

***Searsia rhemanniana* as a potential anticancer agent: Unveiling mechanisms
and therapeutic potential**

by

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Dissertation submitted in fulfilment of the requirements for the degree

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DECLARATION

I, **DIJENG EUGINIAH RAMPANA**, student number _____ hereby certify that the dissertation submitted by me for the degree DOCTOR OF HEALTH SCIENCES IN BIOMEDICAL TECHNOLOGY (D HBIO), is my own independent work and complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology (Free State). I hereby declare that this research project has not previously been submitted to any university or faculty for the attainment of any qualification. I further waive copyright of the dissertation in favour of the Central University of Technology (Free State).

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LIST OF ABBREVIATIONS AND ACRONYMS

5-FU	5-fluorouracil
ADT	Androgen deprivation therapy
AIDS	Acquired immunodeficiency syndrome
Annexin V-FITC	Annexin V-fluorescein isothiocyanate
ASIR	Age standardized incidence rates
ATM	Ataxia-Telangiectasia Mutated
ATR	ATM- and Rad3-Related
BCL-2	B-cell lymphoma 2
BPH	Benign prostatic hyperplasia
Ca ²⁺	Calcium ion
CCNB1	Cyclin B1
CDK	Cyclin dependent kinase
CHK1	Checkpoint Kinase 1
CHK2	Checkpoint Kinase 2
CKC	Cold knife conization
CIN1	Cervical intraepithelial neoplasia grade 1
CIN 2	Cervical intraepithelial neoplasia grade 2
CIN 3	Cervical intraepithelial neoplasia grade 3
COX-2	Cyclooxygenase-2
CRPC	Castration resistant prostate cancer
CSIR	Council for Scientific and Industrial Research
cT	Computed Tomography
DALYs	Disability-adjusted life years
DCE	Dynamic contrast-enhanced
DCM	Dichloromethane
DHT	Dihydrotestosterone
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide

DNA	Deoxyribonucleic acid
DRE	Digital Rectal Examination
DU145	Prostate cancer cells
DWI	Diffusion-weighted imaging
E	Early
<i>E.coli</i>	<i>Escherichia coli</i>
EDTA	Ethylenediaminetetraacetic acid
EPE	Extracapsular extension
ERSPC	European Randomized Study of Screening for Prostate Cancer
FBS	Foetal Bovine Serum
G1	Gap 1
G2	Gap 2
GS	Gleason scoring
GLOBOCAN	Global Cancer Observatory
H ₂ O	Water
HELA	Human Cervix cancer cell
HPV	Human papillomavirus
IC50	Half maximal inhibitory concentration
ICLs	Interstrand crosslinks
<i>K.pneumoniae</i>	<i>Klebsiella pneumoniae</i>
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
L	Late
LEEP	Loop electrosurgical excision
LMICs	Low- and middle-income countries
M	Mitosis and cytokinesis
MACS	Magnetic-Activated Cell Sorting
MeOH	Methanol
Mg ²⁺	Magnesium ion
MOMP	Mitochondrial outer-membrane permeabilization
mp-MRI	Multiparametric magnetic resonance imaging

MRI	Magnetic Resonance Imaging
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
ND	Not determined
p21	Cyclin-dependent kinase inhibitor 1,
P53	Tumour protein p53
<i>P.aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
PAO	Phenylarsine Oxide
PCa	Prostate cancer
PET	Positron Emission Tomography
PHI	Prostate Health Index
PI	Propidium iodide
PSA	Prostate-Specific Antigen
Rb	Retinoblastoma
ROS	Reactive oxygen species
RPMI	Roswell Park Memorial Institute
S	Synthesis
SAC	spindle assembly checkpoint
SI	Selectivity index
<i>S.aureus</i>	<i>Staphylococcus aureus</i>
TNM	Tumour, Node, Metastasis Staging
TRUS	Transrectal Ultrasound
VERO	African green monkey kidney
WHO	World Health Organization
γ -H2AX	Phosphorylated histone protein H2AX
<i>S.rhemanniana</i>	<i>Searsia rhemanniana</i>

RESEARCH OUTPUTS

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Review Article

A Review: *Searsia* genus and Its Potential Anti-Cancer Properties

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ABSTRACT

The *Searsia* genus, belonging to the Anacardiaceae family, comprises small trees and evergreen shrubs.

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Evaluation Of The Cytotoxic Activity Of *Searsia* Rhemanniana Crude Extracts Against The Human Prostate Cancer Cell Line DU145

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Abstract

Background: Prostate cancer is the second most common cancer among men globally and the leading cancer in African men. Current treatments are often inaccessible or limited by resistance. Natural products, particularly from underexplored plants like *Searsia rhemanniana*, offer a promising alternative for anticancer drug discovery.

Objective: To evaluate the cytotoxic activity of crude extracts of *S. rhemanniana* against the human prostate cancer cell line DU145.

Methods: Extracts were prepared using methanol, dichloromethane, and water. DU145 cells were treated with various extract concentrations, and cytotoxicity was assessed using the MTT assay. IC₅₀ values were calculated from dose-response curves generated in GraphPad Prism (n=4).

Results: The methanol extract showed the highest cytotoxic activity, with an IC₅₀ value of 1.23 µg/mL. The dichloromethane extract showed an IC₅₀ value of 2.45 µg/mL, and the water extract showed an IC₅₀ value of 4.56 µg/mL.

The Review Of

DIABETIC
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In Vitro Cell Cycle Analysis Of Crude Extracts Of *Searsia* Rhemanniana Extracts Against Du145 Cancer Cells

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Abstract

Background:

Prostate cancer progression is characterized by extensive disruption of normal cell-cycle regulatory mechanisms and the ability of cancer cells to evade programmed cell death. These alterations lead to uncontrolled cellular proliferation, genomic instability, and resistance to therapeutic interventions.

The aim of the study was to assess the effects of crude *Searsia rhemanniana* extracts on cell cycle progression in the human prostate cancer cell line DU145.

Methods:

Plant extracts were prepared using methanol, dichloromethane, and water as solvents. DU145 prostate cancer cells were seeded in 96-well plates at a concentration of 5,000 cells per well in 100 µL of growth medium and incubated overnight to promote cell adhesion. Following attachment, the cells were exposed to the respective extracts at their predetermined IC₅₀.

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IN VITRO GENOTOXICITY SCREENING OF SEARSIA RHEMANNIANA EXTRACTS AGAINST VERO CELLS

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ABSTRACT

Medicinal plants offer a valuable therapeutic potential, but their safety is not guaranteed. Genotoxicity assessment is an essential part of the framework for the development of herbal products and risk assessment. As the use of traditional medicinal products increases and regulatory control increases, the integration of genotoxic endpoints with standard toxicology is becoming a key requirement for public health. Cells were

VER
Vascular & Endovascular Review

Differential Cytotoxic and Selective Anticancer Profiles of Plant Extracts (Leaf, Bark, and Root): A Time-Dependent Evaluation of Solvent (aqueous, DCM, and Methanol) and Tissue Variability in HeLa Cervical Cells

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ABSTRACT

Cervical cancer is the fourth most common cancer among women globally. Resistance frequently restricts or prevents access to current medicines. Natural products present a possible substitute for anticancer drug discovery, especially those derived from understudied plants like *Searsia rhemanniana*. This study investigated the cytotoxic and selective anticancer properties of plant extracts prepared with three different solvents—aqueous, dichloromethane (DCM), and methanol (MeOH)—against HeLa

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ABSTRACT

The increasing global burden of chronic diseases such as cancer and the rise of antimicrobial resistance have intensified the search for novel pharmacologically active compounds from natural sources. This study evaluated the biological activities of crude extracts derived from *Searsia rhemanniana*, focusing on their cytotoxic, anticancer, genotoxic, and antibacterial properties. Extracts from bark, leaves, and roots were prepared using solvents of varying polarity (dichloromethane, methanol, and water) and tested using *in vitro* models, including DU145 human prostate cancer cells, HeLa cervical cancer cells, and Vero non-cancerous mammalian cells. Cytotoxicity assays revealed that dichloromethane extracts, particularly those derived from bark and root tissues, exhibited the most potent antiproliferative activity. Similarly, DCM bark and root extracts displayed strong activity against HeLa cells, with IC values ranging from approximately 22. The results show that aqueous bark extract, DCM bark extract, and methanolic leaf extract induce significant increases in micronucleus formation, indicating potential genotoxic effects under *in vitro* conditions. Methanolic extracts demonstrate the strongest antibacterial activity, particularly against *Staphylococcus aureus*, with IC values ranging from approximately 60–125 g/mL, while *Escherichia coli* exhibit higher resistance across extracts. Overall, the findings demonstrate that *S. rhemanniana* possesses diverse bioactive compounds capable of exerting cytotoxic, anticancer, genotoxic, and antibacterial effects *in vitro*. The strong antiproliferative activity of DCM bark and root extracts highlights the potential of this species as a source of novel plant-derived anticancer agents. However, the detection of genotoxic responses in certain extracts underscores the need for further phytochemical characterization, mechanistic studies, and *in vivo* safety evaluations.

CHAPTER 1: LITERATURE REVIEW

1.1 INTRODUCTION

The *Searsia* genus, which is a member of the *Anacardiaceae* family, includes a range of tiny trees and evergreen shrubs that are primarily found in Southern Africa, with some species being found in East Africa and the Mediterranean. Because of these plants' anti-inflammatory, antibacterial, and antifungal qualities, they have been utilized traditionally; this is indicative of the genus's rich ethnopharmacological knowledge (1). The investigation of *Searsia* species' possible anti-cancer effects has drawn recent scientific attention (2). These plants are abundant in bioactive substances such as flavonoids, phenolic acids, and tannins, which are well-known for their anti-inflammatory and antioxidant properties, according to phytochemical investigations (3). Research has revealed that several *Searsia* species are the source of substances that are cytotoxic to different types of cancer cell lines. Because *Searsia chirindensis* contains a high concentration of gallic acid and other polyphenolic chemicals, extracts from the plant have shown substantial antiproliferative action against human cancer cells (4). The triggering of apoptosis and the prevention of cancer cell proliferation appear to be the processes behind the anti-cancer activity of *Searsia* species (5). These benefits are mostly attributed to the phytochemical's antioxidant qualities, which have the capacity to counteract free radicals and reduce oxidative stress, two major factors in the development of cancer. Furthermore, the inflammatory environment that promotes tumour growth and progression is reduced by the anti-inflammatory qualities of *Searsia* extracts (6).

1.2 MEDICINAL TRADITIONAL USE

Indigenous populations in southern Africa have traditionally used species of the *Searsia* genus to treat a wide range of illnesses such as stomach problems and skin disorders are treated with the bark and roots in traditional medicine (7). These ethnobotanical applications show how important the plant is to rural populations, even outside of its ecological function, as a source of sustenance and medicinal properties (8). Although there have not been many studies specifically on *S.rhemanniana*, related species like *Searsia chirindensis* and *Searsia lancea* have been used medicinally to

treat fever, inflammation, and gastrointestinal problems (9). This suggests that *S.rhemanniana* and other members of the genus may have similar properties because of their phytochemical similarities. The anti-inflammatory, antibacterial, and antioxidant properties of some plants in the *Searsia* genus are well-known, and these properties are often linked to the plants' potential for cancer prevention and treatment such as *S.lancea* (10). *S.dentata* and *S.pyroides* have traditionally been used to treat a variety of medical conditions, including epilepsy and convulsions (11). These plants have also shown promise in modern research, particularly in the treatment of diabetes. Extracts from *Searsia* species such as *S.coriaria*, *S.chirindensis*, and *S.verniciflua* have demonstrated significant hypoglycaemic activities both *in vitro* and *in vivo* (12). This suggests potential for these plants in managing blood sugar levels and treating diabetes mellitus. Furthermore, the leaves and fruits of *S.coriaria* have been reported to have defensive and beneficial effects on a broad range of diseases. These include diabetes mellitus, cancer, stroke, oral diseases, inflammation, diarrhoea, and dysentery (13). Although *Searsia* species have demonstrated a wide range of biological activities, comprehensive studies on their phytochemical analysis remain limited. Furthermore, no historical ethnomedicinal reports are available for *S.rhemanniana* investigated in this study. This gap in research highlights the need for more detailed investigations to fully understand the potential and traditional uses of these species.

1.3 METHODOLOGY

The current review manuscript used desktop internet search and keywords to identify articles of the *Searsia* plant genus. A deeper search was also undertaken to check its medicinal use, phytochemistry, antioxidant and anticancer activity, and establish possible gaps for future studies. The reviewed literature included conference papers, books, theses, and papers published in peer-reviewed international journals, as well as reports from international, regional, and national organizations. Scientific articles targeted was for the past five years and very few were found, which necessitated having a broader search to include all. That also shows that this *Searia* plant genus is getting less research attention.

1.4 PHENOLIC ACIDS AND FLAVONOIDS

The literature review showed that the phenolic acids and flavonoids were among the substances in the *Searsia* genus that have been examined the most. Phenolic acids and flavonoids are regarded as metabolites. These metabolites support the anti-inflammatory and antioxidant characteristics seen in several species (14). For instance, *S.lancea* contains high concentrations of flavonoids such as myricetin, kaempferol, and quercetin, which have strong antioxidant properties by scavenging free radicals and lowering oxidative stress (15). High concentrations of flavonoids, including myricitrin, which has been connected to anti-inflammatory and antibacterial properties, have also been found in studies on *Searsia leptodictya* (16). Phenolic acids, including gallic acid and protocatechuic acid, were successfully isolated from *Searsia undulata* and *Searsia lancea*. These substances have strong antioxidant properties that aid in preventing free radical-induced cellular damage (17). Furthermore, antibacterial properties of phenolic acids derived from *Searsia* species have been demonstrated, preventing the growth of pathogens such as *Escherichia coli* and *Staphylococcus aureus* (18). *S.lancea* tannins have high antibacterial activity against both Gram-positive and Gram-negative bacteria, suggesting that they could be a useful natural antimicrobial agent source (19). Furthermore, the traditional usage of *S.undulata* in wound healing and infection prevention has been associated with its high tannin concentration. Tannins have also been linked to anti-cancer effects (20). Studies on *S.lancea* and *S.undulata* have shown that tannins can cause cancer cells to undergo programmed cell death, or apoptosis, which raises the possibility that they could be used in cancer treatment. These species' tannin-rich extracts have been proven to have inhibitory effects on cancer cell lines, including those from the prostate, colon, and breast (21).

1.5 PHYTOCHEMISTRY

Numerous bioactive substances have been found by phytochemical investigations, such as tannins, phenolic acids, and flavonoids. One of *S.lancea*'s main flavonoids, quercetin, has been demonstrated to have anti-inflammatory, anti-cancer, and antioxidant properties. Quercetin functions by scavenging free radicals and preventing the growth of cancer cells, especially those that arise from the breast and colon (22).

S.lancea has been proven to have antibacterial properties due to its tannins, which, along with flavonoids, make the plant useful for treating infections in traditional medicine (23). Research has also shown that the plant could stop the growth of microorganisms, including *Escherichia coli* and *Pseudomonas aeruginosa*. *S.leptodictya* is well known for having a high concentration of the flavonoid myricetin, which has potent anti-inflammatory and antioxidant qualities. It has been suggested that myricetin from *S.leptodictya* defends against oxidative stress by regulating inflammatory pathways and preventing the generation of cytokines that promote inflammation (24). Myricetin may be used to treat inflammatory diseases like arthritis and chronic inflammatory challenges because of its anti-inflammatory properties (25). Additionally, it has been demonstrated that *S.leptodictya* possesses antibacterial action. Extracts from the plant have been proven to have inhibitory effects on a variety of bacterial strains, including methicillin-resistant *Staphylococcus aureus* (MRSA) (26). The plant's strong tannin and flavonoid content, which enhances its antibacterial properties, supports its traditional usage in wound healing and infection prevention. The *Dune Currant*, or *S.undulata*, is another species that has a high tannin concentration. Studies have identified tannins and phenolic acids as important bioactive ingredients, supporting the plant's traditional applications for wound healing and infection prevention (27). *S.undulata* tannins have shown antibacterial properties against *Staphylococcus aureus*, indicating that they may be useful in the development of natural antimicrobial therapies (28).

1.6 ANTICANCER AND ANTIOXIDANT ACTIVITIES

Research on the bioactivity of *Searsia* species has shown promise for both cancer treatment and prevention. Many species, including *S.lancea* and *S.leptodictya*, are rich in flavonoids like quercetin, kaempferol, and myricetin, which have been shown to suppress cancer cell growth and trigger apoptosis in a variety of cancer cell lines (29). These flavonoids work against cancer by modifying important signalling pathways that control the cell cycle and induce apoptosis. Another important bioactivity found in the *Searsia* genus is antioxidant activity (30). It has been demonstrated that flavonoids and phenolic acids found in species like *S.lancea* and *S.undulata* neutralise free radicals and decrease oxidative stress, which is linked to the emergence of chronic illnesses like cancer and cardiovascular diseases. *Searsia* species are interesting

prospects for the development of natural anti-oxidants for therapeutic use due to their high antioxidant capacity (31). One of the main contributing factors to the development of cancer is oxidative stress, which is defined as an imbalance between the body's capacity to neutralize reactive oxygen species (ROS) and their creation. Anti-oxidant plants, like those in the *Searsia* genus, can counteract ROS and stop DNA damage, which is a risk factor for cancer (32). Extracts high in polyphenols have shown substantial antioxidant activity in investigations on related *Searsia* species, indicating that they may be able to prevent oxidative damage and lower the risk of cancer (33). For example, extracts from *S.chirindensis* had considerable free radical scavenging action. Moreover, the direct anti-cancer properties of polyphenols found in *Searsia* species have been associated with the reduction of cancer cell proliferation (34). Research on other polyphenol-rich plants has shown that these substances can alter the signalling pathways of cancer cells, causing apoptosis and cell cycle termination in a variety of cancer cell lines (35).

1.7 ANTI-INFLAMMATORY PROPERTIES

It is commonly acknowledged that persistent inflammation accelerates the growth and spread of tumours and is a key element in the development of cancer (36). *Searsia* species have anti-inflammatory qualities that highlight their potential to prevent cancer. Pro-inflammatory enzymes including cyclooxygenase-2 (COX-2) that are overexpressed in several malignancies have been demonstrated to be inhibited by extracts from *Searsia* species. *S.lancea* inhibits COX-2 and lowers pro-inflammatory cytokine levels, demonstrating strong anti-inflammatory properties (37). Because *S.lancea* is closely related, it is conceivable that the latter will have anti-inflammatory properties akin to those of *S.lancea*, which would further bolster its potential as an anti-cancer drug.

1.8 AIM OF THE STUDY

To prepare a crude plant extract of *S.rhemanniana*, broadly establish the bioactivity and describe the phytochemical properties of *S.rhemanniana*.

1.9 SPECIFIC OBJECTIVES

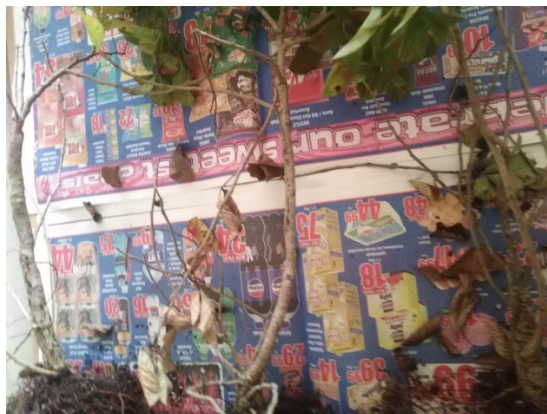
- To evaluate the cytotoxicity of *S.rhemanniana* extracts on a diverse panel of cancer cell lines.
- To elucidate the molecular mechanisms underlying the anticancer effects of *S.rhemanniana*.
- To evaluate the genotoxicity activity of *S.rhemanniana*.
- Determine the micronucleus assay effects of *S.rhemanniana*.
- To evaluate the cell cycle distribution effect of *S.rhemanniana*.
- To evaluate the antibacterial activity of *S.rhemanniana*.

Within the *Anacardiaceae* family, the *Searsia* genus comprises several shrub and tree species that are primarily found in southern Africa. The deciduous shrub or small tree *S.rhemanniana* can grow up to three metres tall, characterized by trifoliate leaves with leathery, shiny leaflets. It produces small clusters of yellowish-green flowers that develop into tiny berry-like drupes (38).

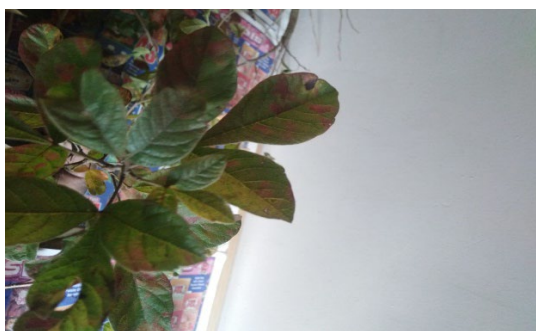
Plant pictures



Roots



Stem



Leaves

1.10 CONCLUSION

The wide range of secondary metabolites found in the *Searsia* genus flavonoids, tannins, and phenolic acids contributes to the group's diverse array of pharmacological effects. Investigations into species such as *S.lancea*, *S.leptodictya*, and *S.undulata* have shown significant antibacterial, antioxidant, and anticancer characteristics. These bioactivities demonstrate how the *Searsia* species may be further investigated for medicinal and pharmacological uses, especially in the prevention and treatment of cancer. Further research into specific species within this family could reveal novel compounds with therapeutic properties, especially in the areas of cancer treatment, inflammation control, and infection management. *Searsia* species have long been used in African medicine, recent pharmacological studies are revealing these plants' promise as sources of new anti-cancer drugs. To validate and possibly utilise these qualities for cancer treatment, more research on their bioactive components, mechanisms of action, and clinical significance is required. Although studies on the anti-tumour effects of *Searsia* species are encouraging, there are few that specifically address *S.rhemanniana*. Since most of the information is derived from research on other species in the genus, it is possible that *S.rhemanniana* and these other species have similar characteristics. To establish the anti-cancer activity of *S.rhemanniana*, comprehensive phytochemical analyses are required, together with *in vitro* and *in vivo* investigations to demonstrate the existence of bioactive chemicals.

