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Cytotoxicity and cell cycle analysis of *Asparagus laricinus Burch.* and *Senecio asperulus DC.* on breast and prostate cancer cell lines

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ABSTRACT

Aims: Medicinal plants play an important role in our African communities for treatment and prevention of various diseases including cancer. This study was aimed on evaluating the cytotoxicity activities of *Asparagus laricinus Burch.* and *Senecio asperulus DC.*

Main methods: In vitro cytotoxicity screening was carried out using fluorescent cellular stains on human prostate cancer (PC3), human breast cancer (MCF-7) and the non-cancerous African green monkey kidney (Vero) cell lines. The cells were imaged with the ImageXpress Micro XLS Widefield fluorescent Microscope, and the acquired images were analysed using the MetaXpress software and the Multi-Wavelength cell scoring application module. Melphalan was used as a positive control in all experiments.

Key findings: Asparagus laricinus methanol and *Senecio asperulus DC.* dichloromethane extracts exhibited cytotoxicity activity against breast cancer cells with IC_{50} values of 97.6 μg/mL and 69.15 μg/mL, respectively. Cell cycle analysis suggested that *Asparagus laricinus* methanol extract induced cell death selectively through apoptosis observed from Annexin V-FITC and PI stain. Cell cycle analysis also showed that *Senecio asperulus DC.* dichloromethane extracts induced breast cancer cells death through cell arrest at the synthesis phase and G2 phase. *Senecio asperulus DC.* dichloromethane extracts further showed cytotoxicity activity against prostate cancer cells with IC_{50} values of 69.25 μg/mL due to cell arrest at the G2 and early mitotic (G2/M) phase.

Significance: We, therefore, propose that the methanol extract of *Asparagus laricinus* is a suitable aspirant for future breast cancer chemotherapeutic drug, due to its selective cytotoxicity on cancer cells and not on non-cancerous cells.

1. Introduction

Cancer is a serious public health problem and it continues to be the leading cause of mortality and morbidity worldwide (Lee et al., 2014; Ogbole et al., 2017). In the African region, the most common cancers are breast, cervical, liver and prostate cancer (World Health Organization, 2017). Prostate cancer is leading cancer in males: with nearly 1 in 5 men to be diagnosed with this illness during their lifetime and more than 4000 men being diagnosed with prostate cancer every year in South Africa (Sylla and Wild, 2012; Seigel et al., 2018). Breast cancer is also a growing health problem in sub-Saharan Africa (Akarolo-Anthony et al., 2010; Jemal et al., 2012; Sylla and Wild, 2012) and has now surpassed cervical cancer as the leading cause of death in many countries, with 94 378 new cases of breast cancer diagnosed annually (Akarolo-Anthony et al., 2010; Seigel et al., 2018). Additionally, the number of other types of cancer cases and death are estimated to increase over the next two decades (World Health Organization, 2017), despite current advancements in scientific knowledge. Several chemotherapeutic agents are available and in use for the management of cancer, nevertheless, the problem of indiscriminate toxicity and serious adverse events still exist (Ogbole et al., 2017). Thus, there is a significant increase in scientific and commercial interest in the continued discovery of novel anticancer agents from natural product sources.

African countries still depend greatly on traditional medicinal remedies for the treatment of different types of cancers as access to western medicine is limited (WHO, 2013). In Limpopo Province, South Africa, unexpected improvements have been observed on

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patients who had been terminally ill due to advanced prostate cancer and had used a decoction of *Asparagus laricinus Burch.* (Mashele and Kolesnikova, 2010). *Asparagus laricinus Burch.* (Asparagaceae) is a very hardy, evergreen, shrubby *Asparagus* with fine, feathery foliage and silvery, zigzag branchlets (Van der Merwe et al., 2001). It has myriads of tiny white, nectar-rich flowers that develop during spring and summer. These flowers are fragrant and attract insects and birds (Wildlife Wholesale Nursery, 2018). *A. laricinus Burch.* bears attractive red and blackberries that also attract birds. This species has alternate cladodes which grow in clusters of 4–6. *Asparagus laricinus Burch.* can grow up to 1.5 m. This plant species is used medicinally to treat tuberculosis, sores, red water, uterine infection, general alignments, umbilical cord inflammation, and serves as a diuretic (Van der Merwe et al., 2001). *A. laricinus Burch.* is native to Botswana and South Africa, Lesotho and Swaziland. *A. laricinus Burch.* is known as cluster-leaf asparagus in English, as Lesitwane in Setswana and Lerara tau in Sesotho (Van der Merwe et al., 2001; Mugomeri et al., 2014).

