D, Curtis B, Gelderblom WCA. Cancer incidence in selected municipalities of the Eastern Cape Province, 1998-2002: PROMEC Cancer Registry Technical Report. Cape Town: South African Medical Research Council (2007).

[4] Talip Q, Theron G, Steyn W, Hall D. Total perinatally related losses at Tygerberg Hospital - a comparison between 1986, 1993 and 2006. *S. Afr. Med. J.* (2010)100 (4): 250-3.

[5] Van Wyk BE, Albrecht C. A review of the taxonomy, ethnobotany, chemistry and pharmacology of *Sutherlandia frutescens*. *J. Ethnopharmacol.* (2008)119: 620-629.

[6] Moshe D, Bank HV, Bank MV, Van Wyk BE. Lack of genetic differentiation between 19 populations from seven taxa the *sutherlandia* Tribe. *Biochem. Syst. Ecol.* (1998) 26: 595–609.

[7] Mncwangi, Viljoen. Devil's Claw—A review of the ethnobotany, phytochemistry and biological activity of *Harpagophytumprocumbens*. *J. EthnoPharmacol.* (2012) 143: 755-771.

[8] Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J. Nat. Prod.* (2012) 75(3):311-35.

[9] Gurib-Fakim A. Medicinal plants: traditions of yesterday and drugs of tomorrow. *Mol. Aspects Med.* (2006) 27(1):1-93.

[10] Rowinsky EK, Onetto N, Canetta RM, Arbuck SG. Taxol: the first of the taxanes, an important new class of antitumor agents. *Semin. Oncol.* (1992) 19 (6): 646-62.

[11] Legarza K, Yang LX. Novel camptothecin derivatives. *In Vivo.* (2005) 19 (1): 283-92.

[12] Choene M, Motadi LR. Validation of the Antiproliferative Effects of *Euphorbia tirucalli* Extracts in Breast Cancer Cell Lines. *MOL. BIOL.* (2016) 50 (1): 115-127.

[13] Cheone M, Motadi LR. Anti-Proliferative effects of methanolic extract of *K. Foetidissima* in breast

cancer cell lines MCF7 and YMB1. *Mol. Biol.* (2012) 102.

[14] Thafeni MA, Sayed Y, Motadi LR. Euphorbia mauritanica and Kedrostis hirtella extracts can induce anti-proliferative activities in lung cancer cells. *Mol. Biol. Rep.* (2012) 39 (12): 10785-94.

[15] Blázquez C, Carracedo A, Barrado L, Real PJ, Fernández-Luna JL, Velasco G, Malumbres M, Guzmán M. Cannabinoid receptors as novel targets for the treatment of melanoma. *FASEB. J.* (2006) 20 (14): 2633-5.

[16] Marcu JP, Christian RT, Lau D, Zielinski AJ, Horowitz MP, Lee J, Pakdel A, Allison J, Limbad C, Moore DH, Yount GL, Desprez PY, McAllister SD. Cannabidiol enhances the inhibitory effects of delta9-tetrahydrocannabinol on human glioblastoma cell proliferation and survival. *Mol. Cancer Ther.* (2010) 9 (1): 180-9.

[17] Karasawa K, Matsumoto F, Ito S, Oba S, Furuya T, Hirowatari H, Izawa H, Ito K, Sasai K. Hyperfractionated radiotherapy with concurrent docetaxel for advanced head and neck cancer: a phase II study. *Anticancer Res.* (2012) 32(9): 4013-8.

[18] Mthembu NN, Motadi LR. Apoptotic potential role of Agave palmeri and Tulbaghia violacea extracts in cervical cancer cells. *Molecular Biology Reports. Mol. Biol. Rep.* (2014) 41(9): 6143-55.

[19] Tan DS, Kaye S. Ovarian clear cell adenocarcinoma: a continuing enigma. *J. Clin. Pathol.* (2008) 60 (4): 355-60.

[20] Tsujimoto Y. Apoptosis and necrosis: intracellular ATP level as a determinant for cell death modes. *Cell Death Differ.* (1997) 4(6): 429-34.

[21] Sakagami H, Satoh K, Hakeda Y, Kumegawa M. Apoptosis-inducing activity of vitamin C and vitamin K. *Mol. Cell Biol.* (2000) 46(1):129-43.

[22] Matsumoto K, Okano J, Nagahara T, Murawaki Y. Apoptosis of liver cancer cells by vitamin K2 and enhancement by MEK inhibition. *Int. J. Oncol.* (2006) 29 (6): 1501-8.

[23] Yokoyama T, Miyazawa K, Naito M, Toyotake J, Tauchi T, Itoh M, Yuo A, Hayashi Y, Georgescu MM, Kondo Y, Kondo S, Ohyashiki K. Vitamin K2 induces autophagy and apoptosis simultaneously in leukemia cells. *Autophagy.* (2008) 4(5): 629-40.