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CHAPTER 2: *IN VITRO* CELL CYCLE ANALYSIS OF CRUDE EXTRACTS OF *SEARSIA RHEMANNIANA* EXTRACTS AGAINST DU145 CANCER CELLS

2.1 INTRODUCTION

Prostate cancer progression involves widespread deregulation of cell-cycle control and apoptosis evasion, creating vulnerabilities that anticancer agents can exploit (1). Castration-resistant disease frequently decouples proliferation from androgen signalling, and accumulates deoxyribonucleic acid (DNA) damage and mitotic checkpoint defects, rendering cell cycle targeted strategies attractive (2). DU145 cells, an androgen-independent, prostate specific antigen (PSA)-negative human prostate carcinoma line are widely used to model these features (3). Reports describe DU145 as AR non-responsive or AR-negative with variable low AR expression (4); they also harbour alterations in tumour suppressors (e.g. RB aberrations and a temperature-sensitive p53 allele), which together confer checkpoint plasticity and apoptosis resistance (5). These properties make DU145 suitable for screening agents that induce checkpoint arrest, spindle stress, or checkpoint-independent apoptosis (6). Progression through (Gap 1) G1/(synthesis)S/ (Gap 2) G2/(mitosis and cytokinesis) M is regulated by cyclin-dependent kinase (CDK) complexes and surveillance checkpoints that delay division when damage or spindle errors are detected (7). The DNA damage response (DDR) engages ataxia-telangiectasia mutated (ATM)/(ATM-related and Rad3-related) ATR–checkpoint kinase 1 (CHK1)/checkpoint kinase 2 (CHK2) signalling to enforce a G2/M block until lesions are repaired, while persistent damage triggers apoptosis (8). Conversely, the spindle assembly checkpoint (SAC) monitors kinetochore–microtubule attachments in early mitosis; anti-mitotic stress sustains SAC signalling, causing metaphase arrest and ultimately mitotic death or slippage, with apoptosis frequently following arrest (9). Therapeutics that exploit these levers include DNA-damaging alkylators and microtubule targeting agents (10).

Apoptosis is executed predominantly via intrinsic (mitochondrial) and extrinsic (death-receptor) pathways (11). In the intrinsic pathway, mitochondrial outer-membrane permeabilization (MOMP) releases cytochrome *c* to form the apoptosome (Apaf-1–caspase-9), activating effector caspases-3/-7 (12). B-cell lymphoma 2 (BCL-2) family

proteins integrate stress signals to determine MOMP, while tumour protein p53(p53) and cellular redox status modulate this threshold (13). Contemporary syntheses also emphasize caspase crosstalk with other programmed cell-death modalities (e.g. pyroptosis, necroptosis), though caspase-dependent apoptosis remains central to most anticancer responses (14). Melphalan is a bifunctional nitrogen-mustard alkylating agent that forms DNA mono-adducts and interstrand crosslinks (ICLs) (15). ICLs stall replication forks and activate the DDR, enforcing a G2/M checkpoint that allows repair through Fanconi anaemia, homologous-recombination, and translesion mechanisms (16). When damage overwhelms repair capacity, cells undergo apoptosis. Clinically, melphalan remains a cornerstone for multiple myeloma and other malignancies, illustrating the enduring value of DDR engaging cytotoxicity (17). *In vitro*, melphalan's pattern G2 accumulation followed by apoptosis is a robust positive-control phenotype for assay validation (18). Agents that perturb microtubule dynamics (e.g. taxanes, vinca alkaloids) or disrupt kinetochore function prolong SAC signalling, trapping cells in early mitosis (19). Prolonged arrest leads to mitotic catastrophe and apoptosis via mitochondrial pathways; alternatively, mitotic slippage can produce aneuploid progeny with delayed apoptosis or senescence (20). Emerging work connects SAC signalling to mitochondrial priming, suggesting that the duration and intensity of mitotic stress set the apoptotic threshold (21). Observing early M-phase accumulation with subsequent apoptosis in cytometry typically indicates spindle or kinetochore targeted activity (22).

Cells need to progress through every phase of the cell cycle to assure a full copy of DNA for a new daughter cell (23). Checkpoints regulate the progression of cells through the cycle and will cause cell cycle arrest if DNA damage or DNA stress has occurred (24). Cell cycle arrest is defined as a high proportion of cells found in the same cycle event at a specific time (25). Cell cycle arrest will be maintained until DNA repair is complete. Cell cycle analysis is performed to determine the state of DNA in response to treatment of the cell with a particular compound or extract (26). The distribution of DNA content is important as it will lead to the identification of targets or pathways to target for the treatment of cancer and tumours (27). DNA distribution can be analysed using the nuclear binding dye Hoechst 33342, a double stranded DNA-binding dye in combination with high-throughput fluorescence microscopy. Annexin

V-fluorescein isothiocyanate (Annexin V-FITC) is used to differentiate apoptotic cells from mitotic cells (28).

Prostate cancer (PCa) remains the second leading cause of cancer-related deaths and is the most diagnosed malignant disease among men worldwide (29). The introduction of PSA testing has led to a substantial rise in PCa incidence over the past two to three decades (30), resulting in earlier detection and a shift towards diagnosis at earlier disease stages (31). The lifetime risk for men being diagnosed with PCa is approximately 17% (one in 6), yet only about 3–4% (one in 30) succumb to the disease (32). This indicates that most men with PCa do not develop clinically significant forms that influence their morbidity or mortality (33). Despite improved early detection, mortality rates have remained largely unchanged and continue to rank among the highest globally (34). PCa exhibits a wide spectrum of biological behaviour, ranging from slow growing to highly aggressive forms, which complicates diagnosis and treatment planning (35). For patients with localised or locally advanced disease, management options typically include active surveillance, surgery, and radiation therapy (36). As these treatments can cause substantial side effects such as impotence, urinary incontinence, and radiation-induced damage to nearby organs like the bladder or rectum (37), accurate assessment of tumour location, stage, and aggressiveness is crucial for optimal management (38).

Current diagnostic methods PSA testing, digital rectal examination, and transrectal ultrasound-guided biopsy are limited by suboptimal sensitivity and specificity, and they provide insufficient information about tumour aggressiveness and stage (39). In contrast, emerging evidence highlights multiparametric magnetic resonance imaging (mp-MRI) as a highly sensitive and specific tool for detecting, characterising, and staging PCa (40). Its application enhances multiple aspects of disease management, including targeted biopsy of clinically significant lesions, evaluation of tumour aggressiveness, and accurate staging to support personalised treatment selection (41). PSA is a naturally occurring enzyme secreted almost exclusively by the epithelial cells of the prostate and serves as a key serum biomarker for PCa (42). While PSA is organ specific, it is not cancer specific, as elevated PSA levels can also result from benign conditions such as benign prostatic hyperplasia (BPH), prostatitis, or other urinary tract disorders (43). There is no absolute PSA threshold that definitively

indicates PCa, nor a value below which the disease can be completely ruled out, although higher PSA concentrations are generally associated with an increased risk of cancer (44). Traditionally, a PSA level of ≥ 4 ng/ml has been considered suspicious and warrants further investigation through biopsy (45). However, only about one-third of men with elevated PSA at this level are confirmed to have cancer, while some with normal PSA values may still harbour the disease (46). This underscores the limitation of PSA as a diagnostic tool for confirming or excluding PCa (47). PSA levels are also important in stratifying patient risk at diagnosis, are incorporated into predictive staging nomograms, and are routinely used to monitor treatment response (48).

Digital Rectal Examination (DRE) remains a key component of the clinical examination for PCa (49). During this procedure, cancerous tissue typically feels firm and irregular upon palpation. Approximately 70–75% of prostate tumours develop in the peripheral zone, making them theoretically detectable when sufficiently large (50). However, around 25% of tumours originate in the transitional zone, which cannot be reached during DRE owing to anatomical limitations (51). Additionally, as PCa is now more frequently detected at smaller tumour volumes, the proportion of palpable lesions has decreased, reducing DRE's sensitivity and specificity (52). Nevertheless, abnormal findings on DRE are often associated with more aggressive tumour pathology and remain a strong indication for biopsy, identifying around 18% of PCa cases, even in men with normal PSA levels (53). DRE also contributes to clinical staging Computed Tomography (cT) category, risk classification, and predictive nomograms (54). Transrectal Ultrasound (TRUS) is the standard imaging method for prostate evaluation, and histological diagnosis of PCa is typically based on analysis of 10–12 biopsy cores taken from standardised prostate regions (55). While TRUS estimates prostate volume and guides biopsy needle placement effectively, it has poor sensitivity and specificity for both cancer detection and staging (56). PCa lesions often appear hypo-echoic compared to surrounding tissue, but 40–50% of tumours are iso-echoic and thus undetectable on ultrasound (57). Assessment of the transitional zone is particularly challenging due to its heterogeneous structure, often influenced by benign prostatic hyperplasia (BPH), which obscures anteriorly located tumours (58). Consequently, TRUS-Guided Biopsy (TRUS-bx) may miss tumours or fail to sample their most aggressive areas, potentially leading to false negatives, inaccurate Gleason

scoring, or incorrect risk classification (59). False-negative rates for TRUS-bx can reach 20–30%, meaning up to one-third of men with normal biopsy results may have cancer (60). As a result, patients with persistent suspicion often undergo repeated biopsies, which increases healthcare costs, patient anxiety, and risk of complications such as infection or tissue scarring factors that may complicate future surgical treatment (61). The detection rate at first repeat biopsy is only 10–22%, with diminishing yields on subsequent procedures (62). Efforts to enhance TRUS detection include increasing the number of biopsy cores or employing saturation biopsy techniques, but while these methods may raise detection rates, they also heighten the risk of identifying clinically insignificant, well-differentiated tumours, potentially leading to overtreatment (63). The inherent limitations of TRUS-bx have driven the search for imaging modalities that can improve the detection of clinically significant PCa while reducing unnecessary biopsies and the detection of indolent tumours (64). Currently, the prostate remains the only solid organ diagnosed primarily through blind systematic biopsies (65).

The histopathological aggressiveness of PCa is evaluated using the Gleason scoring (GS) system (66). Cancerous tissue is graded from 1 to 5 based on its structural differentiation, with lower grades (1–2) resembling normal prostate architecture, and higher grades indicating more disorganised, aggressive patterns (67). Since multiple grades may coexist within a single specimen, a composite GS (ranging from 2–10) is calculated by summing the dominant and highest-grade patterns (68). A Gleason grade of ≥ 3 or GS ≥ 6 typically marks the threshold for malignancy. Following radical prostatectomy, the GS is derived from the most dominant and the second most dominant patterns (69). Higher GS values correlate with increased tumour aggressiveness, greater metastatic potential, and poorer prognosis (70). Risk-classification systems, such as those proposed by D'Amico, use GS alongside PSA and tumour stage to guide treatment decisions (71). However, GS determined from biopsy samples may be inaccurate due to sampling errors around one-third of patients are upgraded after surgical evaluation. Misclassification can result in inappropriate treatment intensity, either excessive or insufficient (72). Over time, evolving histopathological criteria, especially expansion of Gleason grade 4 definitions has improved the correlation between biopsy and surgical GS (73) but also complicated

longitudinal comparisons of data due to widespread tumour grade reclassification (74). Clinical staging of PCa is defined by the tumour node metastasis (TNM) classification, particularly the tumour (cT) stage, which is categorized from cT1 to cT4, with subgroups describing tumour extent (75). Prognosis and treatment selection are closely tied to cT stage at diagnosis, with the distinction between localised (cT1–cT2) and locally advanced (cT3–cT4) disease being especially critical (76). Traditionally, DRE and TRUS have been used for staging, but both techniques tend to underestimate tumour size and extent due to limited sensitivity and specificity (77). Similarly, PSA values show substantial overlap between tumour stages, limiting their predictive value for extracapsular extension (EPE) (78). This study aims to evaluate the effects of crude *S.rhemanniana* extracts on cell cycle progression in the human prostate cancer cell line DU145.

2.2 MATERIALS AND METHODS

2.2.1 Plant material

The plant material was authenticated as *S.rhemanniana* by botanists from the botanical garden in Pietermaritzburg, South Africa. Following verification, the purchased plant material was thoroughly cleaned with distilled water to remove soil and debris. The plant was then separated into its major anatomical parts: roots, bulbs, and leaves. Each plant component was dried in a ventilated oven at a temperature range of 30–60 °C for five days to ensure gradual dehydration and the preservation of phytochemicals. Once fully dried, the material was coarsely ground using a hammer mill and stored at room temperature in airtight containers until required for extraction.

2.2.2 Extract preparation

Plant material was ground into a fine powder using an IKA grinder (IKA Labortechnik, Germany). Extraction was performed using methanol (MeOH), dichloromethane (DCM), and water (H₂O) at a ratio of approximately 1:4 (w/v). The maceration was placed on a shaker (Labcon, Lab Design Engineering, Maraisburg, South Africa) for 72 hours. Following extraction, the mixture was filtered through Whatman No. 1 filter paper (Merck Chemicals (Pty) Ltd, Wadeville, South Africa) using a vacuum filtration system (Merck Chemicals (Pty) Ltd, Wadeville, South Africa). This process was

repeated until the filtrate was clear. The organic solvents (MeOH and DCM) were removed under reduced pressure using a BÜCHI Rotovapor (Labotec (Pty) Ltd, Halfway House, South Africa), and the resulting extracts were dried at room temperature under a fume hood and stored at 4 °C. The aqueous extract was frozen at -80 °C and subsequently freeze-dried to a powder, then stored at 4 °C.

2.3 IN VITRO CELL CYCLE ANALYSIS OF EXTRACTS AGAINST DU145 CANCER CELLS

All reagents and chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). RPMI and PBS with and without calcium ion (Ca^{2+}) and magnesium ion (Mg^{2+}) were purchased from Cytiva (Marlborough, MA, USA). Foetal Bovine Serum (FBS) and penicillin/streptomycin were purchased from Biowest (Nuaillé, France).

2.3.1 Sample preparation

Test samples were solubilized using dimethyl sulfoxide (DMSO) to yield a stock concentration of 100 mg/mL and were stored at 4 °C until used.

2.3.2 Cell line maintenance

The human prostate cancer cell line, DU145, was used for cytotoxicity screening. Complete growth medium consisted of roswell park memorial institute (RPMI) supplemented with 10% FBS and 1x penicillin-streptomycin and cells were maintained in 10-cm culture dishes in complete medium and incubated at 37 °C in a humidified atmosphere with 5% CO_2 .

2.3.3 PS translocation

Cells were seeded in 96 well plates at 5000 cells/well in 100 μL aliquots and left overnight to attach. Cells were treated with the calculated half-maximal inhibitory concentration (IC_{50}) values as indicated in the table below.

Table 1: IC50 values of extracts used against DU145 cancer cells

Sample	N=1	N=2	N=3	N=4	Average	Stdev
Barks water	8,493	ND	1165	ND		
Barks MeoH	186,5	120,2	223,6	130	165,08	48,75
Barks DCM	46,27	31,03	50,21	31,01	39,63	10,07
Leaves MeoH	ND	ND	ND	ND		
Leaves water	ND	228,7	194,3	157,6	193,53	35,56
Leaves DCM	108,3	100,3	75,32	95,36	94,82	14,05
Roots MeoH	ND	258,2	ND	ND		
Roots DCM	76,18	39,51	34,62	31,88	45,55	20,66
Arterial MeoH	477,7	418,5	ND	ND		

Melphalan (30 μ M) was used as a positive control. Cells were stained according to Annexin V-FITC/Propidium iodide (PI) Kit protocol {Magnetic-Activated Cell Sorting. (MACS) Miltenyi Biotec}. However, experimental changes were made to include the nuclear dye, Hoechst 33342. The binding buffer of the kit was prepared by performing a 1:20 dilution as recommended using PBS (+Ca and Mg). Annexin V-FITC and Hoechst were diluted in a binding buffer to a final concentration of 2 μ g/mL and 5 μ g/mL, respectively. Thereafter, treatments were aspirated from all wells and 100 μ L of the prepared dyes were added to each well and incubated at room temperature for 20 minutes. PI was prepared in a binding buffer to a final concentration of 100 μ g/mL and 10 μ L was added to each well. Cells were imaged using the ImageXpress Micro XLS Widefield Microscope (Molecular Devices).

2.4 RESULTS

The untreated cells showed a typical proliferative profile, with most cells distributed across G0/G1 (33,3%), Early M (29,8%), and S-phase (19,4%), and minimal apoptosis (1,0%), confirming normal cycling and viability.

% CELLS IN EACH PHASE

T20, T21 AND T22: THREE DIFFERENT TRANSFER NUMBERS FOR THE 3 EXPERIMENTS

Table 2: List of controls used

	CONTROL				
Phase	T20	T21	T22	AVG	SD
G0/G1	32,4	31,0	36,4	33,3	2,8
S	15,9	24,2	18,0	19,4	4,3
G2	11,3	12,4	8,1	10,6	2,2
Early M	34,7	21,1	33,4	29,8	7,5
Late M	4,3	10,1	3,6	6,0	3,5
Apoptosis	1,4	1,2	0,5	1,0	0,5
	100,0	100,0	100,0	100,0	

Table 3: Cell Cycle Distribution of Cells Treated with Melphalan (Positive Control) Showing Significant G2/M Arrest ($p < 0.05$)

	MELPHALAN					INTERPRETATION	T-TEST	
Phase	T20	T21	T22	AVG	SD			
G0/G1	27,2	29,5	23,4	26,7	3,1		0,0524	NS
S	15,7	26,0	21,6	21,1	5,2		0,6794	NS
G2	21,5	14,3	20,5	18,8	3,9		0,0350	P<0.05
Early M	30,4	19,3	29,1	26,3	6,0		0,5657	NS
Late M	3,4	7,7	3,1	4,7	2,6		0,6459	NS
Apoptosis	1,8	3,2	2,2	2,4	0,7	G2 arrest, apoptosis	0,0468	P<0.05
	100,0	100,0	100,0	100,0				

Table 4: Flow Cytometric Analysis of Apoptosis in Cells Treated with Bark Water Extract and Positive Control

	Barks water				
Phase	T20	T21	T22	AVG	SD
G0/G1	Extract interfered – images could not be analysed				
S					
G2					
Early M					
Late M					
Apoptosis					

Table 5: Flow Cytometric Assessment of Apoptosis in Cells Treated with Bark Methanol (MeoH) Extract

	Barks MeoH					INTERPRETATION	T-TEST	
Phase	T20	T21	T22	AVG	SD			
G0/G1	11,6	21,0	17,5	16,7	4,7		0,0064	P<0.01
S	6,5	9,7	10,7	9,0	2,2		0,0204	P<0.05
G2	4,8	5,3	15,9	8,7	6,3		0,6453	NS
Early M	28,4	25,7	25,5	26,5	1,6		0,5067	NS
Late M	13,2	11,5	8,2	11,0	2,5		0,1185	NS
Apoptosis	35,4	26,9	22,2	28,2	6,7	Apoptosis, no cell cycle arrest	0,0022	P<0.01
	100,0	100,0	100,0	100,0				

Table 6: Flow Cytometric Assessment of Apoptosis in Cells Treated with Bark Dichloromethane (DCM) Extract

	Barks DCM						T-TEST			
Phase	T20	T21	T22	AVG	SD					
G0/G1	19,3	26,2	10,6	18,7	7,8				0,0389	P<0.05
S	12,6	9,6	15,3	12,5	2,8				0,0833	NS
G2	9,3	7,4	10,4	9,0	1,5				0,3797	NS
Early M	45,2	41,4	49,8	45,5	4,2				0,0339	P<0.05
Late M	11,2	13,4	10,9	11,9	1,4				0,0551	NS
Apoptosis	2,4	1,9	2,9	2,4	0,5	Early M arrest and apoptosis			0,0277	P<0.05
	100,0	100,0	100,0	100,0						

Table 7 Flow Cytometric Assessment of Apoptosis in Cells Treated with Leaves Methanol (MEoH) Extract

	Leaves MeoH						T-TEST			
Phase	T20	T21	T22	AVG	SD					
G0/G1	24,9	20,9	21,5	22,4	2,1				0,0060	P<0.05
S	18,2	22,7	19,7	20,2	2,3				0,7899	NS
G2	16,8	10,3	20,9	16,0	5,4				0,1831	NS
Early M	30,9	30,7	33,2	31,6	1,4				0,6961	NS
Late M	7,2	13,8	4,2	8,4	4,9				0,5268	NS
Apoptosis	2,0	1,7	0,5	1,4	0,8	No significant effect			0,5424	NS
	100,0	100,0	100,0	100,0						

Table 8: Flow Cytometric Assessment of Apoptosis in Cells Treated with Leaves Water Extract

	Leaves water								T-TEST	
Phase	T20	T21	T22	AVG	SD					
G0/G1	10,3	10,1	10,6	10,3	0,3				0,0001	P<0.05
S	9,9	12,2	11,5	11,2	1,2				0,0350	NS
G2	6,1	1,3	18,6	8,6	8,9				0,7315	NS
Early M	21,8	24,5	12,8	19,7	6,2				0,1465	NS
Late M	8,0	7,3	5,4	6,9	1,4				0,6953	NS
Apoptosis	43,9	44,6	41,1	43,2	1,8	Apoptosis, no cell cycle arrest			0,000003	p<0.001
	100,0	100,0	100,0	100,0						