Senecio asperulus DC. belongs to the genus *Senecio* and is from the daisy family, Asteraceae, that includes ragworts and groundsels (Brummitt, 1992). *Senecio asperulus DC.* is known as Moferefere or Letapisa in Sesotho (Kose et al., 2015). *Senecio asperulus DC.* is a very aromatic plant with yellow flowers when young which turns to woolly flowers as the plant gets old and known to be highly toxic (Quattrocchi, 2016). In South Africa, leaves of this plant are medicinally used as an infusion mixture with other medicinal plants for the treatment of rheumatoid arthritis, sore throat, mouth ulcers, flu and cold (Quattrocchi, 2016). The plant is very common in the Eastern Cape province, KwaZulu-Natal, and Western Cape province of South Africa. In the kingdom of Lesotho, *S. asperulus DC.* is abundant and widely spread across the country. According to traditional medicinal plants practices in Mohale's Hoek, Lesotho, *Senecio asperulus DC.* is used on its own or in combination with other medicinal plants for the treatment of various ailments by laypeople (Kose et al., 2015; Mugomeri et al., 2014). Roots of *S. asperulus DC.* are used mostly in Lesotho for a treatment of various diseases such as treat herpes, syphilis, itching feet, arthritic joints, colds and flu, sore throat, sore joints, swollen gums, and used as a vasodilating agent, thus, improves circulation (Kose et al., 2015; Mugomeri et al., 2014).

Herbal medicine has always been one of the main components of healthcare systems for ages. However, most ethnobotanical claims have not yet been investigated scientifically. The investigation of traditionally used medicinal plants is valuable as a source of potential chemopreventative and chemotherapeutic agents since there are dissatisfactions with current anticancer treatment options due to their limitations. Plant-derived natural products provide an interesting source for screening and ultimately isolate novel potent molecules to combat a variety of ailments, including the modern disease "cancer". Thus, the cytotoxicity of both *Senecio asperulus DC.* and *Asparagus laricinus Burch.* was determined against breast cancer cell line (MCF-7), prostate cancer cell line (PC3) and non-malignant African green monkey kidney cell line (Vero).

2. Materials and methods

2.1. Plant material collection

Plant material was collected from Mohale's Hoek district, Lesotho and Free State Province, South Africa (SA). Plant identification was authenticated by scientists at the National Botanical Gardens in Bloemfontein, South Africa by Dr Zietsman and Mr Nenugwi. Plant materials voucher numbers were PHM02 for *Senecio asperulus DC.* (from Lesotho) and MAS001 for *Asparagus laricinus Burch.* (from SA) and were delivered at National Botanical Gardens herbarium. Plant names were checked with http://www.theplantlist.org. The roots of *Senecio asperulus DC.* and cladodes *Asparagus laricinus Burch.* were washed, air dried at room temperature (25 °C) and then ground into fine powder using an electric blender.

2.2. Plant extraction methods

For extraction, maceration method was used as adapted from Azwanida (2015). Powdered plant materials were soaked separately in purified water, 100% methanol (MeOH), 1:1 (v/v) absolute methanol: dichloromethane (MeOH: DCM), 100% dichloromethane (DCM) and 100% Hexane for 72 hours with occasional stirring. The extracts were then filtered, and aqueous extracts were concentrated in a freeze dryer and organic solvents with a rocket evaporator.

2.3. Sample preparation

The plant extracts were reconstituted in dimethyl sulphoxide (DMSO) to give a final concentration of 100 mg/mL, respectively. Stock solutions were sonicated if necessary were solubility was an issue. All samples were then stored at 4 °C.

2.4. Cell culture

The human prostate cancer cell line, PC3; human breast cancer cell line, MCF7 and the African green monkey kidney cell line, Vero were cultured in DMEM (Dulbecco's Modified Eagle's medium) and 10% FBS (Fetal bovine serum). PC3 cell lines were purchased from Japanese Collection of Research Bioresources Cell Bank, while, MFC7 and Vero cells were purchased from ATCC Cell Biology collection. Suspensions of monolayer cultures of cells were seeded into 96 well microtiter plates at a density of 6000 cells/well using a volume of 100 µl in each well. The microtiter plates with cells were incubated at 37 °C, 5% CO_2, and 100% relative humidity for 24 hours prior to addition of test compounds to allow for cell attachment (Freshney, 2005).

2.5. In vitro cytotoxicity assay and IC_{50} determination

Cytotoxicity was studied using a high content screening approach, and nuclear morphological changes were visualized by Hoechst 33342 staining (Crowley et al., 2016), after cells were treated with 50 and 200 µg/mL of each extract respectively. One hundred microliter aliquots of the diluted extract in fresh medium were used to treat cells. Cells were incubated at 37 °C in a humidified 5% CO_2 incubator for 48 hours. Melphalan was used as a positive control. Treatment medium was aspirated from all wells and replaced with 100 µL of Hoechst 33342 nuclear dye (5 µg/mL in phosphate buffered saline) and incubated for 10 minutes at room temperature. Thereafter, cells were stained with propidium iodide (PI) at 100 µg/mL to enumerate the proportion of dead cells within the population. Cells were imaged immediately after addition of PI using the ImageXpress Micro XLS Widefield Microscope (Molecular Devices). DNA distribution is analysed using propidium iodide (PI), a double-stranded DNA-binding dye. Cells first need to be fixed and permeabilized to allow the entry of PI stain into the cell (Krishan, 1975). For the *in vitro* IC_{50} determination, cells were treated with increasing concentrations of each extract prepared from serial drug dilutions, 6.25–300 µg/mL. Concentration to inhibit 50 percent of the cell population was determined and only those obtained IC_{50} value concentrations were used for cell cycle analysis.

2.6. In vitro cell-cycle analysis

Cell cycle analysis was performed by propidium iodide (PI) based measurements of the DNA content of the cells by flow cytometry using the method by Crowley et al. (2016) and Moore et al. (1997). One hundred microliters aliquots of the diluted compound in fresh medium was used to treat cells. Cell lines were incubated at 37 °C in a humidified 5% CO_2 for 48 hours. Melphalan was used as a positive control. Treatment medium was removed from all wells and replaced with 100 µL of Hoechst 33342 nuclear dye (5 µg/mL) and Annexin V-FITC and then incubated for 10 minutes at room temperature. The plates were then inspected under an inverted microscope to guarantee growth and images were then acquired on the ImageXpress Micro XLS Widefield microscope (Molecular Devices). Thereafter, cells were stained with propidium iodide (PI) at 100 µg/mL to enumerate the proportion of dead cells within the population. Cells were imaged immediately after addition of PI using the ImageXpress Microscope.

2.7. Image quantification and analysis

Quantification of live and dead cells for the screening assay was performed using the ImageXpress Micro XLS Widefield Microscope (Molecular Devices). Acquired images were analysed using the MetaXpress software and Multi-Wavelength Cell Scoring Application Module and the Cell Cycle Application Module. Acquired data was transferred to Microsoft Excel spreadsheet and data was analysed and processed using GraphPad Prism version 4 for IC_{50} determination and Microsoft Excel for cell cycle analysis.

2.8. Statistical analysis

All experiments were performed in triplicated and data represent the mean ± standard deviation (SD). The statistical significance was measured by one-way ANOVA and p-values less than 0.05 were considered significant.

3. Results

Aqueous, methanol, dichloromethane, hexane and methanol: dichloromethane extracts of *Senecio asperulus DC.* and *Asparagus laricinus Burch.* were screened at 2 concentrations (50 and 200 µg/mL) for cytotoxicity against prostate (PC3) and breast (MCF-7) cancer cells and against non-cancerous kidney (Vero) cells, respectively. Since the assay protocol used could provide quantitative data, the total number of cells were compared with the number of live and dead cells using a dual staining cytotoxicity assay. All activities were compared with both negative (medium only) and positive (melphalan) controls and only p-values < 0.005 were statistically significant. The results, as shown in Figs. 1, 2, and 3, indicated that methanolic extracts of *A. laricinus Burch.* had some cytotoxic effect against breast cancer (MCF-7) cells, with very little effect on non-cancerous (Vero) cells. The hexane extract of *S. asperulus DC.* as well as the methanol: dichloromethane extract of *A. laricinus Burch.* which were cytotoxic against all the cell lines used. While dichloromethane extracts of *S. asperulus DC.* showed a dose-dependent cytotoxic effect against prostate (PC3) cells (Fig. 2) and against breast (MCF-7) cells (Fig. 3), it had little cytotoxic effect against non-cancerous kidney (Vero) cells (Fig. 1). The rest of the extracts from *S. asperulus DC.* and *A. laricinus Burch.* did not show any concentration-dependent cytotoxicity in any of the tested cell lines (PC3, MCF-7, and Vero).