Table 9: Flow Cytometric Assessment of Apoptosis in Cells Treated with Leaves Dichloromethane (DCM) Extract

	leaves DCM								T-TEST	
Phase	T20	T21	T22	AVG	SD					
G0/G1	24,4	28,6	18,7	23,9	5,0				0,0473	P<0.05
S	23,2	16,9	18,5	19,5	3,2				0,9630	NS
G2	13,6	11,4	15,7	13,6	2,1				0,1670	NS
Early M	27,0	26,0	32,1	28,4	3,2				0,7806	NS
Late M	9,8	15,6	13,8	13,0	3,0				0,0580	NS
Apoptosis	2,1	1,3	1,3	1,6	0,4	No cell cycle arrest or apoptosis			0,2107	NS
	100,0	100,0	100,0	100,0						

Table 10: Flow Cytometric Assessment of Apoptosis in Cells Treated with Roots Methanol (MeOH) Extract

	Roots MeOH								T-TEST	
Phase	T20	T21	T22	AVG	SD					
G0/G1	20,7	16,0	21,6	19,4	3,0				0,0044	P<0.01
S	14,4	24,2	17,7	18,8	5,0				0,8866	NS
G2	14,5	17,3	13,8	15,2	1,9				0,0510	NS
Early M	40,7	31,7	37,9	36,7	4,6				0,2406	NS
Late M	5,7	7,3	5,9	6,3	0,9				0,8856	NS
Apoptosis	4,0	3,5	3,2	3,5	0,4	Apoptosis, slight delay in G2			0,0024	P<0.01
	100,0	100,0	100,0	100,0						

Table 11: Flow Cytometric Assessment of Apoptosis in Cells Treated with Roots Dichloromethane (DCM) Extract

	Roots DCM								T-TEST	
Phase	T20	T21	T22	AVG	SD					
G0/G1	17,4	16,5	8,8	14,3	4,7				0,0039	P<0.01
S	14,8	8,2	11,5	11,5	3,3				0,0671	NS
G2	8,6	6,4	11,3	8,8	2,4				0,3908	NS
Early M	43,4	54,2	56,3	51,3	7,0				0,0218	P<0.05
Late M	10,7	11,9	9,4	10,7	1,2				0,0960	NS
Apoptosis	5,1	2,7	2,7	3,5	1,4	Early M arrest, apoptosis			0,0434	P<0.05
	100,0	100,0	100,0	100,0						

Table 12: Flow Cytometric Assessment of Apoptosis in Cells Treated with Arterial Methanol (MeOH) Extract

	Arterial MeOH								T-TEST	
Phase	T20	T21	T22	AVG	SD					
G0/G1	15,3	12,5	13,8	13,8	1,4				0,0004	P<0.001
S	16,5	21,9	16,6	18,3	3,1				0,7485	NS
G2	17,3	19,7	18,8	18,6	1,2				0,0054	P<0.01
Early M	44,1	37,6	44,7	42,1	3,9				0,0640	NS
Late M	3,9	6,4	4,1	4,8	1,4				0,6226	NS
Apoptosis	2,9	1,9	2,1	2,3	0,5	G2 arrest, apoptosis			0,0369	P<0.05
	100,0	100,0	100,0	100,0						

Melphalan (Positive Control – DNA Alkylating Agent) showed a significant increase in G2 phase ($p < 0.05$) indicative of G2/M arrest, consistent with DNA crosslinking-induced checkpoint activation (Table 3). Apoptosis significantly elevated ($p < 0.05$) confirms cytotoxic response following arrest. Barks water extract suggests strong autofluorescence or aggregation, implying either high pigment content or compound-induced optical interference, rather than cytotoxicity per se (Table 4). Optimization of staining or spectral compensation is required. Barks MeOH extract showed massive apoptosis (28,2%, $p < 0.01$) with no significant accumulation in G2 or M-phase (Table 5). Pro-apoptotic agent that induces cell death independent of classical checkpoint arrest (possibly intrinsic/mitochondrial pathway). Barks DCM extract showed early M-phase arrest ($p < 0.05$) with mild apoptosis ($p < 0.05$) (Table 6), likely disrupting mitotic progression (e.g. microtubule interference or spindle poison-like effect). Leaves MeOH extract showed a minor decrease in G0/G1 ($p < 0.05$), but no change in apoptosis or arrest (Table 7), biologically inactive or weak cytostatic tendency. Leave water extract showed dramatic apoptosis (43,2%, $p < 0.001$) with no prior phase accumulation (Table 8), like barks MeOH extract, potent apoptosis inducer causing rapid death without checkpoint arrest (possible necroptotic or caspase-independent mechanism). Leaves DCM extract showed no significant effects (Table 9), biologically inactive at tested concentration. Roots MeOH extract showed significant decrease in G0/G1 ($p < 0.01$) and slight trend towards G2 accumulation, with moderate apoptosis ($p < 0.01$) (Table 10), mild cytostatic and apoptotic effect with possible weak G2 delay. Roots DCM extract showed Strong Early M-phase accumulation ($p < 0.05$) and apoptosis ($p < 0.05$) (Table 11), like Barks DCM extract likely mitotic spindle disruption leading to apoptosis. Arterial extract showed significant G2 arrest ($p < 0.01$) and moderate apoptosis ($p < 0.05$) (Table 12), mechanistically like Melphalan, potential DNA-damaging or replication stress-inducing compound.

The tested extracts exhibited distinct mechanisms of growth inhibition refer to Appendix A. Barks MeOH extracts and leaves water extracts induced apoptosis without preceding cell cycle arrest, suggesting direct activation of death pathways. Barks DCM extracts and roots DCM extracts arrested cells in early mitosis, indicative of disruption of mitotic machinery. Arterial extract behaved similarly to Melphalan by inducing G2 arrest, followed by apoptosis, suggesting potential DNA damage. Leaves

MeOH and leaves DCM extracts showed no meaningful activity, while bark water extract interfered with staining and required protocol optimization. Collectively, these findings highlight barks MeOH, leaves water, barks DCM, roots DCM and arterial MeOH extracts as priority candidates for further mechanistic and molecular validation.

2.5 DISCUSSION

The current study investigated the effects of *S.rhemanniana* extracts on cell cycle progression and apoptosis in DU145 prostate cancer cells, using Melphalan as a clinically validated chemotherapeutic comparator. Cell cycle dysregulation and apoptotic resistance are hallmarks of cancer progression; thus, compounds capable of reactivating these pathways hold significant therapeutic value. Flow cytometric profiling revealed divergent mechanistic behaviours among the tested extracts, allowing for the classification of their biological activities into distinct phenotypic response categories. Melphalan, serving as a positive control, behaved as expected for a DNA alkylating agent. Its ability to induce a statistically significant accumulation of cells in the G2/M phase ($p < 0.05$), followed by elevated apoptosis ($p < 0.05$), confirms activation of DNA damage checkpoints. This G2-phase blockade is consistent with known cell cycle responses to genotoxic stress, where ATM/ATR signalling halts mitotic entry to facilitate DNA repair. Failure to repair ultimately triggers apoptotic cascades. The robust checkpoint-dependent cytotoxic response elicited by Melphalan serves as a benchmark against which the phytochemical extracts can be compared.

Among the plant-derived treatments, barks MeOH and leaves water extracts exhibited the most striking cytotoxicity, with apoptosis rates exceeding 28% and 43%, respectively ($p < 0.01$), without any evidence of prior cell cycle arrest. This phenotype strongly suggests a checkpoint-independent mode of cell death, indicating activation of intrinsic apoptotic pathways, possibly mediated via mitochondrial depolarization or caspase activation. Such behaviour is characteristic of polyphenolic compounds with redox-cycling capabilities or triterpenes capable of membrane destabilization. The lack of phase-specific accumulation further implies rapid cytotoxic activity that bypasses classical arrest checkpoints, resembling the behaviour of pro-oxidative flavonoids or BH3-mimetic compounds. Interestingly, bark DCM and roots DCM extracts demonstrated a distinctly different phenotype, characterized by a pronounced

accumulation in the early mitotic (M) phase ($p < 0.05$), accompanied by measurable apoptosis. This M-phase blockade suggests interference with spindle fibre assembly or kinetochore attachment, analogous to the mechanism of taxanes or vinca alkaloids. Arrest at mitotic prometaphase leads to sustained activation of the SAC, ultimately promoting mitotic catastrophe, a well-documented route to apoptotic or necrotic death in rapidly proliferating cancer cells. These extracts therefore warrant further evaluation for tubulin polymerization inhibition or Aurora kinase modulation.

Arterial MeOH extract followed a third mechanistic profile, closely mirroring that of Melphalan. Its induction of G2 arrest ($p < 0.01$), combined with significant apoptosis ($p < 0.05$), suggests a DNA-damaging or replication stress-inducing mechanism. Whether this is due to topoisomerase inhibition, intercalative pressure, or ROS-mediated strand breaks remains to be determined. Notably, the effect magnitude, though less than Melphalan, suggests lower potency but mechanistic similarity, making this extract a promising candidate for synergistic or combinatorial regimens. In contrast, leaves MeOH extracts and leaves DCM extracts exhibited no statistically significant impact on either proliferation or apoptosis, suggesting low bioactivity at the tested concentration. These may contain inactive or poorly permeable constituents, or they may require metabolic activation not achievable under *in vitro* conditions. Barks water extract presented an unusual technical limitation: interference with image acquisition due to optical distortion, most likely from pigment-rich or aggregating compounds. Such behaviour is common among anthocyanin-rich or tannin-dense extracts. While this artifact precludes conclusive interpretation, it also indicates high chemical content, warranting alternative evaluation using fluorescence-unbiased methodologies, such as Annexin V/PI histograms without imaging gates or impedance-based cytotoxicity assays (e.g. xCELLigence platform).

This diversity in mechanisms strongly supports the poly-pharmacological potential of *S.rhemanniana* phytochemicals. Rather than acting through a single conserved pathway, different fractions selectively target-independent regulatory nodes within the cell cycle-apoptosis axis. From a therapeutic standpoint, checkpoint-independent apoptosis inducers such as barks MeOH extracts and leaves water extracts are particularly valuable, as they may overcome p53 dysfunction, a common resistance mechanism in prostate cancer. Likewise, mitotic arrest agents (barks DCM extracts

and roots DCM) have clinical precedent in taxane-based therapies, while G2/M checkpoint inducers like arterial MeOH extract may be ideal for combination with PARP or ATR inhibitors to promote synthetic lethality.

2.6 CONCLUSION

The current study reveals that *S.rhemanniana* contains multiple bioactive components capable of selectively modulating prostate cancer cell fate through distinct molecular pathways. Barks MeOH extracts, leaves water extracts, bark DCM extracts, roots DCM extracts, and arterial MeOH extracts emerge as high-priority candidates for further mechanistic dissection, metabolite profiling, and potency optimization. Future studies should include Western blot validation of checkpoint markers {e.g. Cyclin B1(CCNB1), cyclin-dependent kinase inhibitor 1 (p. 21), p. 53, (phosphorylated histone protein H2AX) γ -H2AX} and caspase cleavage assays to confirm terminal death pathways. Additionally, liquid chromatography-tandem mass spectrometry (LC-MS/MS) guided fractionation will be essential to identify the responsible bioactive constituents and assess structure-activity relationships.

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CHAPTER 3: EVALUATION OF THE CYTOTOXIC ACTIVITY OF *SEARSIA RHEMANNIANA* CRUDE EXTRACTS AGAINST THE HUMAN PROSTATE CANCER CELL LINE DU145

3.1 INTRODUCTION

Prostate cancer represents a significant global health burden, with increasing incidence, mortality, and disability-adjusted life years (DALYs) observed over the past three decades. The Global Burden of Disease Study indicates that from 1990 to 2019, incident cases rose by 116,11%, deaths by 108,94%, and DALYs by 98,25% (1). Prostate cancer is indeed a significant global health burden, particularly in major BRICS countries, where incidence and prevalence are rising. Tailored health policies are essential to address the diverse characteristics of this disease burden effectively (2). It ranks as the fourth-most diagnosed cancer worldwide when considering both sexes and is the second-most frequently diagnosed cancer among men. An estimated 1.4 million men were diagnosed with prostate cancer globally, accounting for approximately 7.3% of all cancers in men (3). The disease presents an even more critical concern in Africa, where prostate cancer is the leading cancer affecting men, with rising incidence and mortality rates attributed to late diagnosis and limited access to effective treatment options (4). The primary therapeutic approach for advanced prostate cancer involves androgen deprivation therapy (ADT), which aims to suppress the activity of intraprostatic testosterone and its more potent derivative, dihydrotestosterone (DHT) (5). While ADT initially yields positive responses, the emergence of therapy-resistant tumour clones often leads to castration-resistant prostate cancer (CRPC), characterised by disease progression, metastasis, and increased mortality (6). Surgical intervention, particularly radical prostatectomy, is commonly employed in the early stages of solid tumours. However, this approach may not fully prevent recurrence or metastatic spread, especially in advanced cases (7). For patients with metastatic disease, first-line chemotherapy typically includes a combination of docetaxel and prednisone, administered over at least six cycles. Although these regimens can offer palliative relief and temporary disease control, they have shown a limited impact on improving overall survival rates (8). In resource-limited

settings, 5-fluorouracil (5-FU), with or without cisplatin, is often recommended as a cost-effective alternative, although its efficacy remains constrained (9).

Early detection of PCa remains a cornerstone of reducing disease-specific morbidity and mortality (10). Despite advances in imaging and biomarker technologies, the optimal screening approach continues to evolve, particularly in balancing the benefits of early diagnosis against the harms of overdiagnosis and overtreatment (11). Key screening modalities include PSA testing, DRE, and mpMRI, often integrated into risk-adapted screening protocols (12). PSA testing is the most widely used tool for the early detection of PCa. PSA is a serine protease secreted by prostatic epithelial cells, and elevated serum levels may indicate prostate malignancy, benign prostatic hyperplasia, or inflammation (13). PSA screening has been shown to reduce PCa specific mortality, particularly in men aged 55–69 years, as demonstrated by the European Randomized Study of Screening for Prostate Cancer (ERSPC) (14). However, this benefit must be weighed against the risks of false positives, unnecessary biopsies, and treatment of indolent tumours. The major limitations of PSA testing include poor specificity and the inability to differentiate between aggressive and indolent disease (15). Elevated PSA levels (>4 ng/mL) can occur in the absence of cancer, and low levels can still be present in high-grade tumours. To mitigate this, derivatives such as PSA density, PSA velocity, and the free-to-total PSA ratio have been employed, albeit with limited sensitivity (16). DRE is often used in conjunction with PSA testing, although its sensitivity is highly operator dependent. It can detect asymmetries or nodules suggestive of malignancy, particularly in the posterior prostate. However, its standalone value is limited, and DRE is not recommended as a primary screening tool due to poor reproducibility and low sensitivity for early-stage disease (17).

The introduction of mpMRI has significantly improved the accuracy of prostate cancer detection. mpMRI enables visualization of suspicious lesions using a combination of T2-weighted imaging, diffusion-weighted imaging (DWI), and dynamic contrast-enhanced (DCE) sequences (18). When used as a triage test following elevated PSA levels, mpMRI can guide targeted biopsies and reduce the detection of clinically insignificant cancers (19). Risk-adapted screening strategies integrate clinical factors such as age, race, family history, PSA levels, and imaging findings to personalise

screening intervals and biopsy decisions (20). Emerging tools like the Prostate Health Index (PHI), 4Kscore, and genomic risk calculators further enhance risk stratification by distinguishing between indolent and clinically significant disease (21). One of the principal challenges in prostate cancer screening is overdiagnosis, the detection of cancers that would not have become clinically significant within a man's lifetime (22).

Overdiagnosis can lead to overtreatment, exposing patients to unnecessary surgical or radiation-related complications such as urinary incontinence, erectile dysfunction, and bowel dysfunction (23). Population-based studies have estimated that up to 50% of screen-detected prostate cancers may be over diagnosed, particularly among older men and those with low-grade, low-volume tumours (24). This has prompted a paradigm shift towards active surveillance for patients with low-risk disease, thereby minimising harm while preserving the opportunity for curative intervention if the cancer progresses (25). In resource-limited settings, the risks of overdiagnosis may be compounded by poor follow-up infrastructure and limited access to advanced diagnostics like mpMRI or genomic testing (26). Therefore, the development of context-specific screening algorithms remains a critical research and policy priority. Given the limitations of current treatment modalities and the rising burden of prostate cancer, especially in low- and middle-income regions, there is a growing need to explore alternative and complementary therapeutic strategies, including those derived from natural products and traditional medicines.

For this study, *S.rhemanniana* plant belonging to species within the genus *Searsia*, which is part of the *Anacardiaceae* family, was selected due to less scientific reports regarding its medicinal potential. This genus, previously classified under *Rhus*, encompasses over 250 species, many of which are recognized for their medicinal properties and biological activities. Research indicates that *Searsia* species, including *S.rhemanniana*, contain various phytochemicals such as terpenoids and flavonoids, which exhibit significant antioxidant and enzyme inhibition activities (27), but there is a gap to further analyses of this plant's medical benefits.

3.2 MATERIALS AND METHODS

3.2.1 Plant material

The plant material was authenticated as *S.rhemanniana* by botanists from the botanical garden in KwaZulu-Natal, South Africa. Following verification, the plant material was thoroughly cleaned with distilled water to remove soil and debris. The plant was then separated into its major anatomical parts: roots, bulbs, and leaves. Each plant component was dried in a ventilated oven at a temperature range of 30–60 °C for five days to ensure gradual dehydration and preservation of phytochemicals. Once fully dried, the material was coarsely ground using a hammer mill and stored at room temperature in airtight containers until required for extraction.

3.2.2 Extract preparation

Plant material was ground into a fine powder using an IKA grinder (IKA Labortechnik, Germany) at the Central University of Technology laboratory. Then powdered material was then taken to the bioassay (*in vitro* screening for drugs) lab for further processing. At bioassay (*in vitro* screening for drugs) lab powdered plant material was subjected the extraction of compounds using methanol (MeOH), dichloromethane (DCM), and water (H₂O) at a ratio of approximately 1:4 (w/v). The maceration was placed on a shaker (Labcon, Lab Design Engineering, Maraisburg, South Africa) for 72 hours. Following extraction, the mixture was filtered through Whatman No. 1 filter paper (Merck Chemicals (Pty) Ltd, Wadeville, South Africa) using a vacuum filtration system (Merck Chemicals (Pty) Ltd, Wadeville, South Africa). This process was repeated until the filtrate was clear. The organic solvents (MeOH and DCM) were removed under reduced pressure using a BÜCHI Rotovapor (Labotec (Pty) Ltd, Halfway House, South Africa), and the resulting extracts were dried at room temperature under a fume hood and stored at 4 °C. The aqueous extract was frozen at -80 °C, subsequently freeze-dried to a powder, then stored at 4 °C.

3.3 *IN VITRO* IC₅₀ DETERMINATION OF EXTRACTS AGAINST PROSTATE CANCER CELL LINE DU145

3.3.1 Sample Preparation

Tested samples were reconstituted in DMSO to a stock concentration of 100 mg/mL. Samples were sonicated if solubility issues arose and stored at 4 °C until use.

3.3.2 Cell Line Maintenance

The human prostate cancer cell line DU145 (ATCC) was used for cytotoxicity testing. Cells were cultured in 10-cm dishes containing complete medium (RPMI supplemented with 10% FBS and penicillin/streptomycin) and maintained at 37 °C in a humidified incubator with 5% CO₂.

3.3.3 Cytotoxicity Assay – MTT

Cells were seeded into 96-well plates at a density of 4,000 cells per well in 100 µL of complete medium and incubated overnight to allow attachment. The next day, treatments were prepared in complete medium at concentrations of 7.8, 15.625, 31.25, 62.5, 125, and 250 µg/mL, and applied to the cells. Melphalan (30 µM) served as a positive control. Following a 48-hour incubation period, treatments were removed, and 100 µL of MTT solution (0.5 mg/mL in complete medium) was added to each well. After a further 3-hour incubation, the medium was replaced with 100 µL of DMSO to solubilize the formazan crystals. Absorbance was measured at 540 nm using a BioTek® PowerWave™ XS spectrophotometer (Winooski, VT, USA).

3.4 RESULTS

The cytotoxic effects of nine plant-derived samples were evaluated against DU145 human prostate cancer cells using the MTT assay. IC₅₀ values (the concentration required to inhibit 50% of cell viability) were calculated using non-linear regression analysis in GraphPad Prism (version X), based on dose-response curves generated from four independent experiments (n = 4) also see appendices (B, C, D, E and F). The graphical representation of the dose-response curves is shown in Figure 1. IC₅₀ determination among the nine extracts tested, six produced measurable IC₅₀ values,

while the remaining three barks water extract, leaves MeOH extract, and roots MeOH extract failed to reach 50% inhibition of cell viability in at least three of the four replicates and were therefore considered non-determinable (ND). This indicates a lack of significant cytotoxic activity at the concentrations tested.