For all instances (Figs. 1, 2, 3, 4, 5, 6, and 7), the plant extracts are represented by the abbreviations in brackets. Thus, for *Senecio asperulus DC.* the extracts are methanol (L1); aqueous (L2); dichloromethane (L3); hexane (L4); and methanol: dichloromethane (L5). *Asparagus laricinus Burch.* extracts are methanol (AL1); aqueous (AL2); dichloromethane (AL3); hexane (AL4) and methanol: dichloromethane (AL5). Similarly, the controls are Medium only (MO) and melphalan (melph) at a concentration of 40 µg/mL.

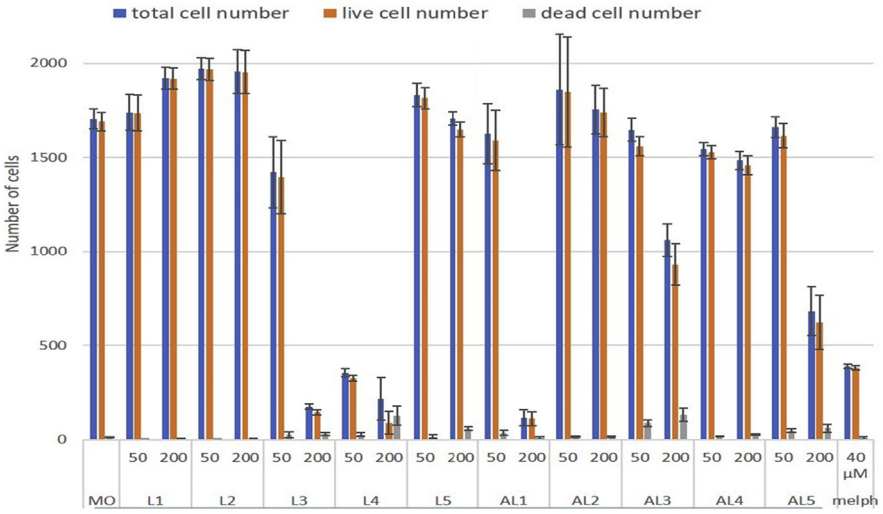

Fig. 1. Cytotoxicity effect of *Senecio asperulus DC.* and *Asparagus laricinus Burch.* extracts on normal kidney (Vero). Error bars indicate standard deviation of triplicate values.

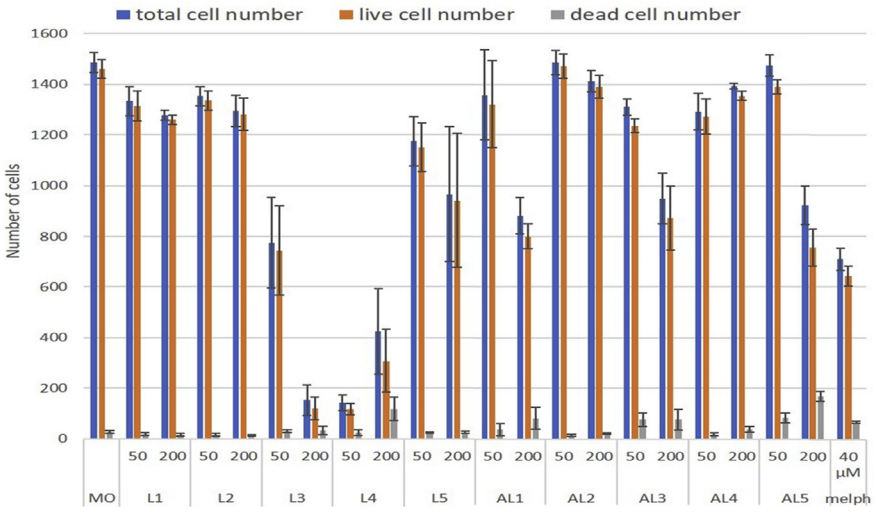

Fig. 2. Cytotoxicity effect of *Senecio asperulus DC.* and *Asparagus laricinus Burch.* extracts on the prostate cancer cell line (PC3). Error bars indicate standard deviation of triplicate values.

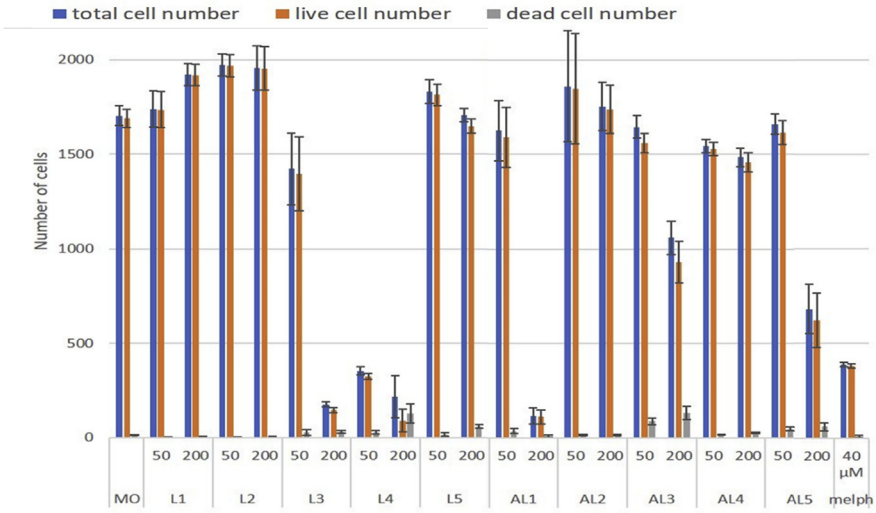

Fig. 3. Cytotoxicity effect of *Senecio asperulus DC.* and *Asparagus laricinus Burch.* extracts on breast cancer cell line (MCF-7). Error bars indicate standard deviation of triplicate values.

From the cytotoxicity screening findings, two plant extracts (*Senecio asperulus DC.* dichloromethane and *Asparagus laricinus Burch.* methanol extracts), showed these characteristics when compared to controls. Thus, their respective concentrations required to reduce 50% (IC_{50}) of prostate and or breast cell viability were determined and illustrated in Fig. 4A and B.

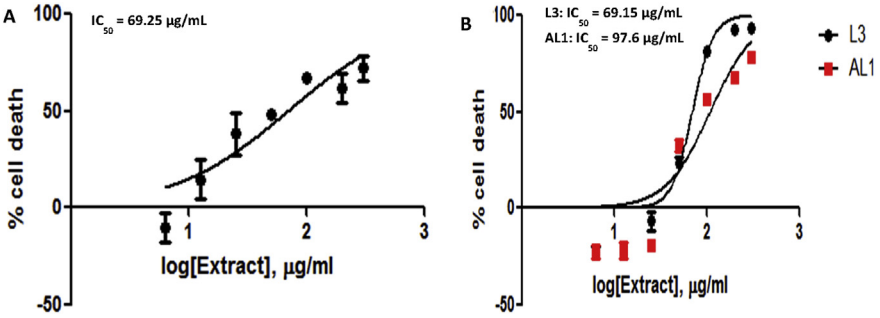

Fig. 4. IC_{50} values of *Senecio asperulus DC.* dichloromethane (L3) on prostate cancer cell lines (**A**). IC_{50} values of *Asparagus laricinus Burch.* methanol (AL1) and *Senecio asperulus DC.* dichloromethane (L3) extracts on breast cancer cell lines (**B**).

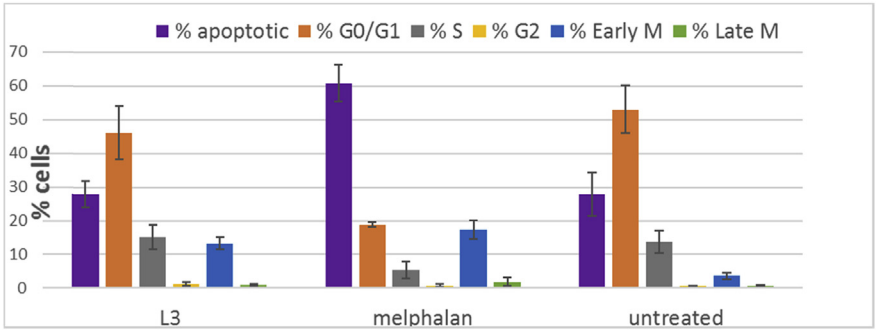

Fig. 5. Distribution of PC3 cells in the different phases of the cell cycle when treated with *Senecio asperulus DC.* dichloromethane (L3). Melphalan (40 μM) was used as a positive control. Error bars indicate the standard deviation of quadruplicate values. G0/G1: Gap 0/Gap1 phase; S: synthesis phase; M: mitotic phase.