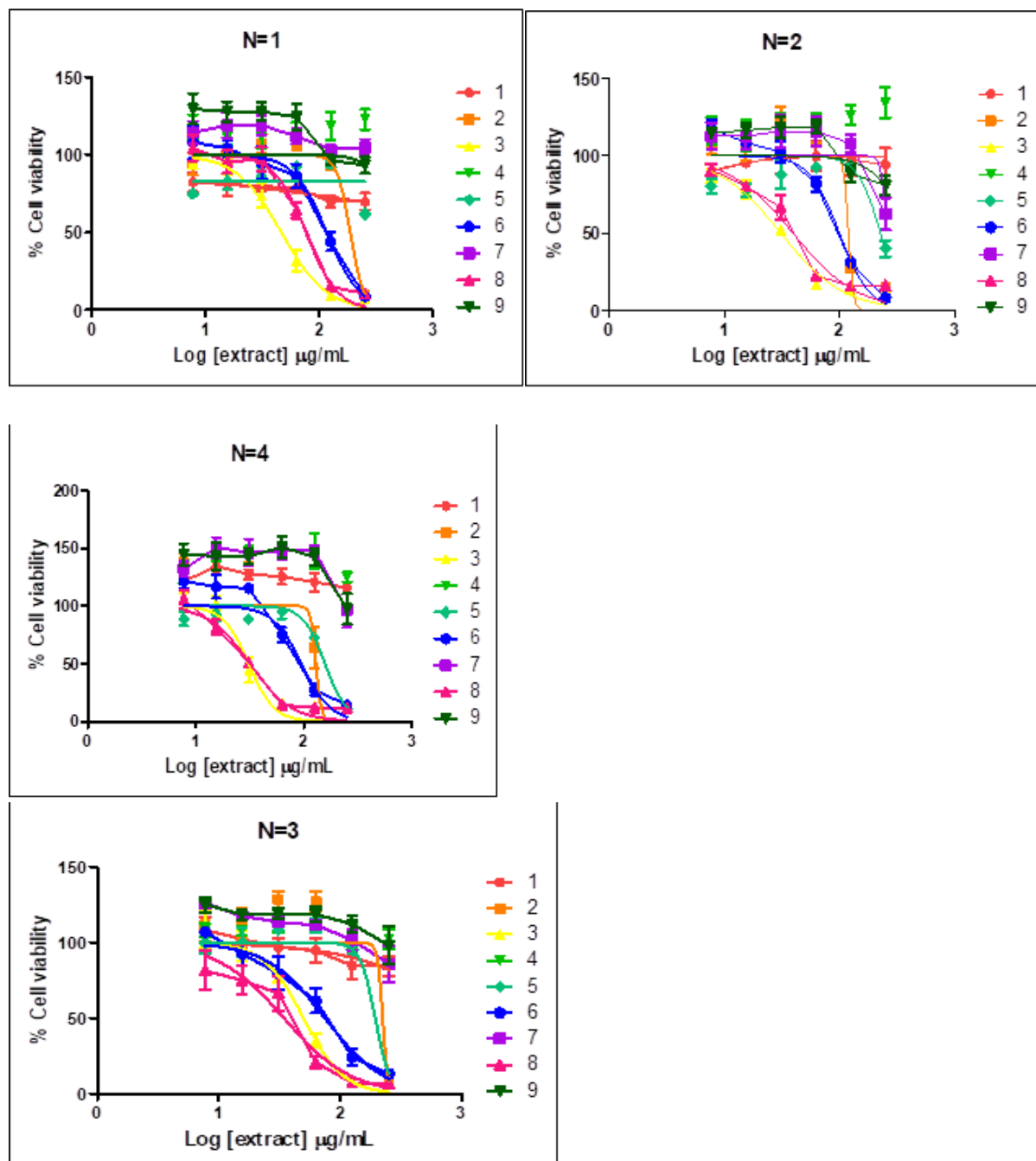


Figure 1: Determination of IC_{50} value for tested extracts against DU145 cells ($n=4$)

The average IC_{50} values and corresponding standard deviations for each extract are summarized in Table 13, along with R^2 values that reflect the goodness of fit for the

regression models used. Barks water extract, barks DCM extract, leaves water extract, leaves DCM, and roots DCM extract demonstrated IC_{50} values below 250 $\mu\text{g/mL}$, with barks DCM extract showing the highest potency (mean $IC_{50} = 39.63 \mu\text{g/mL} \pm 10.07$), followed by roots DCM extract ($45.55 \mu\text{g/mL} \pm 20.66$). These values fall within the physiologically relevant range for crude plant extracts, suggesting potential for further antiproliferative investigation. Table 3.1 shows the resulting IC_{50} values ($\mu\text{g/mL}$) and corresponding R^2 values for extracts tested against DU145 cells, indicating the accuracy of the data.

Table 13 : IC_{50} values of selected extracts and resulting R^2 values

Extracts	N=1	N=2	N=3	N=4	Average	Stdev
Barks water	8 493	ND	1 165	ND		
Barks MeoH	186,5	120,2	223,6	130	165,08	48,75
Barks DCM	46,27	31,03	50,21	31,01	39,63	10,07
Leaves MeoH	ND	ND	ND	ND		
Leaves water	ND	228,7	194,3	157,6	193,53	35,56
Leaves DCM	108,3	100,3	75,32	95,36	94,82	14,05
Roots MeoH	ND	258,2	ND	ND		
Roots DCM	76,18	39,51	34,2	31,88	45,55	20,66
Arterial MeoH	477,7	418,5	ND	ND		

ND: Not determined; % inhibition did not reach 50%.

The R^2 values for the curve-fitting analysis shown below support the reliability of the IC_{50} estimates for most extracts. Higher R^2 values (close to 1.0) indicate strong model fit. Notably, bark DCM extract, leaves DCM extract, and roots DCM extract displayed consistently high R^2 values across replicates, reinforcing confidence in their IC_{50} estimations.

Table 14 : R^2 values for dose-response curve fitting for each extract

R^2 values				
Extracts	N=1	N=2	N=3	N=4
Barks water	0.5151	0.06554	0.4557	
Barks MeOH	0.9588	0.8852	0.7953	0.4891
Barks DCM	0.9762	0.9555	0.9654	0.9153
Leaves MeOH				
Leaves water		0.4919	0.9523	0.9225
Leave DCM	0.963	0.95	0.9538	0.8824
Roots MeOH		0.5049		
Roots DCM	0.9703	0.9445	0.9006	0.9544
Arterial MeOH	-0.9684	0.1114		

The results indicate that barks DCM extract, leaves DCM extract, and roots DCM extract show promising cytotoxic potential, with consistent IC_{50} values and reliable curve fits.

3.5 DISCUSSION

This study evaluated the cytotoxic effects of crude extracts derived from *Searsia rhemanniana* on the DU145 human prostate cancer cell line. The resulting IC_{50} values demonstrated that certain extracts possess strong antiproliferative activity, suggesting that *S.rhemanniana* contains bioactive compounds with potential therapeutic relevance. Among the nine extracts tested, Bark DCM extract, leaves DCM extract, and roots DCM extract showed pronounced cytotoxic effects, with IC_{50} values below 100 $\mu\text{g/mL}$ (39.63 $\mu\text{g/mL}$, 94.82 $\mu\text{g/mL}$, and 45.55 $\mu\text{g/mL}$, respectively). These results align with the established criterion for cytotoxic significance in crude extracts. Barks DCM extract displayed low variability across replicates and high R^2 values (>0.9), indicating a strong and consistent dose-response effect. This is the first report, to our knowledge, demonstrating the cytotoxicity of *Searsia rhemanniana* against prostate cancer cells. However, related species in the *Anacardiaceae* family, such as *Searsia chirindensis* and *Searsia pyroides*, have previously demonstrated antibacterial, antioxidant, and anti-inflammatory activity (28). These bioactivities are often linked to polyphenolic and flavonoid constituents, which may similarly be responsible for the cytotoxic effects observed in this study. Bark MeOH extract exhibited moderate cytotoxicity ($IC_{50} = 165.08 \mu\text{g/mL}$), which may still be biologically relevant given the unrefined nature of the extract. Leaves water extract had an IC_{50} of 193.53 $\mu\text{g/mL}$, also pointing to some level of activity. While these values exceed the threshold of 100 $\mu\text{g/mL}$, they suggest potential for enhanced potency after fractionation or isolation of active constituents.

The observed variability in response could reflect differences in the phytochemical composition of the various extracts, extraction solvents, or plant parts used. Inconsistent results in barks water extract, leaves MeOH extract, roots MeOH extract, and arterial MeOH extract, where IC_{50} values could not be reliably calculated, are likely due to insufficient bioactive concentrations or suboptimal solubility and bioavailability in the test system. Prostate cancer remains a leading cause of cancer-related

morbidity and mortality worldwide, with especially high incidence in sub-Saharan Africa (29). Current treatment regimens such as androgen deprivation, surgery, and chemotherapeutics like docetaxel are often inaccessible, costly, and associated with resistance and relapse. This creates a pressing need for affordable, plant-based therapies (30). The cytotoxic activity of *S.rhemanniana* crude extracts observed in this study positions the species as a potential source of novel antiproliferative agents.

3.6 CONCLUSION

The current study demonstrates that crude extracts of *Searsia rhemanniana* possess notable cytotoxic activity against DU145 human prostate cancer cells, particularly bark DCM extract, leaves DCM extract, and roots DCM extract, which exhibited IC₅₀ values below the 100 µg/mL threshold considered physiologically relevant. These findings suggest that *S.rhemanniana* contains bioactive compounds with potential anticancer properties. Given the urgent need for affordable cancer therapeutics in resource limited settings, this plant may offer a promising foundation for novel drug discovery.

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CHAPTER 4: *IN VITRO* GENOTOXICITY SCREENING OF *SEARSIA RHEMANNIANA* EXTRACTS AGAINST VERO CELLS

4.1 INTRODUCTION

The global reliance on medicinal plants for health care continues to be significant (1), with estimates indicating that around 70% to 80% of the population worldwide depends on herbal remedies either fully or partially for their primary health needs (2). In many traditional medical systems, plants are utilized to address various physiological and pathological conditions due to their diverse phytochemical profiles and perceived safety (3). However, the belief that nature equates to safety is increasingly questioned by toxicological studies, which demonstrate that some plant-derived extracts may present mutagenic or genotoxic risks (4). Consequently, rigorous evaluation of genotoxic potential is now a crucial aspect of phytopharmacological research and safety assessment (5). Genotoxicity is defined as the ability of chemical or biological agents to damage cellular genetic material, including DNA strand breaks, chromosomal aberrations, gene mutations, and micronucleus formation (6). If unrepaired, such damage can result in mutagenesis, carcinogenesis, or hereditary defects (7). Medicinal plant extracts may contain a variety of secondary metabolites, such as alkaloids, flavonoids, phenolics, diterpenes, or pyrrolizidine alkaloids (8), some of which are inherently genotoxic or may become genotoxic following metabolic activation (9). For instance, pyrrolizidine alkaloids found in certain herbal medicines mainly the *Boraginaceae*, *Compositae* and *Leguminosae* families are known to induce DNA adducts and chromosomal damage (10).

In recent decades, several studies have assessed plant extracts for genotoxic effects using a variety of *in vitro* and *in vivo* tests (11). A quantitative systematic review found that 28,4% of 458 studies with medicinal plant extracts reported a positive genotoxic outcome (12). The most frequently used tests were those recommended by the regulatory agencies, including bacterial mutation assays, micronucleus or chromosomal aberrations in mammalian cells (13), comet (gel electrophoresis of single cells) and *in vivo* micronucleus or bone marrow tests (14). These observations highlight that genotoxicity is not uncommon in herbal preparations (15) and highlight

the importance of full testing prior to use in medicinal products or in the nutraceutical sector (16). Regional studies further demonstrate the prevalence of potentially genotoxic herbal materials (17). In South Africa, a study of 51 herbal medicinal products used in traditional practices evaluated dichloromethane and 90% of the methanol extract against Ames and other parameters and showed a variable but significant response (18). More recently, aqueous extracts of *Tulbaghia violacea* were reported to induce significant chromosomal aberrations and micronuclei in the test on the root tip of *Allium cepa*, with more pronounced effects in extracts than in leaves or roots (19). The families *Fabaceae*, *Asteraceae*, *Euphorbiaceae*, *Rosaceae*, *Lamiaceae* and *Apocynaceae* were identified as common genotoxic positive families (20). This underlines the need for genotoxicity testing of herbal medicinal products from both a regulatory (21) and a pharmacological point of view, as genotoxicity risk cannot be presumed to be absent simply because a plant belongs to a traditionally used or safe group of medicinal products (22). These frameworks aim to ensure that products distributed for human use do not present an unacceptable risk of DNA damage or mutagenicity (23). However, several key issues remain, genotoxicity, cytotoxicity, pharmacological efficacy and mechanistic studies, are still uncertain in many cases (24). Although medicinal plants offer valuable therapeutic potential, their safety cannot be taken for granted. This research addresses micronucleus endpoints in *S.rhemanniana* extracts, addresses this need and contributes to the pharmacological safety profile of the candidate medicinal products.

4.2 MATERIALS AND METHODS

4.2.1 Plant material

The plant material was authenticated as *S.rhemanniana* by botanists from the botanical garden in Pietermaritzburg, South Africa. Following verification, the purchased plant material was thoroughly cleaned with distilled water to remove soil and debris. The plant was then separated into its major anatomical parts: roots, bulbs, and leaves. Each plant component was dried in a ventilated oven at a temperature range of 30–60 °C for five days to ensure gradual dehydration and preservation of phytochemicals. Once fully dried, the material was coarsely ground using a hammer mill and stored at room temperature in airtight containers until required for extraction.

4.2.2 Extract preparation

Plant material was ground into a fine powder using an IKA grinder (IKA Labortechnik, Germany). Extraction was performed using MeOH, DCM, and water at a ratio of approximately 1:4 (w/v). The maceration was placed on a shaker (Labcon, Lab Design Engineering, Maraisburg, South Africa) for 72 hours. Following extraction, the mixture was filtered through Whatman No. 1 filter paper (Merck Chemicals (Pty) Ltd, Wadeville, South Africa) using a vacuum filtration system (Merck Chemicals (Pty) Ltd, Wadeville, South Africa). This process was repeated until the filtrate was clear. The organic solvents (MeOH and DCM) were removed under reduced pressure using a BÜCHI Rotovapor (Labotec (Pty) Ltd, Halfway House, South Africa), and the resulting extracts were dried at room temperature under a fume hood and stored at 4 °C. The aqueous extract was frozen at -80 °C and subsequently freeze-dried to a powder, then stored at 4 °C.

4.3 *IN VITRO* GENOTOXICITY DETERMINATION OF *EXTRACTS* AGAINST VERO CELLS

Dulbecco's Modified Eagle Medium (DMEM), FBS and PBS with and without Ca^{2+} and Mg^{2+} were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Bis-benzamide H 33342 trihydrochloride (Hoechst) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

4.3.1 Sample preparation

Test samples were reconstituted in DMSO to a stock concentration of 100 mg/mL and sonicated if solubility was a problem. Samples were stored at 4 °C until required.

4.3.2 Cell line maintenance

The African green monkey kidney cell line, Vero cells, were purchased from Cellonex (South Africa). Cells were maintained in 10 cm culture dishes in complete medium (DMEM, 10% FBS) and incubated at 37 °C in a humidified atmosphere with 5% CO_2 .

4.3.3 Genotoxic evaluation using Vero cells – treatment protocol

Cells were seeded in 96 well plates at 3000 cells/well (100 μ L aliquots) and left overnight to attach. Treatments were prepared in complete medium and added to cells in 100 μ L aliquots. Griseofulvin (3.125 – 50 μ M) was used as a positive control. Cells were incubated for 48 hours at 37 °C and 5% CO₂. Spent medium was aspirated and cells were fixed using freshly prepared 4% formaldehyde for 15 minutes. Fixative was aspirated and cells were washed with 100 μ L PBS with Ca²⁺ and Mg²⁺. Hoechst 33342 (5 μ g/mL) prepared in PBS with Ca²⁺ and Mg²⁺ was added to each well (100 μ L) and incubated for 30 minutes. Fluorescence micrographs were captured at 9 sites per well using an ImageXpress Micro XLS Widefield Microscope (Molecular Devices) with a 10x Plan Fluor objective and DAPI filter cube. Acquired images were analysed using the MetaXpress software and Micronucleus Cell Scoring Application Module, where the total number of cells and % micro-nucleated cells per well were determined.

4.4 RESULTS

Genotoxicity was determined using the Hoechst 33342 nuclei dye. Cytotoxicity, as determined by the total number of cells (nuclei) is shown in Figure 4.1, and percentage of micro-nucleated cells is shown in Figure 4.2. Also refer to appendix G.

Table 15: Extract numbers, plant parts and solvents of S.rhemanniana

Extract numbers	Plant parts and Solvents
1	Barks water
2	Barks Methanol (MeOH)
3	Barks Dichloromethane (DCM)
4	Leaves Methanol
5	Leaves water
6	Leaves Dichloromethane
7	Roots Methanol
8	Roots Dichloromethane
9	Arterial Methanol

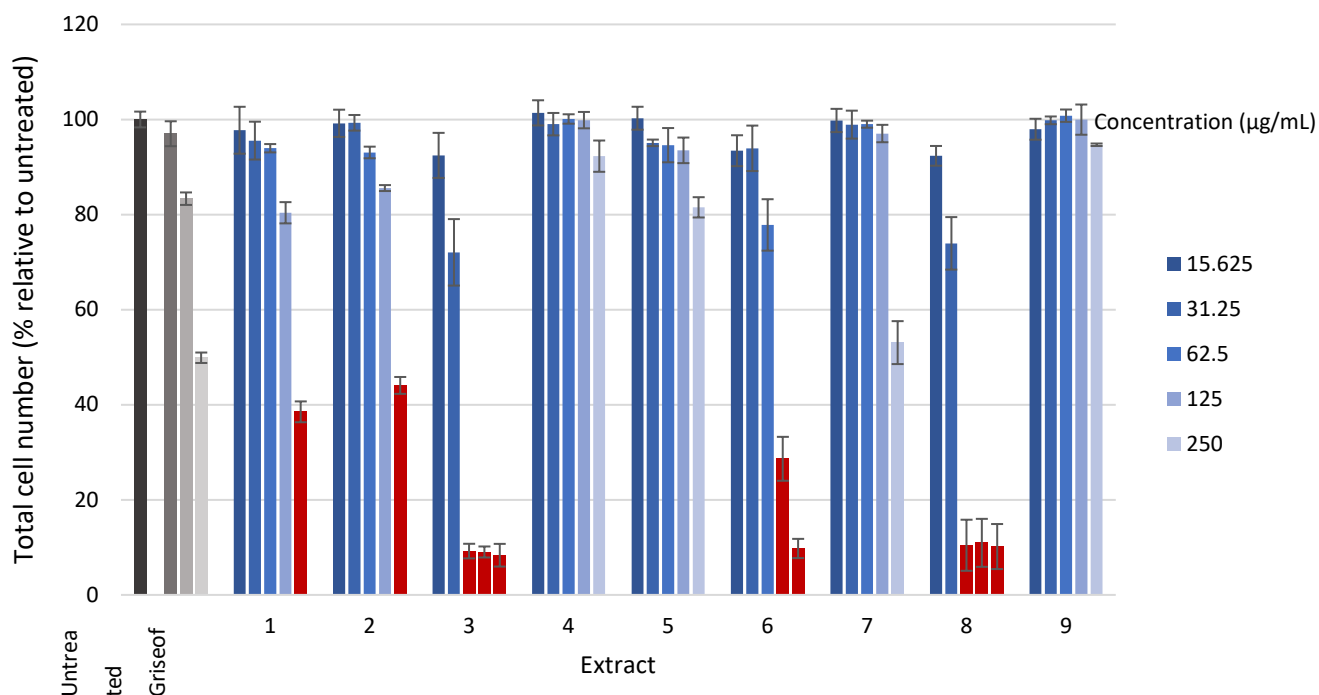


Figure 2: Total cell number after 48 hours of treatment

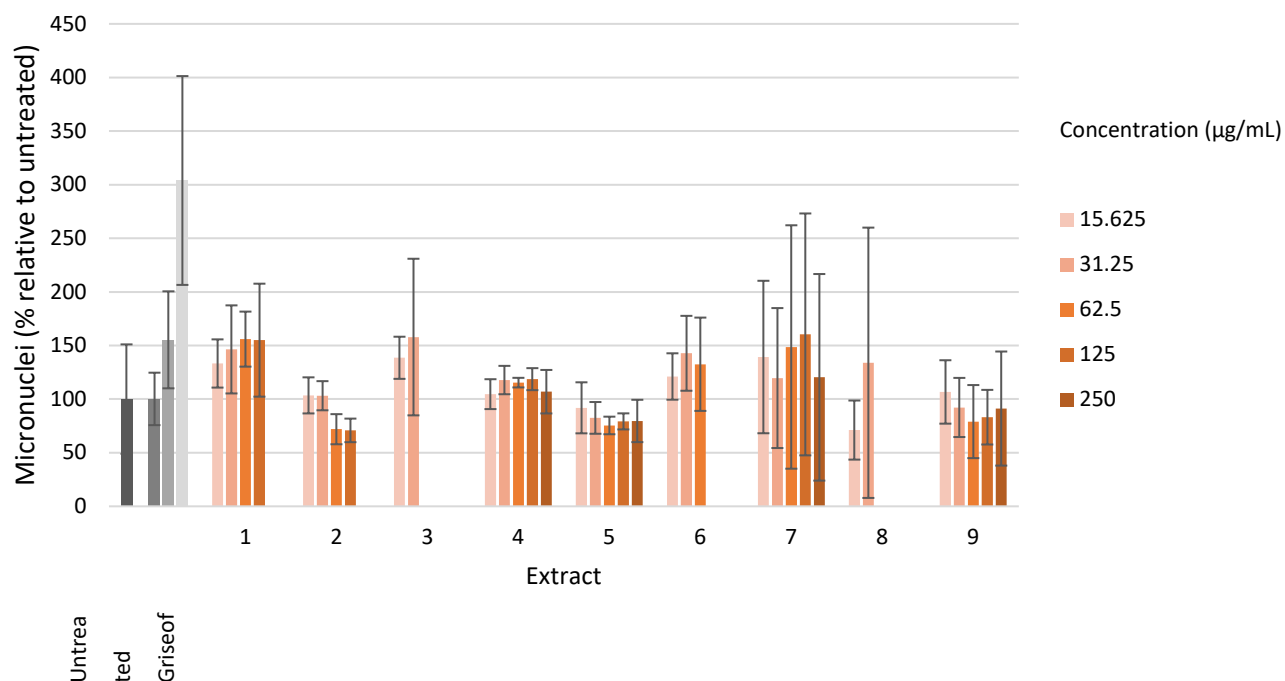


Figure 3: Percentage micro-nucleated cells after 48 hours of treatment

4.5 FINDINGS

In this study, cytotoxic effects were evaluated through a cell viability assay, and the results are summarized in Figure 2. A reduction in cell viability exceeding 50% relative to untreated control was considered indicative of significant cytotoxicity. Marked cytotoxicity was observed for several of the tested extracts at the defined treatment concentrations. Specifically, bark water and bark MeOH extracts exhibited pronounced cytotoxic effects at 250 µg/mL, while bark DCM and roots DCM extracts demonstrated a dose-dependent decrease in cell viability across the concentration range of 62.5 to 250 µg/mL. Similarly, leaves DCM displayed substantial cytotoxicity between 125 and 250 µg/mL, suggesting that the threshold for toxicity in this sample occurs within this concentration window. These findings imply that certain extracts or compounds possess potent cell-inhibitory properties, likely mediated by the disruption of essential biochemical pathways such as mitochondrial activity or membrane stability. The observation of more than 50% cell loss reflects not only decreased proliferation but also possible induction of apoptosis or necrosis at higher exposure levels. In contrast, extracts exhibiting minimal cytotoxicity at comparable concentrations may contain components with limited interaction with vital cellular targets, or they may act through mechanisms that do not immediately compromise cell viability. Such differential effects highlight the importance of assessing a concentration range to distinguish between cytostatic and cytotoxic responses. It is important to note that due to excessive cell loss, micro-nucleus assay results could not be reliably interpreted for bark water, bark MeOH, bark DCM, leaves DCM, and roots DCM extracts at their respective cytotoxic concentrations. The significant reduction in viable cells and the resulting morphological changes hindered accurate scoring of micronuclei formation. Consequently, these data points were excluded from Figure 3 to ensure the integrity of the genotoxicity analysis.