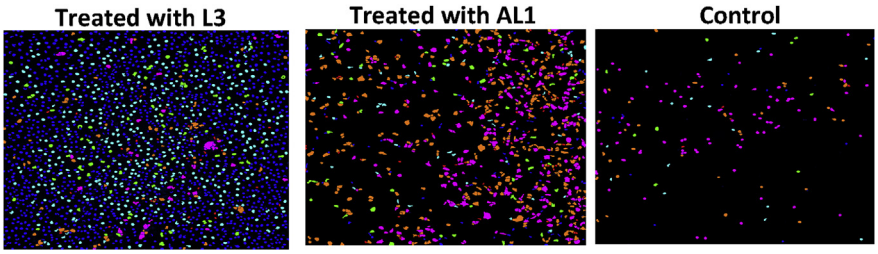

Fig. 6. Morphological changes observed after Hoechst 33342, Annexin V-FITC and PI staining of MCF-7 cells treated with L3, AL1, and melphalan at determined IC_{50} concentrations.

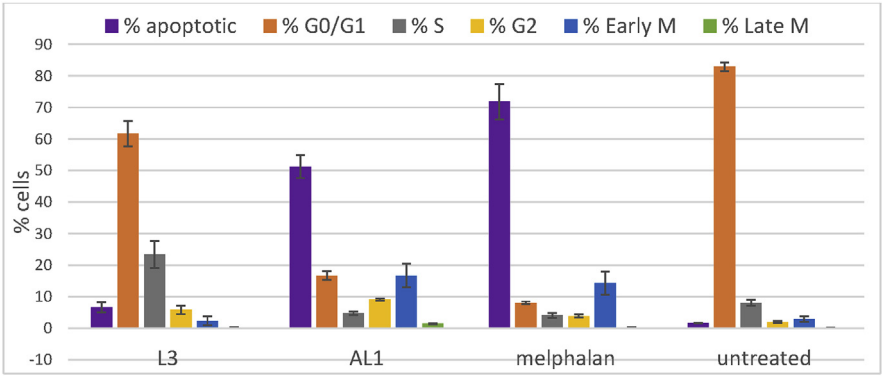

Fig. 7. Distribution of MCF7 cells in the different phases of the cell cycle when treated with *Senecio asperulus DC.* dichloromethane (L3) and *Asparagus laricinus Burch.* methanol (AL1). Melphalan (40 μM) was used as a positive control. Error bars indicate the standard deviation of quadruplicate values. G0/G1: Gap 0/Gap1 phase; S: synthesis phase; M: mitotic phase.

It is evident from the results in Fig. 4B, the IC_{50} value of *Senecio asperulus DC.* dichloromethane (L3) extract in both PC3 and MCF-7 cell lines was two-fold higher compared to those of *Asparagus laricinus Burch.* methanol (AL1) on MCF-7 cells. Thus, further studies to investigate whether the cytotoxic effects were due to apoptosis or necrosis were undertaken with both extracts L3 and AL1, at their IC_{50} concentrations. Furthermore, to evaluate the mechanism of action of these plant extracts on cancer cells, cell cycle analysis was also performed. At the end of the analysis, deoxyribonucleic acid (DNA) contents in different cell cycle phases were determined using Hoechst 33342, Annexin V-FITC and PI multiplex staining technique (Fig. 6). The results were presented in the form of percentages as showed in Figs. 5 and 7.