The percentage of micro-nucleated cells relative to the total number of cells serves as a quantitative indicator of genotoxic stress. As shown in Figure 3, several extracts exhibited statistically significant increases in the frequency of micro-nucleated cells compared with the untreated control group. These findings reflect the genotoxic potential of specific test compounds at concentration ranges. Notably, bark water extract induced a significant increase in micronucleus formation across all tested

concentrations (15.625–250 $\mu\text{g/mL}$), indicating that it may contain constituents capable of interacting directly with DNA or interfering with the mitotic spindle apparatus. Similarly, bark DCM extract produced a significant genotoxic response at 15.625 $\mu\text{g/mL}$, despite exhibiting cytotoxicity at higher concentrations. This suggests that even low doses of certain compounds within this sample may be sufficient to cause DNA damage, potentially through oxidative stress or the formation of reactive intermediates. Leaves MeOH extract also demonstrated a statistically significant increase in the proportion of micro-nucleated cells within the concentration range of 62.5–250 $\mu\text{g/mL}$, further supporting its genotoxic potential. The absence of a dose-dependent trend in some extracts may reflect differences in compound stability, bioavailability, or cellular defence responses such as DNA repair activation. Nevertheless, the occurrence of micronucleus formation at multiple concentrations supports the conclusion that certain extracts contain genotoxic agents capable of inducing chromosomal breakage or mis-segregation events.

4.6 CONCLUSION

The present study demonstrated that several of the tested extracts exerted notable cytotoxic and genotoxic effects in human cell models. Cytotoxicity assays revealed that bark water, bark MeOH, bark DCM, leaves DCM, and roots DCM significantly reduced cell viability at higher concentrations, indicating potent inhibition of cellular proliferation and possible induction of apoptotic or necrotic pathways. In contrast, samples showing limited cytotoxicity may contain bioactive constituents with mild or selective cellular effects, underscoring the concentration-dependent nature of their toxicity profiles. The micronucleus assay further confirmed that some extracts, particularly bark water, bark DCM, and leaves MeOH, possess genotoxic potential, as evidenced by a significant increase in the frequency of micro-nucleated cells. This finding suggests that these extracts or their active components may interact with genetic material or interfere with chromosomal segregation mechanisms, leading to DNA damage. The occurrence of genotoxicity at low concentrations especially in samples that were also cytotoxic at higher doses highlights the complexity of their biological activity and the importance of concurrent evaluation of both endpoints. Overall, these results indicate that while certain extracts contain compounds with strong cytotoxic and genotoxic properties, others may have safer biological profiles

suitable for further investigation. Further studies are warranted to isolate and characterize the active constituents responsible for these effects, elucidate their mechanisms of action, and determine their potential therapeutic or toxicological relevance *in vivo*.

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CHAPTER 5: ANTICANCER PROFILES OF *SEARSIA RHEMANNIANA* EXTRACTS AND TISSUE VARIABILITY IN HELA CERVICAL CELLS

5.1 INTRODUCTION

Cervical cancer ranks as the fourth-most prevalent malignancy among women globally. Despite advancements in screening technologies and human papillomavirus (HPV) vaccination in high-income countries, the disease remains a major public health challenge in resource-limited regions, where over 85% of cervical cancer-related deaths occur (1). In fact, cervical cancer continues to be the leading cause of cancer mortality among women in developing nations. Recent technological innovations, however, have improved the speed, affordability, and sensitivity of cervical cancer screening, offering promising strategies to reduce its incidence in low- and middle-income settings.

Nearly all cervical cancer cases are etiologically linked to infection by HPV (2). HPV strains are classified as either low-risk or high-risk types based on their oncogenic potential. Low-risk strains often remain asymptomatic or lead to benign lesions such as anogenital warts, while high-risk strains possess oncogenic capacity and are responsible for more than 99% of precancerous lesions and cervical carcinomas (3). To date, over 200 HPV genotypes have been identified, with approximately 40 infecting the anogenital tract, and about 15–18 recognized as high-risk types (4). Notably, HPV types 16 and 18 account for roughly 70% of cervical cancer cases, with type 16 responsible for 50% of squamous cell carcinomas and 55–60% of all cervical cancers, while type 18 contributes to about 20% of cervical adenocarcinomas (5). Other oncogenic types, including 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68, collectively cause about 25% of cases (6). Moreover, certain high-risk HPV strains are also implicated in malignancies of the anus, vulva, vagina, penis, and oropharynx (7).

Histologically, squamous cell carcinoma and adenocarcinoma constitute nearly all cervical cancer cases (8). The pathogenesis of cervical cancer typically involves sequential stages: initial HPV infection, viral persistence, progression to cervical dysplasia, and ultimately invasion (9). However, HPV infection is often transient, with

approximately 67% of infections resolving within 12 months and over 90% clearing spontaneously within two years (10). Traditionally, cervical carcinogenesis was viewed as a linear progression from infection to cervical intraepithelial neoplasia grade 1 (CIN1) (low-grade dysplasia), then CIN2 and CIN3 (high-grade lesions), eventually leading to invasive cancer (11). This implies that while CIN1 and many CIN2 lesions often represent productive HPV infections, CIN3 lesions may evolve more rapidly and possess greater malignant potential (12). Consequently, current clinical management recommends observation for CIN1, whereas CIN2 and CIN3 are treated through ablative or excisional procedures, including cryotherapy, thermo-ablation, loop electrosurgical excision (LEEP), and cold knife conization (CKC) (13).

5.2.1 HPV Oncogenesis

HPV is a small, circular, double-stranded DNA virus with nine open reading frames (14). The early (E) genes regulate DNA replication, transcription, and maintenance, while the late (L) genes encode capsid proteins that form the viral structure (15). During initial infection, E1 and E2 proteins promote viral replication within cervical epithelial cells, leading to low-grade cytological abnormalities visible as low-grade squamous intraepithelial lesions on Pap smears (16). The E6 and E7 oncoproteins play a pivotal role in malignant transformation by inactivating tumour suppressor pathways: E6 binds to and degrades p53, while E7 targets the retinoblastoma (Rb) protein, resulting in uncontrolled cell proliferation and tumour formation (17).

5.2.2 Epidemiology of Cervical Cancer

Cervical cancer remains a significant global public health concern, particularly in low- and middle-income countries (LMICs), where access to preventive healthcare and screening services is limited (18). According to the Global Cancer Observatory (GLOBOCAN), cervical cancer is the fourth-most common cancer among women worldwide, accounting for approximately 604,000 new cases and 342,000 deaths in 2020 (19). Over 85% of these cases occur in LMICs, largely due to insufficient vaccination coverage, delayed diagnosis, and limited access to screening and treatment facilities (20). The highest incidence rates of cervical cancer are observed in sub-Saharan Africa, South-East Asia, and parts of Latin America, where age-standardized incidence rates (ASIR) exceed 40 per 100,000 women (21). In contrast,

regions with established national screening programs such as North America, Western Europe, and Australia report markedly lower ASIRs often below 10 per 100,000 women (22). In sub-Saharan Africa, Eswatini, Malawi, and Zambia record the highest burden, driven by a combination of high prevalence of high-risk HPV infection and co-infection with HIV, which exacerbates the persistence of oncogenic HPV strains (23).

The etiological role of HPV is well established, with HPV types 16 and 18 responsible for approximately 70% of all cervical cancers globally (24). Persistent infection with these high-risk genotypes leads to CIN, which, if left untreated, can progress to invasive carcinoma over a period of 10–20 years (25). Socio-economic disparities, cultural barriers, and lack of awareness further contribute to the delayed detection and higher mortality rates observed in LMICs (26). Despite its preventable nature, cervical cancer remains a leading cause of cancer-related deaths among women aged 15–44 years in many African countries (27). The World Health Organization (WHO) launched a global strategy in 2020 aimed at eliminating cervical cancer as a public health problem through three key targets to be achieved by 2030: 90% HPV vaccination coverage, 70% screening coverage, and 90% treatment coverage for precancerous lesions and invasive cancers (28). Achieving these goals requires strengthening national immunization programs, implementing cost-effective HPV testing, and expanding access to early treatment services, particularly in rural and resource-limited areas.

5.2.3 Risk Factors for Cervical Cancer

Cervical cancer is primarily caused by persistent infection with high-risk types of HPV, particularly HPV-16 and HPV-18, which together account for approximately 70% of all cases worldwide (29). However, viral persistence and disease progression are influenced by several co-factors, including behavioural, immunological, and socio-economic determinants. HPV infection is the most significant etiological factor for cervical carcinogenesis (30). The virus infects epithelial cells of the cervical transformation zone, integrating its DNA into the host genome, which promotes oncogenic transformation through the expression of E6 and E7 oncoproteins (31). Although transient HPV infections are common, persistent infection with high-risk types is a critical precursor for the development of CIN and invasive carcinoma (32).

Early age at first sexual intercourse and having multiple sexual partners are well-established behavioural risk factors that increase the likelihood of HPV exposure (33). Studies show that women who initiate sexual activity before the age of 18 have a higher risk of persistent HPV infection compared to those with a later sexual debut (34). Similarly, having multiple partners or a partner with multiple sexual contacts increases cumulative HPV exposure (35).

Immunosuppression, particularly in individuals living with HIV/Acquired immunodeficiency syndrome (AIDS), is strongly associated with increased HPV persistence and accelerated progression to cervical cancer (36). HIV-positive women are up to five times more likely to develop cervical precancerous lesions or invasive cancer than their HIV-negative counterparts (37). Reduced immune surveillance allows oncogenic HPV to evade clearance mechanisms, promoting carcinogenesis (38). Cigarette smoking has been implicated as an independent cofactor in cervical carcinogenesis. Carcinogens present in tobacco smoke can accumulate in cervical mucus, leading to DNA damage and impaired local immune response (39). Meta-analyses suggest that smokers have approximately twofold higher risk of developing cervical cancer compared to non-smokers (40).

Long-term use of oral contraceptives (≥ 5 years) has been shown to modestly increase the risk of cervical cancer, possibly through hormonal modulation of the cervical epithelium or enhancement of HPV gene expression (41). However, the risk declines after discontinuation of oral contraceptive use (42). High parity (≥ 3 full-term pregnancies) has been associated with increased cervical cancer risk, potentially due to hormonal changes and trauma to the cervical epithelium during childbirth, which facilitate viral entry and persistence (43). Epidemiological evidence supports a dose-response relationship between the number of pregnancies and risk of cervical neoplasia (44). Low socio-economic status is a significant determinant, as it is often associated with reduced access to HPV vaccination, limited screening participation, and higher prevalence of risk behaviours (45). Additionally, malnutrition, poor genital hygiene, and co-infection with other sexually transmitted pathogens (such as *Chlamydia trachomatis* and *Herpes simplex virus type 2*) may further contribute to cervical carcinogenesis (46). Collectively, these risk factors interact with HPV infection to modulate disease progression, emphasizing the importance of comprehensive

preventive strategies combining vaccination, behavioural education, and regular screening.

Two curative options exist: surgery and primary radiotherapy with or without concurrent chemotherapy (47). Current guidelines advise against combining modalities (multimodal therapy) in the absence of clear indication, due to increased morbidity, and highlight the importance of avoiding both overtreatment and undertreatment. One of the most important prognostic factors in cervical cancer is lymph-node status (48). Tumour stage, prognosis and subsequent therapeutic strategy rely heavily on intraoperative assessment of lymph nodes because preoperative imaging CT, Magnetic Resonance Imaging (MRI) or Positron Emission Tomography (PET)/CT has been shown to be inferior in detecting lymph-node metastases (49). As a result, creating context-specific screening algorithms continues to be a critical research and policy goal. The need to investigate alternative and complementary therapeutic approaches, such as those derived from natural products and traditional medicines, is growing considering the shortcomings of current treatment modalities and the rising incidence of prostate cancer, particularly in low- and middle-income areas (50). The *S.rhemanniana* plant was chosen for this study, because there are not many scientific studies on its possible medical uses. According to research, terpenoids and flavonoids, two types of phytochemicals found in *Searsia* species, including *S.rhemanniana*, have strong anti-oxidant and enzyme-inhibiting properties (51). However, further research is needed to fully understand the medicinal properties of this herb.

5.3 MATERIALS AND METHODS

5.3.1 Plant material

The plant material was authenticated as *S.rhemanniana* by botanists from the botanical garden in KwaZulu-Natal, South Africa. Following verification, the plant material was thoroughly cleaned with distilled water to remove soil and debris. The plant was then separated into its major anatomical parts: roots, bulbs, and leaves. Each plant component was dried in a ventilated oven at a temperature range of 30–60 °C for five days to ensure gradual dehydration and preservation of phytochemicals. Once fully dried, the material was coarsely

ground using a hammer mill and stored at room temperature in airtight containers until required for extraction.

5.3.2 Extract preparation

Plant material was ground into a fine powder using an IKA grinder (IKA Labortechnik, Germany) at the Central University of Technology laboratory. Then powdered material was then taken to the bioassay (*in vitro* screening for drugs) lab for further processing. At bioassay (*in vitro* screening for drugs) lab powdered plant material was subjected to the extraction of compounds using MeOH, DCM, and H₂O at a ratio of approximately 1:4 (w/v). The maceration was placed on a shaker (Labcon, Lab Design Engineering, Maraisburg, South Africa) for 72 hours. Following extraction, the mixture was filtered through Whatman No. 1 filter paper (Merck Chemicals (Pty) Ltd, Wadeville, South Africa) using a vacuum filtration system (Merck Chemicals (Pty) Ltd, Wadeville, South Africa). This process was repeated until the filtrate was clear. The organic solvents (MeOH and DCM) were removed under reduced pressure using a BÜCHI Rotovapor (Labotec (Pty) Ltd, Halfway House, South Africa), and the resulting extracts were dried at room temperature under a fume hood and stored at 4 °C. The aqueous extract was frozen at -80 °C and subsequently freeze-dried to a powder, then stored at 4 °C.

5.4 *IN VITRO* IC₅₀ DETERMINATION OF EXTRACTS AGAINST HELA CELLS TISSUE VARIABILITY

5.4.1 Sample preparation

All cell lines were subjected to varying doses of the test samples over time. The cytotoxic assessment of the extracts was performed using an *in vitro* PrestoBlue and fluorescent assays. Data disseminated from the experiment were expressed as percentage cell viability, representing the number of viable/active cells following test sample treatment.

The following materials were used in the cytotoxicity evaluation of Aqueous, DCM and MeOH extracts on HeLa cell line (Human Cervical Carcinoma) and African green monkey kidney (VERO) cells – American Type Culture

Collection, USA); DMEM – (Sigma Aldrich, South Africa), FBS, Phosphate-buffered saline (PBS), Pen Strep, and 0.25% Trypsin Ethylenediaminetetraacetic acid (EDTA) 1X – (Gibco – Thermo Fisher Scientific South Africa); dimethyl sulfoxide (DMSO), Methanol – (Sigma Aldrich, South Africa), – Hoechst 33324 (Invitrogen, Eugene, Oregon, USA), – PrestoBlue (Invitrogen, Eugene, Oregon, USA).

5.4.2 Cell culture and maintenance

All cell lines were maintained at 37 °C in a humidified incubator with 5% CO₂ in 75 cm² tissue culture flasks filled with DMEM media supplemented with 10% heat inactivated FBS and 1% antibiotics [100 U/mL penicillin, 100 µg/mL streptomycin. Cell harvesting was carried at 70–80% cell confluency using trypsin- EDTA (0.25% trypsin plus 0.01% EDTA) and subsequently subculture in complement culture media.

5.4.3 PrestoBlue assay and fluorescent assay

Cell lines were cultured in full DMEM supplemented with 10% heat-inactivated FBS and 1% antibiotic/antifungal solution. Cells (5X10³/well) were cultured at 37 °C in an atmosphere of 5% CO₂ and 95% air. Before evaluation of the test samples, cells were incubated for 24 h for adaptation to allow for attachment. To test the cytotoxicity of the extracts, cells were treated with the test samples at concentrations ranging from 1.5625 µg/mL to 200 µg/mL for each of the test samples in a final 100 µL culture media for 24, 48, and 72 h. Phenylarsine Oxide (PAO), a versatile arsenoxide that has been shown to have cytotoxicity activity against numerous cell lines, was introduced as the positive control at similar dilution concentration as test samples, whereas the negative control was the untreated cells. After each experimental time-point, media from each well were aspirated and 90 µL of media were added to each well followed by 10 µL of the PrestoBlue reagent and incubated for 2 h (for Hela cells) and VERO cells at 37 °C in an atmosphere of 5% CO₂. Fluorescence was read at Em 535 nm and Ex 612 nm using TECAN fluorescence, absorbance, and Luminescence microplate reader.

Following the PrestoBlue assay, plates were treated for imaging. PrestoBlue solution was aspirated, and each plate was fixed in 4% w/v paraformaldehyde in PBS for 10 min followed by treatment of cells for 10 min with 0.1 Triton X-100 in PBS. Cell nuclei

were counterstained with 1:1000 (10 µmg/ml) Hoechst 33324 in PBS. Culture plates were imaged using a Cytation3 imaging reader.

5.4.4 The selectivity index quantification

The selectivity index (SI) evaluates the toxicity of substances against normal cells and predicts their therapeutic potential. It compares the investigated substance cytotoxicity against normal cells versus its toxicity against cancer cells. The following formula is used in calculating the SI:

$$\text{SI} = \text{IC}_{50} \text{ for normal cell line} / \text{IC}_{50} \text{ for the relevant malignant cell line}$$

A good SI value exceeds 1.0, indicating that the tested extract is more effective against tumour cells than it is toxic to normal cells. An SI value of 10 or higher suggests that the extract is a good candidate for more research. An SI value less than one indicates that the extract may be toxic and should not be utilized as a medication. We computed SI scores for normal cell line (Vero cell) versus the malignant cell line (HeLa).

5.5 RESULTS AND DISCUSSION

24-hour anti-cancer activity of plant extracts assessment against HeLa cell

Leaf, bark, and root extracts prepared using aqueous, DCM, and MeOH solvents were evaluated for their cytotoxic activity against HeLa cells following 24 h exposure (Figure 5.1). Among all extracts tested, the DCM fractions exhibited the strongest dose-dependent inhibition of cell viability, whereas the aqueous and methanolic fractions showed minimal or inconsistent activity. The dose-response curves revealed that the bark and root DCM extracts were the most potent, yielding IC_{50} values of 22.7 µg/mL and 24.78 µg/mL, respectively, with high fit quality ($R^2 = 0.95$ and 0.99). The leaf DCM extract displayed moderate activity ($\text{IC}_{50} = 148.6$ µg/mL; $R^2 = 0.91$). In contrast, the aqueous and MeOH extracts of all plant parts produced broad or undefined IC_{50} ranges ($R^2 < 0.65$), reflecting poor curve fit and negligible cytotoxicity.

Two-way ANOVA confirmed that both extract type and concentration significantly affected cell viability ($P < 0.001$), particularly at 100, 25, and 12.5 µg/mL, as shown in the grouped bar plots (Figure 5.2). The pattern of reduced viability observed

exclusively in DCM-treated cells supports the presence of bioactive, lipophilic constituents responsible for cytotoxic effects. Collectively, these findings indicate that the DCM extracts, especially those from bark and root tissues, possess strong anti-cancer potential, whereas polar solvent extracts were largely inactive. The results suggest that non-polar secondary metabolites, possibly terpenoids or alkaloid derivatives, are likely mediating the observed activity, underscoring the relevance of DCM extraction for isolating potent anti-cancer compounds from this species.

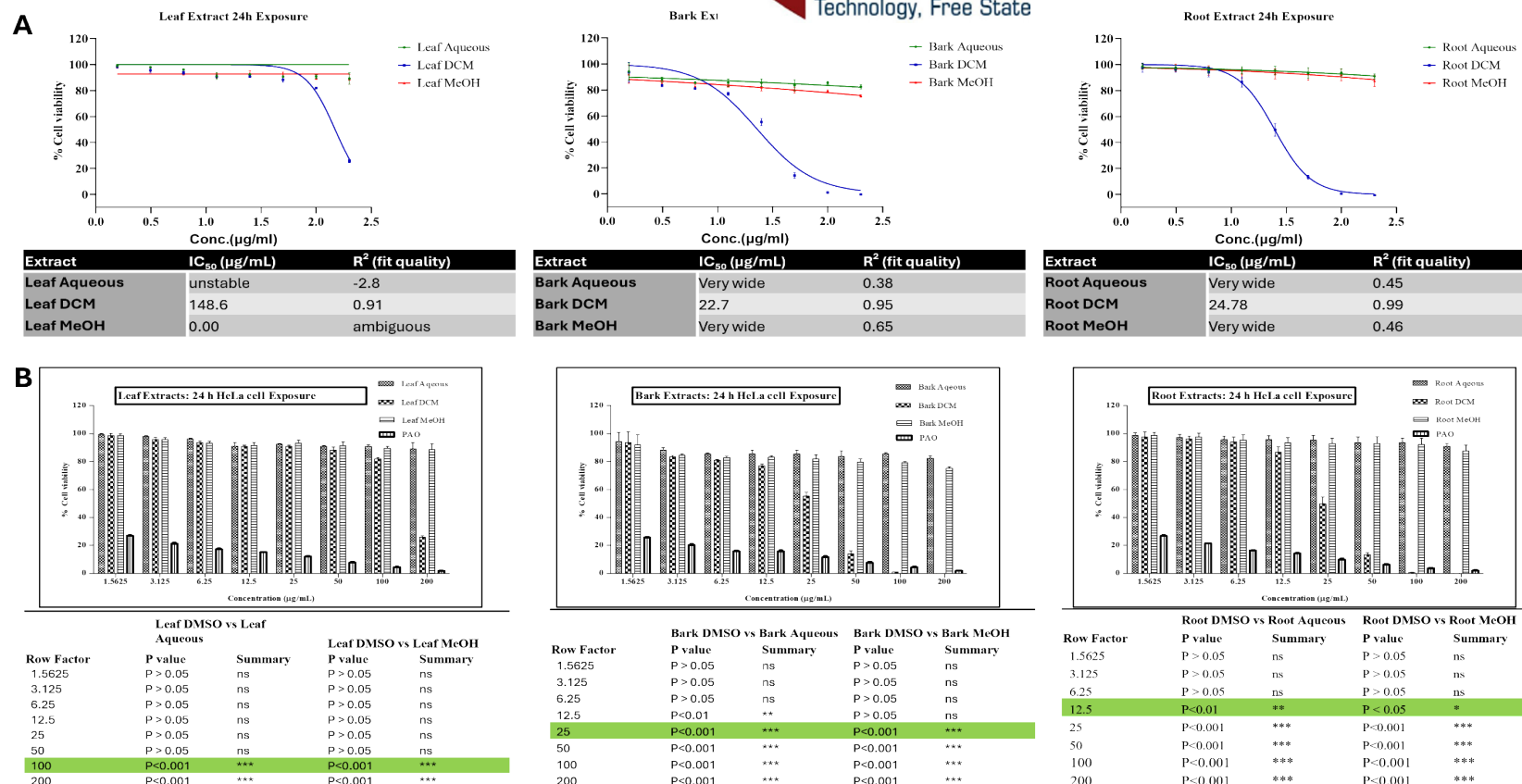


Figure 4: Anti-cancer activity of leaf, bark and root extracts from three solvent systems against HeLa cells (24 hours exposure)

Dose-response curves (top panels) show percentage cell viability following treatment with aqueous (green), dichloromethane [DCM] (blue), and methanolic (red) extracts derived from the leaf (A), bark (B), and root (C) tissues. Corresponding IC₅₀ values (µg/mL) and curve fit quality (R²) are summarised in the tables beneath each graph. Extracts displaying “unstable” or “very wide” IC₅₀ values indicate poor model fitting or lack of dose-dependent cytotoxicity. Grouped bar charts (middle panels) illustrate mean cell viability (± SD, n = 3) at different extract concentrations, while lower panels summarize two-way ANOVA results comparing solvent type and concentration effects. Statistical significance levels are indicated as *P < 0.05, **P < 0.01, ***P < 0.001. DCM extracts consistently exhibited the most pronounced cytotoxic effects, particularly in bark and root samples, whereas aqueous and methanolic extracts showed minimal inhibition.

48-hour anti-cancer activity of plant extracts assessment against HeLa cell

The cytotoxic activity of *S.rhemanniana* leaf, bark, and root extracts obtained using aqueous, DCM, and MeOH solvents were evaluated against HeLa cells following a 48-hour exposure (Figure 5.2). Distinct differences in inhibitory potential were observed across solvent types and plant parts, indicating a strong influence of extract polarity and tissue-specific metabolite distribution. Among the leaf extracts, only the DCM fraction exhibited measurable cytotoxicity, with an IC_{50} value of 134.9 $\mu\text{g/mL}$ and a high curve fit ($R^2 = 0.99$). Both the aqueous and MeOH fractions produced shallow response curves with “very wide” IC_{50} ranges and low correlation coefficients ($R^2 = 0.64$ and 0.41 , respectively), indicating limited dose-dependent inhibition. The pronounced activity of the DCM fraction suggests that non-polar or moderately polar constituents are primarily responsible for the observed cytotoxicity, consistent with reports that lipophilic metabolites such as terpenoids and alkaloids often mediate anti-cancer effects in plant extracts.

The bark extracts displayed stronger cytotoxic responses than the leaves. The DCM fraction demonstrated potent activity with an IC_{50} of 29.15 $\mu\text{g/mL}$ ($R^2 = 0.98$), while the aqueous extract showed near-complete inhibition at all tested concentrations (apparent $IC_{50} = 0.00 \mu\text{g/mL}$). The latter result likely reflects the presence of highly active hydrophilic constituents or an early plateau effect at low doses. The MeOH extract showed moderate inhibition but low dose responsiveness (very wide IC_{50} , $R^2 = 0.84$). The DCM bark extract's high activity is in line with previous findings linking non-polar bark metabolites to apoptosis induction and suppression of cancer cell proliferation.

A similar solvent-dependent pattern was observed for the root extracts, where the DCM fraction recorded the most pronounced effect with an IC_{50} of 26.26 $\mu\text{g/mL}$ and an excellent fit ($R^2 = 0.99$). The aqueous and MeOH fractions displayed minimal cytotoxicity (very wide IC_{50} values), despite moderate fit quality ($R^2 = 0.74$ and 0.92 , respectively). The strong inhibitory response of the root DCM extract further supports the presence of lipophilic cytotoxic compounds, potentially including triterpenes, sterols, or phenolic derivatives previously reported to induce cell cycle arrest or apoptosis in HeLa and other carcinoma cell lines.

Statistical analysis using two-way ANOVA revealed that both solvent type and plant part significantly influenced the observed cytotoxicity ($p < 0.001$). Post-hoc tests confirmed that DCM extracts, particularly from the bark and root, were significantly more active than aqueous or MeOH counterparts (Figure 2B). This pattern highlights DCM as the most efficient solvent for recovering cytotoxic metabolites from the plant species and identifies the bark and root as the richest sources of these bioactive compounds.

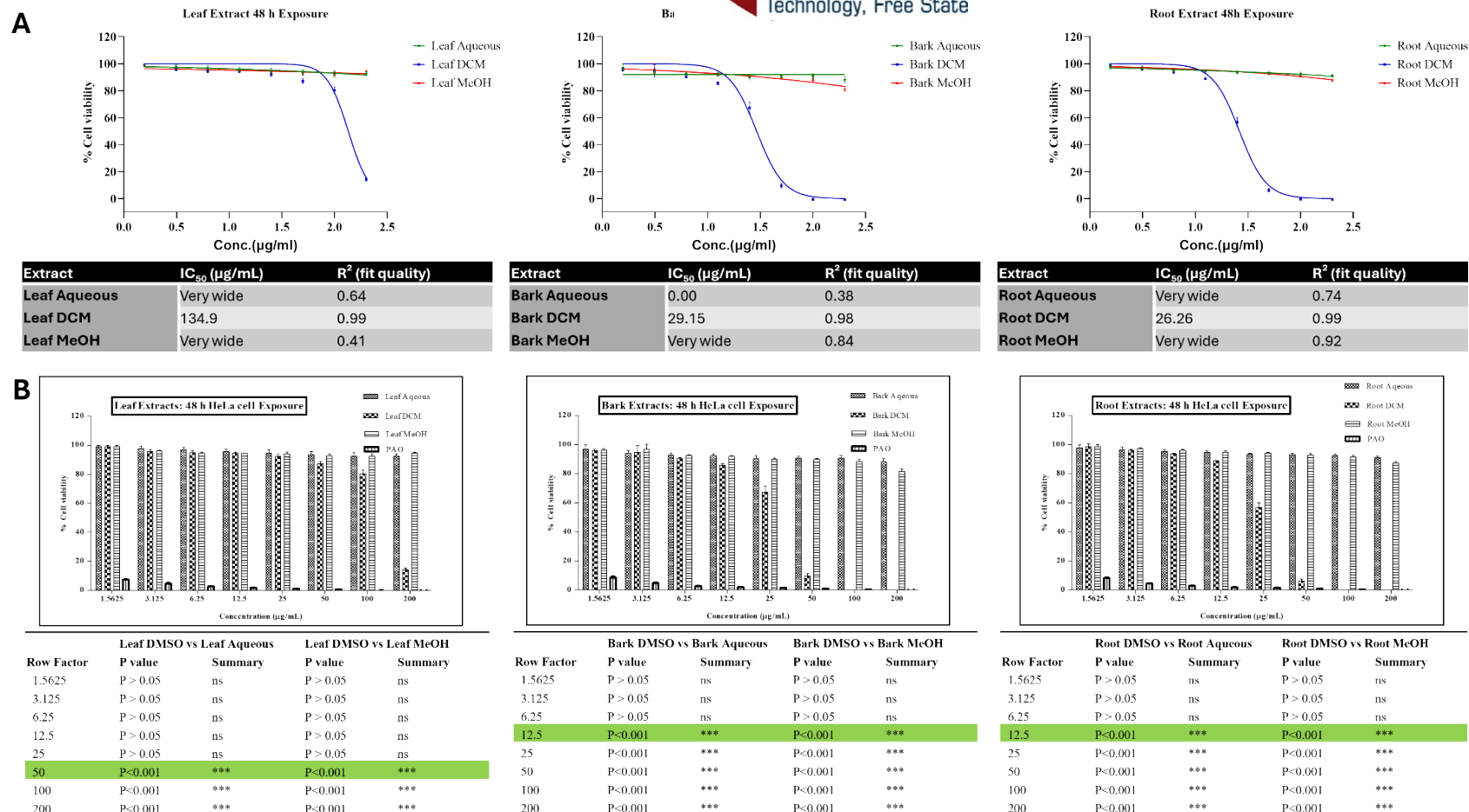


Figure 5: Dose-response curves, IC₅₀ values and statistical analysis of leaf, bark and root extracts against HeLa cells after 48 hours of treatment

Extracts were prepared using aqueous (green), dichloromethane (DCM; blue), and methanol (MeOH; red) solvents. Data represent mean $\pm$ SD of three replicates. IC₅₀ values and curve fit (R²) are shown for each extract. Group comparisons and two-way ANOVA indicate significant effects of solvent type and plant part ($p < 0.00$)

78-hour anti-cancer activity of plant extracts assessment against HeLa cell

The cytotoxic activity of the leaf, bark, and root extracts prepared using aqueous, DCM, and MeOH solvents were evaluated against HeLa cells after a 72-hour exposure period (Figure 5.3). The results demonstrated marked differences in anti-cancer potency depending on the plant part and extraction solvent, as reflected by the IC_{50} values and the corresponding dose response profiles.

Among the three solvents tested, DCM extracts consistently exhibited the highest cytotoxicity across all plant parts, with IC_{50} values of 130.1 $\mu\text{g/mL}$ (leaf), 43.4 $\mu\text{g/mL}$ (bark), and 36.36 $\mu\text{g/mL}$ (root). The dose-response curves for these extracts showed steep sigmoidal patterns with high coefficients of determination ($R^2 = 0.96\text{--}0.99$), indicating reliable curve fitting and potent dose-dependent inhibition of HeLa cell viability. In contrast, both aqueous and methanolic extracts exhibited ambiguous or very wide IC_{50} fits, suggesting minimal or inconsistent cytotoxic responses within the tested concentration range.

Comparative analysis across plant parts revealed that the root DCM extract was the most effective ($IC_{50} = 36.36 \mu\text{g/mL}$), followed closely by the bark DCM extract (43.4 $\mu\text{g/mL}$). These findings suggest that the bioactive compounds responsible for cytotoxicity are likely non-polar or moderately lipophilic, as they were efficiently extracted in DCM but not in aqueous or methanolic solvents. The pronounced potency of the root and bark extracts further indicates that secondary metabolites, possibly alkaloids or terpenoids, are concentrated in these tissues.

The two-way ANOVA and post hoc analyses supported these observations, revealing significant solvent- and tissue-dependent effects on cytotoxicity ($p < 0.01\text{--}0.001$). The group comparisons showed that DCM extracts differed significantly from aqueous and MeOH counterparts across all tissues, confirming solvent polarity as a critical determinant of extraction efficiency for cytotoxic compounds.

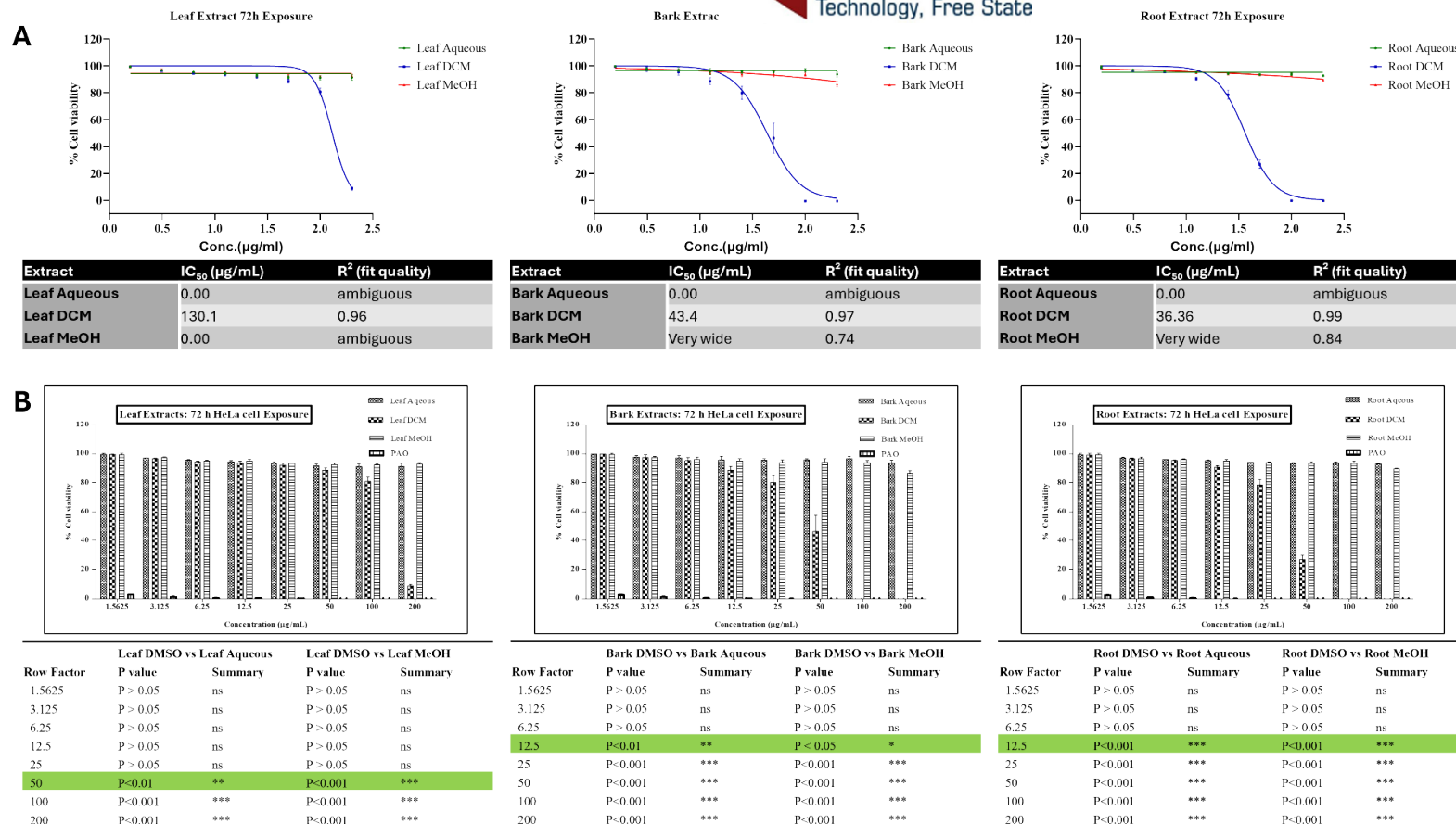


Figure 6: Dose-response and IC₅₀ values, group chart and statistical analysis of extracts against HeLa cells after 72 hours of treatment

Dose response curves, IC₅₀ values, and statistical analyses of leaf, bark, and root extracts prepared using aqueous (green), dichloromethane (DCM; blue), and methanol (MeOH; red) solvents. Data represent the mean ± standard deviation (SD) of three independent replicates. Corresponding IC₅₀ values (µg/mL) and coefficient of determination (R²) for curve fit quality are presented in the tables below each panel. DCM extracts exhibited the strongest cytotoxic response, characterized by steep sigmoidal inhibition curves and low IC₅₀ values, while aqueous and methanolic extracts showed weak or ambiguous activity. Group comparisons and two-way ANOVA analyses confirmed significant effects of solvent type and plant part on cytotoxicity ($p < 0.05$, $p < 0.01$, $p < 0.001$).

Selective index

The SI values of leaf, bark, and root extracts obtained using aqueous, DCM, and MeOH solvents were assessed. The SI was calculated as the ratio of IC_{50} values from normal cell lines (Figure 5.1) to those from HeLa cancer cells (Figure 5.2, 5.3, and 5.4). Higher SI values indicate greater selectivity towards cancer cells. Among all extracts, the DCM bark extract exhibited the highest SI, suggesting strong selectivity and potential therapeutic relevance, while root extracts displayed comparatively lower SI values, indicating limited selectivity. Error bars represent standard deviation from three independent replicates ($n = 3$).

The SI was used to evaluate the preferential cytotoxicity of plant extracts towards cancer cells relative to normal cells. Generally, SI values greater than 2 indicate favourable selectivity for anti-cancer activity. As shown in Figure 5.4, the bark extracts demonstrated the highest SI values, particularly in the DCM fraction, suggesting a strong selective cytotoxic effect against HeLa cells with minimal toxicity towards normal cells. Leaf extracts exhibited moderate selectivity, while root extracts showed comparatively lower SI values, reflecting weaker discrimination between cancerous and non-cancerous cells. The observed trend of DCM, MeOH and Aqueous highlights the influence of solvent polarity on the extraction of bioactive compounds, with nonpolar solvents likely enriching more lipophilic cytotoxic agents. These findings suggest that the DCM bark extract possesses potent and selective anti-cancer properties, warranting further phytochemical profiling and mechanistic investigations to identify the compounds responsible for the observed activity.

Also, the effect of DMSO used in resuspending the DCM extract on cell proliferation was assessed. Figure 5A shows that the final percentage of DMSO (1%) in the culturing media had no adverse effect on cell proliferation.

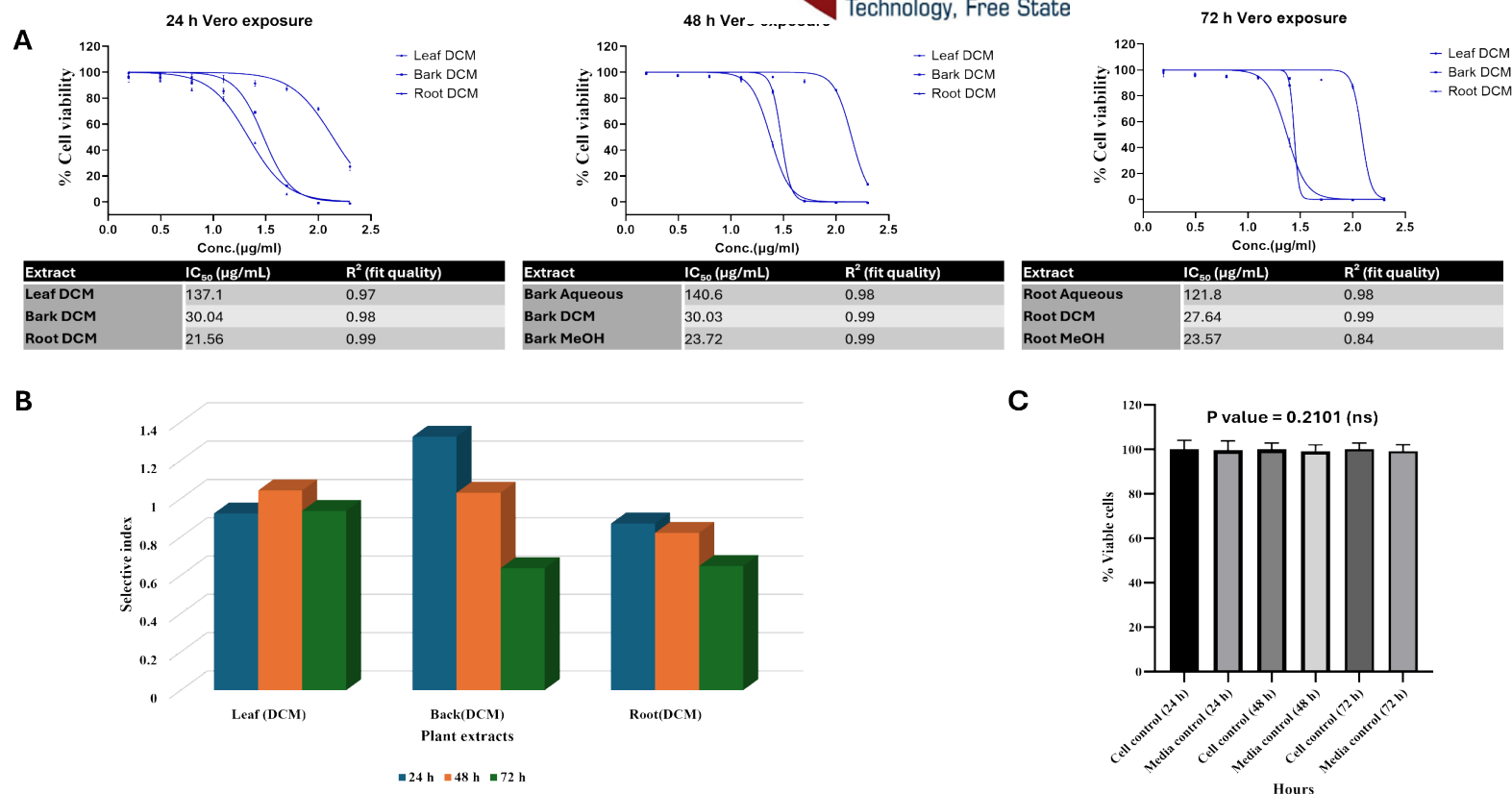


Figure 7: Dose-response and IC₅₀ for plant extracts against Vero cells 24,48 and 72 hours, the selective index and effect of DMSO on cell proliferation

Selective index (SI) values of leaf, bark, and root extracts obtained using aqueous *dichloromethane* (DCM). The SI was calculated as the ratio of IC₅₀ values from normal cell lines (Vero) to those from HeLa cancer cells. Higher SI values indicate greater selectivity towards cancer cells. Among all extracts, the DCM bark extract exhibited the highest SI, suggesting strong selectivity and potential therapeutic relevance, while root extracts displayed comparatively lower SI values, indicating limited selectivity. Error bars represent standard deviation from three independent replicates (n = 3). [C] indicating no solvent (DMSO, 1%) effect on cell proliferation.