4. Discussion

The current study investigated the cytotoxicity activity of indigenous medicinal plants, *Senecio asperulus DC.* and *Asparagus laricinus Burch.*, against human breast (MCF-7) and prostate (PC3) cancer cells. The non-cancerous African green monkey kidney cell line (Vero) was used as a control as well as melphalan that was used as a positive control. The results showed decreased cell viability of MCF-7 and PC3 cancer cells in a dose-dependent manner (Figs. 1, 2, and 3) when treated with *Senecio asperulus DC.* dichloromethane and *Asparagus laricinus Burch.* methanol extracts for a period of 48 hours. The dose-dependent cytotoxicity effect of the methanol extract of *A. laricinus Burch.* and the dichloromethane extract of *S. asperulus DC.* indicates selectivity for cancer (MCF-3 and PC3) as they had little effect on Vero cells. An interesting anticancer agent should have a more cytotoxic effect on cancer cell lines and less effect on non-cancerous cell lines. Thus, the observed preliminary selectivity is encouraging for further research to fully elucidate the anticancer actions of *A. laricinus Burch.* methanolic extract as potential targeted breast cancer therapy for future drug development studies. According to Ayoub et al. (2014), cytotoxic agents should be effective at concentrations of up to 100 μg/mL. Since both extracts were active below

100 μg/mL, they can be considered as suitable candidates for chemotherapeutic drugs. In an attempt to propose a mechanism of action associated with the cytotoxicity observed, these two plant extracts were studied further to determine whether cell death was due to apoptosis or necrosis.

It is worth mentioning that cell viability can decrease due to necrosis, autophagy or apoptosis. Necrosis is caused by factors external to the cell or tissue, such as infection, toxins, or trauma. Autophagy is triggered by nutrients undernourishment or withdrawal of other essential factors needed for cells to survive. Autophagy leads to the degradation of cytoplasmic materials, and this becomes an evident feature of cell morphology analysis. Although autophagy is not inherently complicated, recent progress has confirmed that it plays a wide variety of pathophysiological and physiological roles which are sometimes multifaceted (Yoshimoto et al., 2004). Apoptosis on the other side can be defined as a highly ordered programmed cell death process of unhealthy cells, where a cell dies as part of its normal programme of development, or due to lack of growth factors, or when the immune system instructs it to die. However, cancer cells typically escape apoptosis by disabling the apoptotic pathways, thus continue to proliferate and even become resistant to the chemotherapeutic drug (Ferreira et al., 2004; Luqmani, 2005). Cell death due to apoptosis can be observed with the morphological changes in the cell shape such as; nuclear condensation, cell body shrinkage, cytoplasm fragmentation, plasma membrane blebs, and externalization of phosphatidylserine. Such changes suggest that cells have committed to a specific mode of cell death resulting in apoptosis (Selvan et al., 2018). Furthermore, it is known that drugs with apoptosis-inducing properties may reduce potential drug resistance.

For this study, apoptosis analysis was done using Hoechst 33342 nuclear staining assay. The apoptosis effect of *Senecio asperulus DC.* dichloromethane and *Asparagus laricinus Burch.* methanol extracts were investigated on MCF-7 and PC3 cells stained with Hoechst 33342, Annexin V-FITC and further stained with PI stain (Crowley et al., 2016). The latter stain is generally for dead cells which have lost their cell integrity, as it intercalates into the DNA strands of the cell and staining its red color when observed under a fluorescent microscope. Therefore, dead cells are identified by this red/fluorescent propidium iodide staining after cells have been treated with test agents. Annexin V-FITC is very selective for cells death due to early apoptosis as it targets and binds to externalized phosphatidylserine (apoptotic cell surface marker) of the plasma membrane. During early stages of apoptosis, phospholipids on the plasma membrane translocate from the inner to the outer leaflet, thus exposing the phosphatidylserine which is recognized by, and fluorescently labeled by PS-binding protein, annexin-V. This stains the cell membrane in green color. Moreover, Hoechst 33342 stains the nuclear region of the cell into a bright blue color. Acquired fluorescence images were then analysed using the MetaXpress software. The obtained morphological changes and also Annexin V-FITC and PI stain uptake observations indicated that *Asparagus laricinus Burch.* methanol extract exhibits significant activity in destroying MCF-7 cells by apoptosis when compared with controls (Figs. 6 and 7). However, *Senecio asperulus DC.* dichloromethane extract didn't show much evidence of apoptosis on PC3 cell line when compared to controls (Fig. 6), this was shown by less red-stained cells. Thus, cells that died with loss of cell membrane integrity, were fewer when compared to melphalan which is known of inducing cell death through apoptosis. This finding was no surprise as plants belonging to the *Senecio* family are known to be toxic (Quattrocchi, 2016), thus this explains its high cytotoxic properties on PC3 cell lines (Fig. 2).

Some cells die through programmed pathway while others die through obstruction accompanied by cell-cycle mechanisms (Dirsch et al., 2004). Cell cycle analysis is performed to determine the state of DNA in response to treatment of the cell with a specific compound or extract. The distribution of DNA content is important as it leads to the identification of targets or pathways to target for the treatment of cancer and tumors (Planchais et al., 2000). During cell development, cells need to progress through every phase of the cell cycle to assure a full copy of DNA for a new daughter cell. There exist checkpoints which regulate progression of cells through the cycle and cause cell cycle arrest if DNA damage or DNA stress has occurred. Faults in the G2-M arrest checkpoint allows a damaged cell to enter mitosis before repair and undergo apoptosis (DiPaola, 2002). Thus, efforts to promote the latter outcome may increase the cytotoxicity of chemotherapy, however, other studies also propose enhanced cytotoxicity to be associated with high cell-cycle arrest (Shapiro et al., 2001; Alimbetov et al., 2018). Cell cycle arrest is defined as a high proportion of cells found in the same cycle event at a specific time. Cell cycle arrest is maintained until DNA repair is complete (Nojima, 2004).

From our study, results (Fig. 6) showed that *Senecio asperulus DC.* dichloromethane extract arrested a significant number of PC3 cells at the early stage of mitosis and MCF-7 cells at a synthesis phase. Several MCF-7 cells arrested at the synthesis phase by *Senecio asperulus DC.* dichloromethane extract was two-fold when compared with the positive control (Figs. 5 and 7). At the G2 phase, as cell approaches mitosis phase, cyclin-dependent protein kinases (Cdk1)/nuclear cyclin B level (B1) complex is essential for entry into and progression through mitosis (Wolf et al., 2007). Thus, activation of this Cdk1/B1 complex plays a key regulatory role in cell proliferation, while down-regulation of this complex expression induced G2/M phase arrest (Chang et al., 2003, Porter and Donoghue, 2003). However, continued and inappropriate overexpression of Cdk1/cyclin B1 plays an opposite role by mediating pro-apoptotic signaling in response to the mitotic arrest and causes non-specific cell death (Eichhorn et al., 2014). An increase in the percentage of MCF-7 cells at G2 phase as well as at the early mitosis phase when treated with *Asparagus laricinus Burch.* methanol extract suggests that the cytotoxicity mechanism was also stimulated by active cyclin B1/CDK1 complex in these cells. However, this can only be confirmed through a study of the regulation of apoptosis-related proteins in MCF-7 cancer cells by *Asparagus laricinus Burch.* methanol extract.

5. Conclusion

This study was carried out to investigate the cytotoxicity of two medicinal plants, *Senecio asperulus DC.* and *Asparagus laricinus Burch.* on prostate and breast cancer cell lines, and to further elucidate the mechanism of action of the active extract/s on the cell cycle of these cancer cell lines. *Senecio asperulus DC.* dichloromethane extract was cytotoxic against PC3 and MCF-7 cancer cell lines, and *Asparagus laricinus Burch.* methanol extract showed cytotoxicity on MCF-7 cells only in a dose-dependent manner. Moreover, both extracts had little cytotoxicity against non-cancerous Vero cells. Their ability to show more cytotoxicity on cancer cells than on non-cancerous cells, their low IC_{50} as well as the cell cycle analysis prompted further investigation. The dichloromethane extract of *Senecio asperulus DC.* appeared to arrest cells in the G2 as well as in the early mitotic (M) phase of the cell cycle on PC3 cells, while on the MCF7 cells, cell arrests were more in the S phase. These findings show that cytotoxicity of this extract against PC3 and MCF-7 cancer cell lines was not due to apoptosis, but rather due to cell arrest at the G2 and early mitosis phase. The methanol extract of *Asparagus laricinus Burch.* revealed cell arrest in the early M phase of the cell cycle but also shows a high percentage of MCF-7 apoptotic cells. Therefore, this extract induced MCF-7 cell death through apoptosis at an acceptable growth inhibition concentration dose and therefore, can be considered as a suitable candidate for future chemotherapeutic drugs.

Declarations

Author contribution statement

MFENGWANA, PH: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

MASHELE, S.S, MANDUNA, IT: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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