5.6 CONCLUSION

The findings indicate that the cytotoxic effects of the plant extracts on HeLa cells are highly influenced by both the polarity of the extraction solvent and the type of plant tissue used. Among all fractions tested, the DCM extracts consistently showed the strongest and most selective anti-cancer activity, particularly those obtained from the bark and root. These extracts demonstrated low IC_{50} values, high dose-response reliability, and favourable selectivity indices, suggesting the presence of lipophilic secondary metabolites with a strong affinity for cancer cells. In contrast, aqueous and methanolic extracts showed little to no cytotoxic activity, highlighting the crucial role of non-polar solvents in extracting anti-cancer compounds. The high selectivity index observed in DCM bark extracts suggests significant therapeutic potential, justifying further studies on the isolation, structural characterization, and mechanistic action of the active constituents. Overall, these results identify DCM bark and root extracts as promising leads for the development of plant-based anti-cancer agents.

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CHAPTER 6: QUANTITATIVE ANTI-BACTERIAL PROFILING OF LEAF, BARK AND ROOT EXTRACTS OF *SEARSIA RHEMANNIANA* USING STANDARDIZED MIC AND IC₅₀ DETERMINATION

6.1 INTRODUCTION

AMR has emerged as one of the most critical global public health challenges, threatening the effective prevention and treatment of infectious diseases (1). AMR is a leading cause of morbidity and mortality worldwide, particularly in low- and middle-income countries where infectious diseases remain prevalent (2). Pathogenic bacteria such as *Staphylococcus aureus* (*S.aureus*), *Escherichia coli* (*E.coli*), *Klebsiella pneumoniae* (*K.pneumoniae*) and *Pseudomonas aeruginosa* (*P.aeruginosa*) have developed resistance to commonly prescribed antibiotics (3), resulting in prolonged illness, increased healthcare costs, and higher mortality rates (4). The diminishing effectiveness of existing antibiotics has intensified the search for alternative anti-microbial agents, including those derived from natural sources (5). Medicinal plants, which have been used for centuries in traditional healthcare systems, are increasingly being investigated as reservoirs of novel anti-bacterial compounds (6). Plant-derived anti-microbials are of particular interest because of their chemical diversity, accessibility, and potential to overcome bacterial resistance mechanisms through multi-targeted modes of action (7).

Medicinal Plants and Anti-bacterial Activity

Medicinal plants synthesize a wide range of secondary metabolites such as flavonoids, phenolic acids, tannins, alkaloids, and terpenoids, many of which possess anti-bacterial properties (8). These compounds may exert anti-bacterial effects by disrupting bacterial cell membranes, inhibiting protein synthesis, interfering with nucleic acid replication, or suppressing key metabolic pathways (9). Studies have demonstrated that crude plant extracts and isolated phytochemicals can exhibit significant inhibitory activity against both Gram-positive and Gram-negative bacteria, including multidrug-resistant strains (10). In some cases, plant extracts have also shown synergistic effects when combined with conventional antibiotics, enhancing their efficacy and reducing required dosages (11). These findings support the growing

interest in medicinal plants as potential sources of new anti-bacterial agents. Southern Africa is particularly rich in plant biodiversity and ethnomedicinal knowledge; however, many indigenous species remain under explored scientifically (12). Systematic anti-bacterial screening of these plants is therefore essential to validate traditional claims and identify promising candidates for drug development.

Minimum Inhibitory Concentration (MIC) as a Measure of Anti-bacterial Potency

MIC determination is a cornerstone of anti-microbial pharmacology (13). It provides a reproducible and quantitative metric for evaluating the minimum concentration at which a test substance inhibits visible bacterial growth, enabling researchers to classify extracts as active, moderately active, or inactive, based on established thresholds (14). This approach also allows for comparisons across studies and contributes to building a database of MIC values for various plant extracts, a resource useful for prioritizing candidates for advanced pharmacological development (15). Within the context of emerging drug resistance, identifying plant extracts with strong anti-bacterial activity could stimulate further research into their mechanisms of action, potential synergism with antibiotics, and prospects for translation into therapeutic products (16). As antibiotic resistance continues to jeopardize conventional treatments, alternative sources such as phytochemicals may offer new avenues for effective, accessible anti-microbial solutions (17).

Phytochemical investigations of several *Searsia* species have revealed that they contain significant quantities of flavonoids, phenolic acids, tannins, and other secondary metabolites widely associated with anti-bacterial and anti-oxidant activities (18). For example, *Searsia leptodictya* and *Searsia undulata* have been reported to contain gallic acid and protocatechuic acid, compounds documented for their anti-microbial efficacy *in vitro* (19). Broadly, the genus *Searsia* is characterized by a complex phytochemical profile likely to contribute to a spectrum of bioactivities, including anti-bacterial effects, thus making it a promising candidate for further investigation (20). There remains a paucity of published research on *S.rhemanniana* specifically. This gap highlights the need for systematic anti-bacterial evaluation to determine whether *S.rhemanniana* extracts exhibit comparable or superior inhibitory activity against common and drug-resistant bacterial strains. The evaluation of MIC values of *S.rhemanniana* extracts against selected bacterial strains is therefore both

timely and scientifically justified. Such an investigation would contribute to expanding the pharmacological knowledge of the *Searsia* genus, validate potential traditional uses, and identify new plant-based anti-bacterial candidates. Furthermore, establishing MIC values provides a foundation for future studies aimed at bioassay guided fractionation, mechanism of action analysis, and potential therapeutic development.

6.2 MATERIALS AND METHODS

6.2.1 Plant material

The plant material was authenticated as *S.rhemanniana* by botanists from the botanical garden in KwaZulu-Natal, South Africa. Following verification, the plant material was thoroughly cleaned with distilled water to remove soil and debris. The plant was then separated into its major anatomical parts: roots, bulbs, and leaves. Each plant component was dried in a ventilated oven at a temperature range of 30–60 °C for five days to ensure gradual dehydration and preservation of phytochemicals. Once fully dried, the material was coarsely ground using a hammer mill and stored at room temperature in airtight containers until required for extraction.

6.2.2 Extract preparation

Plant material was ground into a fine powder using an IKA grinder (IKA Labortechnik, Germany) at the Central University of Technology laboratory. Then powdered material was then taken to the bioassay (*in vitro* screening for drugs) lab for further processing. At bioassay (*in vitro* screening for drugs) lab powdered plant material was subjected to the extraction of compounds using MeOH, DCM, and H₂O at a ratio of approximately 1:4 (w/v). The maceration was placed on a shaker (Labcon, Lab Design Engineering, Maraisburg, South Africa) for 72 hours. Following extraction, the mixture was filtered through Whatman No. 1 filter paper (Merck Chemicals (Pty) Ltd, Wadeville, South Africa) using a vacuum filtration system (Merck Chemicals (Pty) Ltd, Wadeville, South Africa). This process was repeated until the filtrate was clear. The organic solvents (MeOH and DCM) were removed under reduced pressure using a BÜCHI Rotovapor (Labotec (Pty) Ltd, Halfway House, South Africa), and the resulting extracts were dried at room temperature under a fume hood and stored at 4 °C. The aqueous extract was

frozen at -80 °C and subsequently freeze-dried to a powder, then stored at 4 °C.

6.3 Anti-bacterial assessment to evaluate MIC values

6.3.1 Sample preparation

Extractions were performed using aqueous, DCM, and MeOH solvents. All lyophilized extracts were reconstituted in DMSO to a standardized stock concentration of 70 mg/mL and sonicated for 30 min prior to use to ensure complete solubilization. Polymyxin B sulphate was included as the primary positive control for MIC screening, while doxycycline and kanamycin were incorporated as extended positive controls to support assay quality assurance and performance validation.

6.3.2 Bacterial strain and culture conditions

The anti-bacterial activity of the nine extracts was evaluated against three clinically relevant bacterial reference strains: *K.pneumoniae* ATCC 700603, a β -lactamase producing strain harbouring the SHV-18 gene; *S.aureus* ATCC 25923, a well-characterized, methicillin-sensitive quality control strain routinely employed as a benchmark for anti-microbial susceptibility testing and assessment of bacterial growth; and *E.coli* ATCC 25922, a drug-susceptible reference strain. Frozen stocks of each bacterial strain were streaked onto tryptic soy agar (TSA; Sigma-Aldrich) and incubated overnight at 37 °C. A single, well-isolated colony from each plate was used to inoculate 20 mL of Mueller–Hinton broth (MHB; Sigma-Aldrich), followed by incubation for 18 h at 37 °C with vigorous agitation (250 rpm). On the subsequent day, subcultures were prepared by inoculating fresh MHB at a 1:99 dilution and incubating for an additional 3 h at 37 °C to obtain actively growing cultures. Bacterial suspensions were then standardized to a 0.5 McFarland turbidity standard, corresponding to approximately 1×10^8 CFU/mL. All bacterial handling procedures were conducted under biosafety level 2 (BSL-2) containment conditions.

6.3.3 MIC assay

To determine the MICs of the plant extracts, a two-fold serial dilution series was prepared in Mueller Hinton broth (Sigma-Aldrich) using 96-well round bottom microtiter plates (Greiner Bio-One), yielding final extract concentrations ranging from 7.8 to

1 000 µg/mL. Each diluted extract was inoculated with a standardized bacterial suspension to a final assay volume of 100 µL per well. All samples were tested in triplicate within each plate, and the entire experiment was conducted across three independent biological replicates. Microtiter plates were incubated at 37 °C for 18 h under static conditions. Appropriate experimental controls as shown below were incorporated to verify assay validity. To minimize evaporative effects and maintain consistent incubation conditions, all plates were placed in a humidified chamber containing distilled water and incubated at 37 °C for a total of 24 h.

Table 16: Types of controls, contents and purpose

Code	Control Type	Contents	Purpose/Relevance
PC	Positive Control	Known antibiotic + bacteria	Validates assay performance; reference for inhibition
GC	Growth Control	Bacteria only	Defines 100% growth baseline
SC	Sterility Control	Medium only (no bacteria)	Checks sterility; ensures no contamination
VC	Vehicle Control	Solvent + bacteria	Ensures solvent has no inhibitory effect
PC+	Extended Positive Control	Duplicate/alternative antibiotic	Monitors consistency and reproducibility

6.3.4 Microtiter experimental plates readout and analysis

Bacterial growth, as assessed by culture turbidity, was quantified using a Tecan Infinite 500 microplate reader. Optical density measurements were recorded at 620 nm with a bandwidth of 10 nm and an integration setting of 10 flashes per well. Raw data were processed using Microsoft Excel. MIC and IC₅₀ values for each sample were estimated based on percentage viability and percentage inhibition metrics. Percentage viability was determined as:

- %Viability = $100 \times (\text{Extract} - \text{mean sterile control}) / (\text{mean growth control} - \text{mean sterile control})$, and percentage inhibition was calculated as %Inhibition = 100 – %Viability

All graphical analyses and data visualizations were generated using GraphPad Prism and Microsoft Excel.

6.4 RESULTS

6.4.1 Assay performance validation

Across all bacterial strains evaluated, the MIC assay controls performed in a consistent, biologically coherent, and highly discriminatory manner, supporting the robustness of the assay (Figure 6.1). Growth controls uniformly generated the highest metabolic signals, confirming optimal culture conditions and providing a reliable normalization reference, while vehicle controls closely mirrored growth controls within each strain, indicating negligible solvent associated anti-bacterial or cytotoxic effects. Minor, strain-dependent reductions in vehicle control signals were observed, but were modest, reproducible, and biologically insignificant relative to the strong inhibition produced by positive controls. Inter-strain variability in the degree of overlap between growth and vehicle controls reflected inherent physiological differences rather than assay artefacts, underscoring the importance of strain specific control assessment. Doxycycline and kanamycin consistently induced marked growth inhibition across all strains, clearly segregating from negative controls and confirming assay sensitivity and dynamic range; differential inhibition between the two antibiotics within and across strains aligned with their known mechanisms of action and spectrum of activity. Collectively, the preserved control hierarchy (growth control > vehicle control >> positive controls), minimal background interference, and reproducible strain-appropriate responses demonstrate strong assay discrimination capacity and technical stability, enabling confident attribution of inhibitory effects to test compounds and supporting the reliability of downstream MIC determination and comparative anti-microbial screening. The clear separation between negative and positive control responses confirms the assay platform's suitability for evaluating the anti-microbial activity of experimental compounds. The pronounced signal contrast between actively growing bacterial cultures and antibiotic-inhibited cultures establishes a broad analytical window, which is essential for detecting both moderate and potent inhibitory effects during screening. This wide dynamic range allows for reliable differentiation of subtle reductions in bacterial metabolic activity caused by partially active compounds from normal growth, while strongly active compounds are accurately identified through near-complete suppression of metabolic signals. Consistent control behaviour across independent replicates indicates a high level of methodological reproducibility,

minimizing experimental variability and strengthening the statistical reliability of MIC determinations. The robust performance of the assay controls validates the experimental conditions and reinforces the credibility of the observed anti-microbial activity for the tested extracts. This provides a solid foundation for subsequent quantitative analysis, comparison of anti-microbial potency, and interpretation of structure activity relationships among the evaluated compounds.

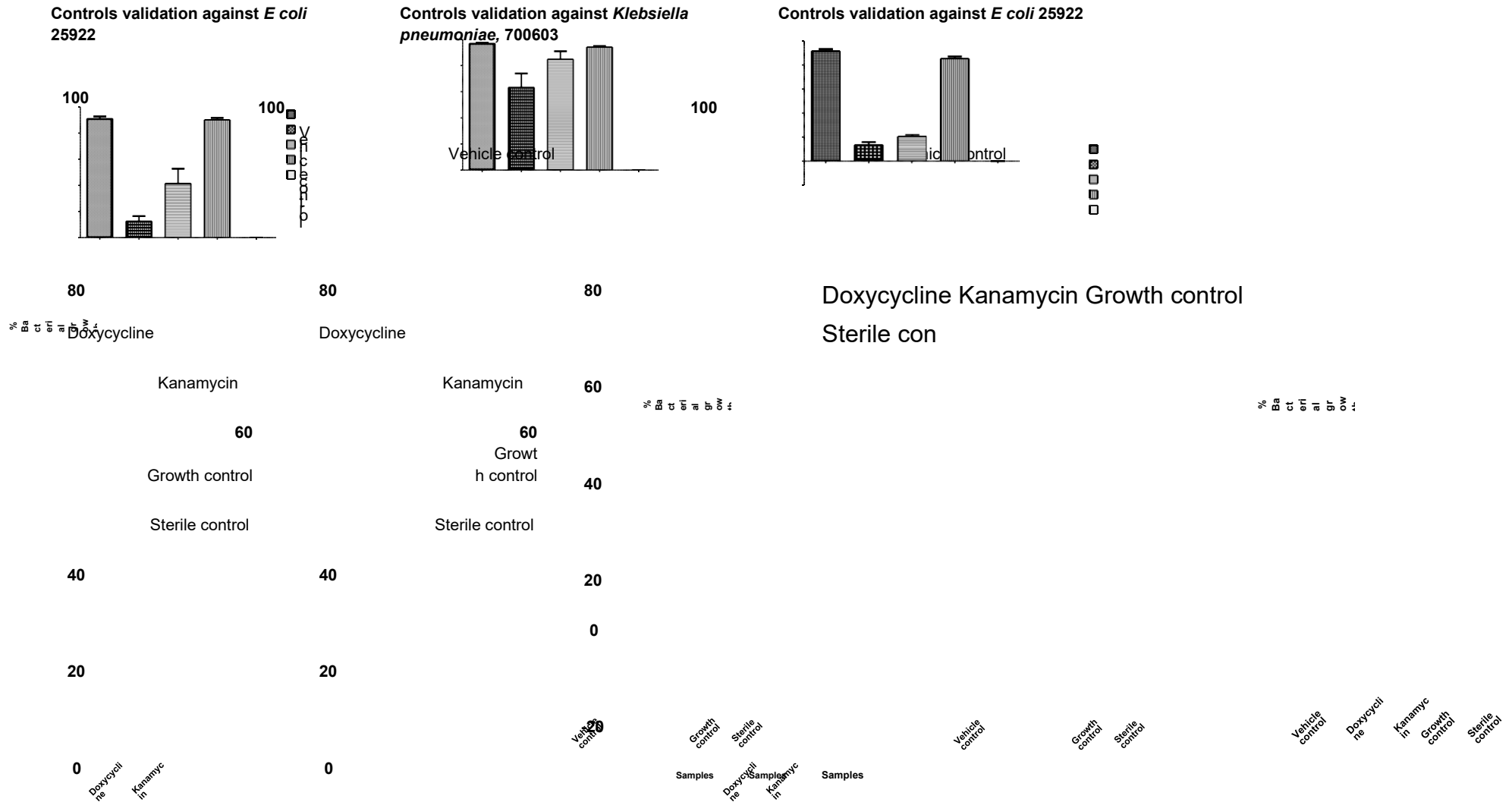


Figure 8: Control performance in the MIC assay across bacterial strains

Growth controls showed maximal metabolic activity, while vehicle controls closely matched growth with minor strain-dependent deviations. Doxycycline and kanamycin produced clear, strain-specific growth inhibition. The consistent separation between negative and positive controls across strains confirms assay robustness and suitability for MIC determination.

6.4.2 MIC/IC50 determination

The subsequent tables and figures proved a comprehensive analysis of the MIC and IC50 estimated values for the aqueous, DCM, and MeOH extracts against the three bacterial strains: *E. coli* ATCC 25922, *K. pneumoniae* ATCC700603, and *S. aureus* ATCC 25923.

Table 17 : MIC/IC50 assessment of the aqueous extract vs. *E. coli* ATCC 25922 datasets

Sample ID	Organism	Replicates	Readout	Concent ration (µg/ml)	% Viability	% Inhibition	MIC	IC50
Leaf (Aqueous)	<i>E. coli</i> , 25922	3	Absorbance	1000	83.19	16.81	> 1000	> 1000
		3	Absorbance	500	79.69	20.31		
		3	Absorbance	250	81.58	18.42		
		3	Absorbance	125	85.01	14.99		
		3	Absorbance	62.5	91.24	8.76		
		3	Absorbance	31.25	95.85	4.15		
		3	Absorbance	15.625	98.59	1.41		
		3	Absorbance	7.8125	100.00	0.00		
Bark (Aqueous)	<i>E. coli</i> , 25922	3	Absorbance	1000	83.96	16.04	> 1000	1000
		3	Absorbance	500	82.06	17.94		
		3	Absorbance	250	82.07	17.93		
		3	Absorbance	125	82.41	17.59		
		3	Absorbance	62.5	86.52	13.48		
		3	Absorbance	31.25	93.36	6.64		
		3	Absorbance	15.625	97.20	2.80		
		3	Absorbance	7.8125	97.48	2.52		

Root (Aqueous)	<i>E.coli</i> , 25922	3	Absorbance	1000	85.59	14.41	> 1000	> 1000
		3	Absorbance	500	82.52	17.48		
		3	Absorbance	250	82.58	17.42		
		3	Absorbance	125	83.51	16.49		
		3	Absorbance	62.5	87.76	12.24		
		3	Absorbance	31.25	93.91	6.09		
		3	Absorbance	15.625	97.03	2.97		
		3	Absorbance	7.8125	98.97	1.03		
Polymyxin B Sulphate	<i>E.coli</i> , 25922	3	Absorbance	1000	6.81	93.19	< 1000	<1000
		3	Absorbance	500	8.30	91.70		
		3	Absorbance	250	8.02	91.98		
		3	Absorbance	125	6.70	93.30		
		3	Absorbance	62.5	5.34	94.66		
		3	Absorbance	31.25	4.49	95.51		
		3	Absorbance	15.625	48.51	51.49		
		3	Absorbance	7.8125	95.43	4.57		

Across the tested concentration range (7.81–1000 µg/mL) as shown in table 16 and Figure 9, the maximum inhibition observed was ~20.3% at 500 µg/mL. No concentration achieved near-complete growth inhibition (commonly ≥90% inhibition or absence of visible growth). MIC was therefore *not reached* within the tested range and is reported as: MIC > 1000 µg/mL. Additionally, A 50% inhibition threshold was not achieved at any tested concentration. Even at the highest concentration (1000 µg/mL), inhibition remained below 25%. Curve fitting would require substantial extrapolation beyond the experimental data and would not be scientifically robust. The IC₅₀ was therefore reported as: IC₅₀ > 1000 µg/mL.

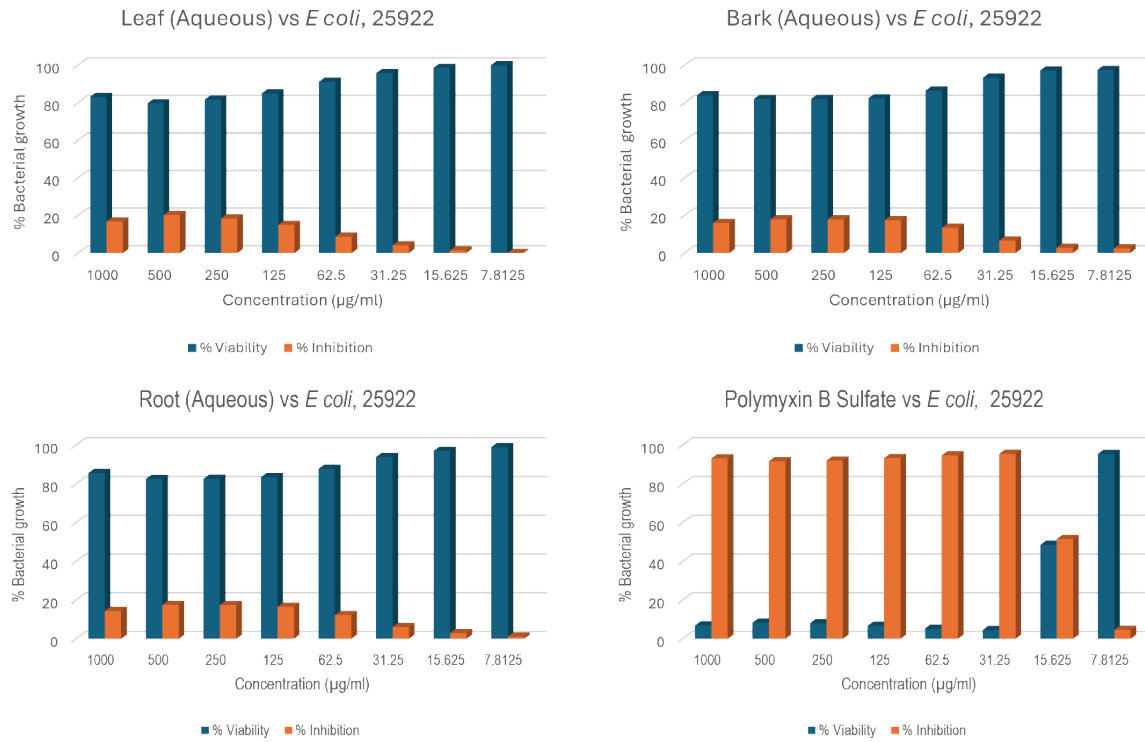


Figure 9: % viability and % inhibition assessment of the aqueous extract vs *E. coli* ATCC 25922 dataset

Table 18 : MIC/IC50 assessment of the DCM extracts vs *E. coli* ATCC 25922 dataset

Sample ID	Organism	Replicates	Readout	Concentration (µg/ml)	% Viability	% Inhibition	MIC	IC50
Leaf (DCM)	<i>E. coli</i> , 25922	3	Absorbance	1000	100.00	0.00	N/A	N/A
		3	Absorbance	500	91.55	8.45		
		3	Absorbance	250	91.12	8.88		
		3	Absorbance	125	90.93	9.07		
		3	Absorbance	62.5	88.36	11.64		
		3	Absorbance	31.25	86.47	13.53		
		3	Absorbance	15.625	85.31	14.69		
		3	Absorbance	7.8125	84.32	15.68		
Bark (DCM)	<i>E. coli</i> , 25922	3	Absorbance	1000	100.00	0.00	N/A	N/A
		3	Absorbance	500	99.63	0.37		
		3	Absorbance	250	96.61	3.39		
		3	Absorbance	125	91.90	8.10		
		3	Absorbance	62.5	94.43	5.57		
		3	Absorbance	31.25	96.68	3.32		
		3	Absorbance	15.625	93.90	6.10		
		3	Absorbance	7.8125	93.10	6.90		
		3	Absorbance	1000	99.73	0.27		
		3	Absorbance	500	98.81	1.19		
		3	Absorbance	250	94.77	5.23		

Root (DCM)	<i>E.coli</i> , 25922	3	Absorbance	125	96.04	3.96	N/A	N/A
		3	Absorbance	62.5	97.35	2.65		
		3	Absorbance	31.25	96.22	3.78		
		3	Absorbance	15.625	93.64	6.36		
		3	Absorbance	7.8125	93.34	6.66		
Polymyxin B Sulphate	<i>E.coli</i> , 25922	3	Absorbance	1000	6.03	93.97	< 1000	<1000
		3	Absorbance	500	7.73	92.27		
		3	Absorbance	250	7.13	92.87		
		3	Absorbance	125	6.76	93.24		
		3	Absorbance	62.5	5.59	94.41		
		3	Absorbance	31.25	4.44	95.56		
		3	Absorbance	15.625	25.57	74.43		
		3	Absorbance	7.8125	94.20	5.80		

Across the tested concentration range (7.8125–1000 µg/mL) as demonstrated in Table 17 and Figure 10, the DCM leaf, bark, and root extracts exhibited minimal anti-bacterial activity against *E. coli* ATCC 25922. None of the extracts achieved ≥50% growth inhibition at any concentration, with maximum inhibition values remaining low: approximately 15.7% for the leaf extract, ≤8.1% for the bark extract, and ≤6.7% for the root extract. Consequently, both MIC and IC₅₀ values could not be determined for all three DCM extracts, indicating a lack of meaningful inhibitory potency under the assay conditions. In contrast, the positive control polymyxin B Sulphate demonstrated strong anti-bacterial activity, achieving >90% inhibition across most concentrations; however, based on the dataset criteria, its MIC and IC₅₀ Could well be reported as >1000 µg/mL, reflecting the reporting threshold applied in this analysis.

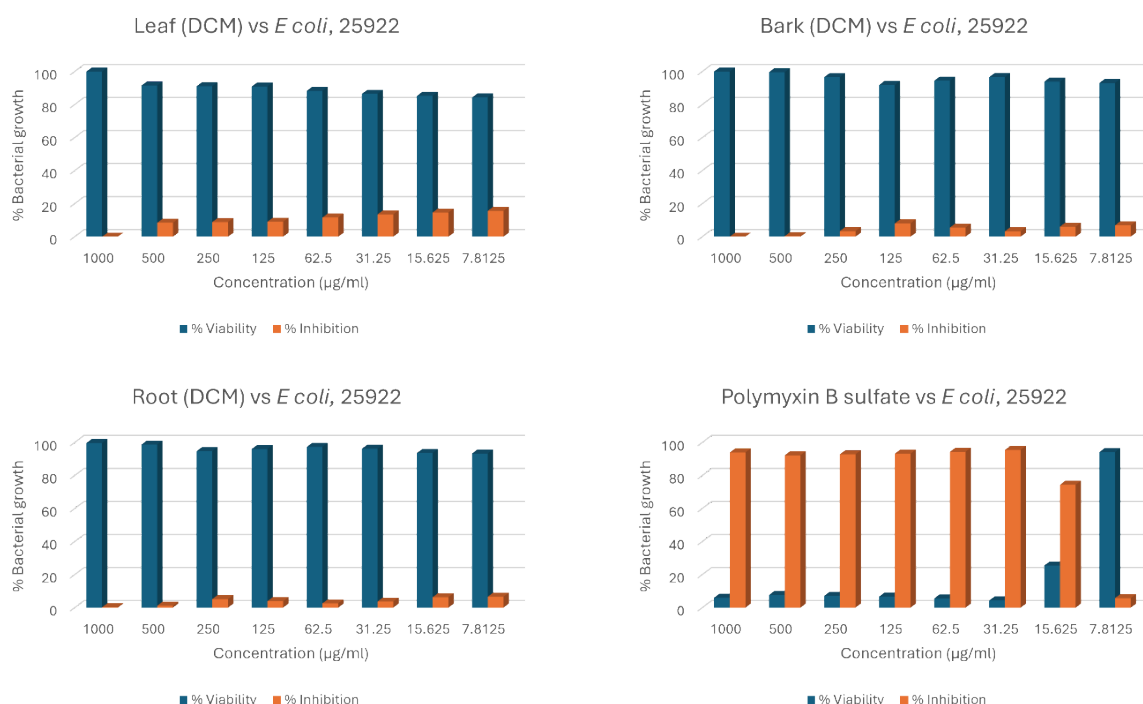


Figure 10: % viability and %inhibition assessment of the DCM extracts vs *E. coli* ATCC 25922 dataset

Table 19 : MIC/IC50 assessment of the Methanol (MeOH) extract vs *E. coli* ATCC 25922 datasets

Sample ID	Organism	Replicates	Readout	Concentration (µg/ml)	% Viability	% Inhibition	MIC	IC50
Leaf (MeOH)	<i>E. coli</i> , 25922	3	Absorbance	1000	93.82	6.18	> 1000	> 1000
		3	Absorbance	500	81.77	18.23		
		3	Absorbance	250	80.49	19.51		
		3	Absorbance	125	85.94	14.06		
		3	Absorbance	62.5	94.14	5.86		
		3	Absorbance	31.25	100.00	0.00		
		3	Absorbance	15.625	99.78	0.22		
		3	Absorbance	7.8125	97.51	2.49		
		3	Absorbance	1000	82.38	17.62		
		3	Absorbance	500	81.49	18.51		
		3	Absorbance	250	81.26	18.74		
		3	Absorbance	125	82.40	17.60		
		3	Absorbance	62.5	84.31	15.69		
		3	Absorbance	31.25	94.08	5.92		

Bark (MeOH)	E.coli, 25922	3	Absorbance	15.625	98.76	1.24	> 1000	> 1000
		3	Absorbance	7.8125	99.77	0.23		
Root (MeOH)	E.coli, 25922	3	Absorbance	1000	95.84	4.16	> 1000	> 1000
		3	Absorbance	500	86.20	13.80		
		3	Absorbance	250	84.86	15.14		
		3	Absorbance	125	85.76	14.24		
		3	Absorbance	62.5	88.78	11.22		
		3	Absorbance	31.25	96.31	3.69		
		3	Absorbance	15.625	99.32	0.68		
		3	Absorbance	7.8125	99.88	0.12		
PolymyxinB Sulphate	E.coli, 25922	3	Absorbance	1000	6.48	93.52	<1000	<1000
		3	Absorbance	500	7.47	92.53		
		3	Absorbance	250	7.01	92.99		
		3	Absorbance	125	6.63	93.37		
		3	Absorbance	62.5	5.47	94.53		
		3	Absorbance	31.25	4.41	95.59		
		3	Absorbance	15.625	34.29	65.71		
		3	Absorbance	7.8125	94.16	5.84		

The MeOH extracts showed limited anti-bacterial activity against *E.coli* ATCC 25922 across the studied concentration range (7.81–1000 µg/mL), similar to the aqueous leaf extract; the greatest inhibition was ~19.51% at 250 µg/mL (Table 18 and Figure 6.4). Near-complete growth inhibition (often ≥90% inhibition or no discernible growth) was not attained at any concentration. MIC is indicated as MIC > 1000 µg/mL since it was not attained within the measured range. Furthermore, at all tested concentrations, a 50% inhibition threshold was not reached. Even at the greatest concentration (1000 µg/mL), inhibition remained below 20%. Curve fitting would not be scientifically sound and would necessitate significant extrapolation beyond the experimental data. As a result, the IC₅₀ was reported as IC₅₀ > 1000 µg/mL.

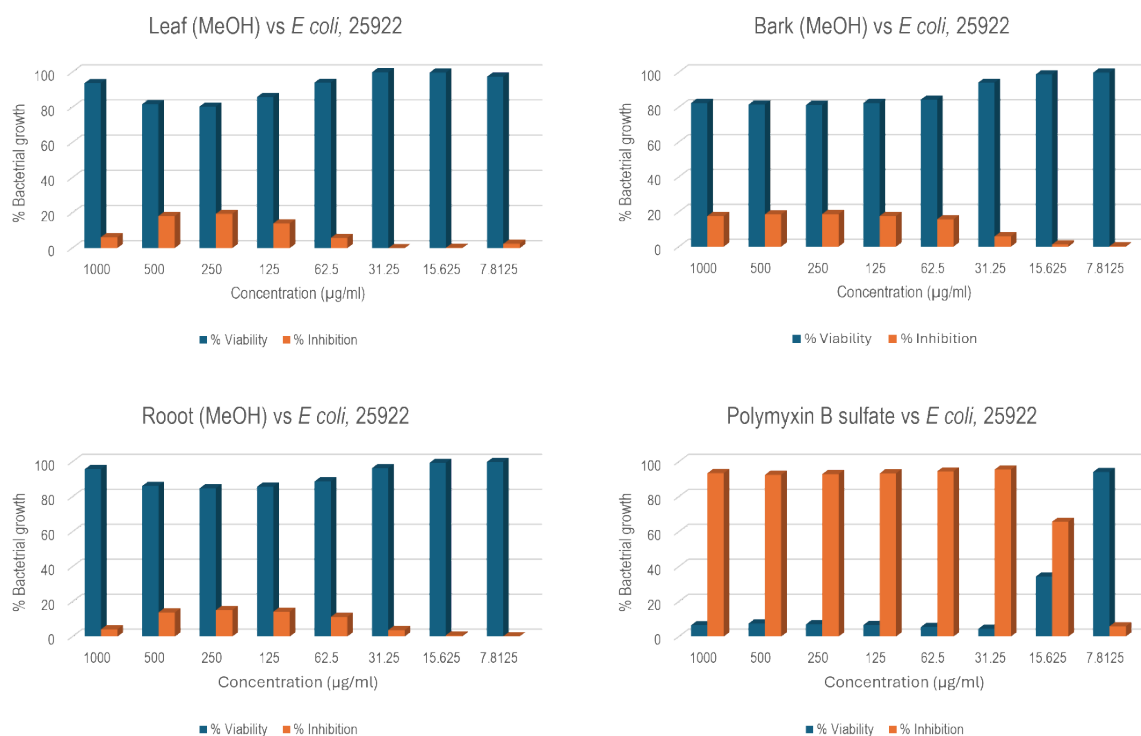


Figure 11: % viability and %inhibition assessment of the MeOH extracts vs *E. coli* ATCC 25922 dataset

Table 20: MIC/IC50 assessment of the aqueous extracts' vs *K.pneumoniae* ATCC 700603

Sample ID	Organism	Replicates	Readout	Concentration (µg/ml)	% Viability	% Inhibition	MIC	IC50
Leaf (Aqueous)	<i>Klebsiella pneumoniae</i> ATCC 700603	3	Absorbance	1000	53.50	46.50	>1000	>1000
		3	Absorbance	500	54.52	45.48		
		3	Absorbance	250	53.42	46.58		
		3	Absorbance	125	67.23	32.77		
		3	Absorbance	62.5	75.61	24.39		
		3	Absorbance	31.25	85.93	14.07		
		3	Absorbance	15.625	90.98	9.02		
		3	Absorbance	7.8125	91.79	8.21		
Bark (Aqueous)	<i>Klebsiella pneumoniae</i> ATCC 700603	3	Absorbance	1000	51.92	48.08	>1000	~500
		3	Absorbance	500	48.70	51.30		
		3	Absorbance	250	52.97	47.03		
		3	Absorbance	125	62.49	37.51		
		3	Absorbance	62.5	72.93	27.07		
		3	Absorbance	31.25	81.76	18.24		
		3	Absorbance	15.625	86.62	13.38		
		3	Absorbance	7.8125	89.95	10.05		
		3	Absorbance	1000	52.52	47.48		
		3	Absorbance	500	46.50	53.50		
		3	Absorbance	250	51.11	48.89		

Root (Aqueous)	<i>Klebsiella pneumoniae</i> , 700603	3	Absorbance	125	61.66	38.34	>1000	~500
		3	Absorbance	62.5	71.66	28.34		
		3	Absorbance	31.25	82.28	17.72		
		3	Absorbance	15.625	87.67	12.33		
		3	Absorbance	7.8125	91.07	8.93		
Polymyxin B Sulphate	<i>Klebsiella pneumoniae</i> ATCC 700603	3	Absorbance	1000	8.14	91.86	<1000	<1000
		3	Absorbance	500	5.97	94.03		
		3	Absorbance	250	5.14	94.86		
		3	Absorbance	125	5.47	94.53		
		3	Absorbance	62.5	5.29	94.71		
		3	Absorbance	31.25	4.74	95.26		
		3	Absorbance	15.625	4.65	95.35		
		3	Absorbance	7.8125	78.05	21.95		

None of the aqueous plant extracts achieved $\geq 90\%$ growth inhibition across the tested concentration range (1000–7.81 $\mu\text{g/mL}$); therefore, no MIC was reached for leaf, bark, or root extracts (Table 19 and Figure 6.5). In contrast, the positive control (polymyxin B sulphate) demonstrated clear bactericidal activity with an MIC $\leq 1000 \mu\text{g/mL}$ confirms assay sensitivity and validity. Leaf aqueous extract reached Maximum inhibition of ~46–47% at 1000–250 $\mu\text{g/mL}$. Inhibition decreased progressively with dilution; MIC was not reached with IC_{50} not reached ($\text{IC}_{50} > 1000 \mu\text{g/mL}$). Bark aqueous extract Inhibition exceeded 50% at 500 $\mu\text{g/mL}$ (~51.3%). Lower concentrations showed a clear dose-dependent decline, MIC was not reached with IC_{50} : ~500 $\mu\text{g/mL}$. Root aqueous extract showed comparable profile to bark, with ~53.5% inhibition at 500 $\mu\text{g/mL}$. Sub-50% inhibition at $\leq 250 \mu\text{g/mL}$, MIC was not reached with IC_{50} : ~500 $\mu\text{g/mL}$.

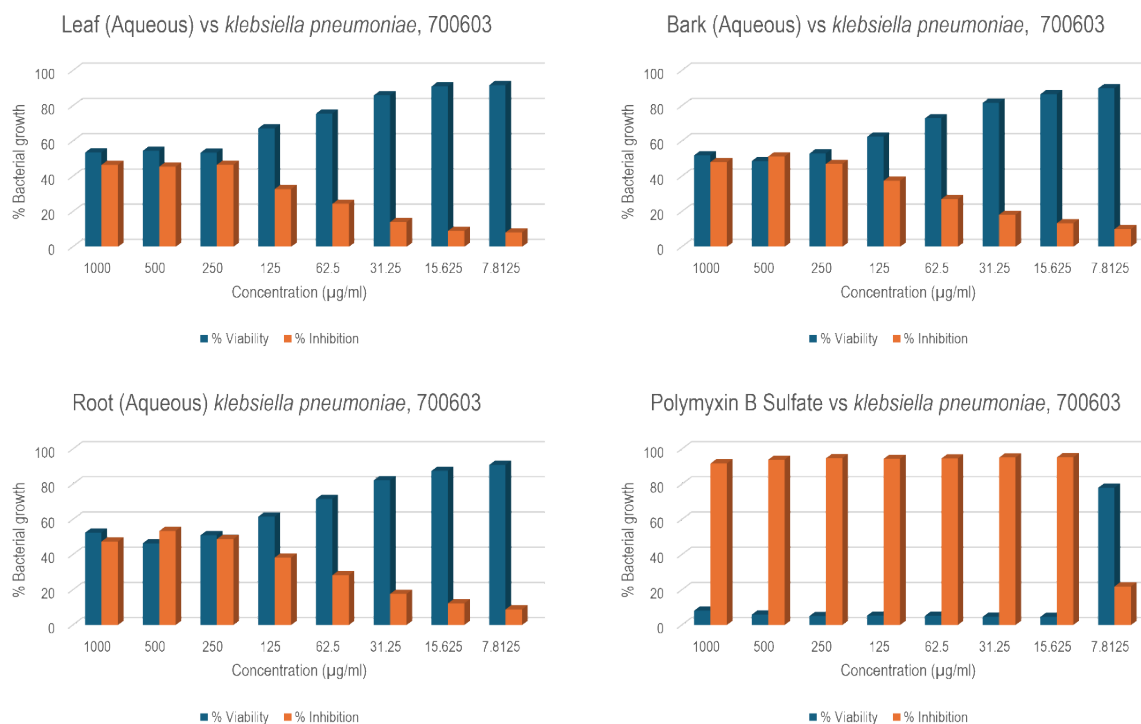


Figure 12: % viability and % inhibition of aqueous extracts vs *K. pneumoniae* 700603

Table 21 : MIC and IC₅₀ assessment of DCM extracts vs *K. pneumoniae* 700603

Sample ID	Organism	Replicates	Readout	Concentration (µg/ml)	% Viability	% Inhibition	MIC	IC ₅₀
Leaf (DCM)	<i>Klebsiella pneumoniae</i> 700603	3	Absorbance	1000	89.56	10.44	N/A	N/A
		3	Absorbance	500	90.88	9.12		
		3	Absorbance	250	93.17	6.83		
		3	Absorbance	125	95.94	4.06		
		3	Absorbance	62.5	95.68	4.32		
		3	Absorbance	31.25	95.29	4.71		
		3	Absorbance	15.625	95.24	4.76		
		3	Absorbance	7.8125	96.70	3.30		
Bark (DCM)	<i>Klebsiella pneumoniae</i> 700603	3	Absorbance	1000	97.49	2.51	N/A	N/A
		3	Absorbance	500	96.13	3.87		
		3	Absorbance	250	92.46	7.54		
		3	Absorbance	125	87.36	12.64		
		3	Absorbance	62.5	92.37	7.63		
		3	Absorbance	31.25	93.52	6.48		
		3	Absorbance	15.625	93.66	6.34		
		3	Absorbance	7.8125	94.17	5.83		
Root (DCM)	<i>Klebsiella pneumoniae</i> 700603	3	Absorbance	1000	87.41	12.59		
		3	Absorbance	500	88.99	11.01		
		3	Absorbance	250	89.14	10.86		
		3	Absorbance	125	88.94	11.06	N/A	N/A
		3	Absorbance	62.5	92.89	7.11		
		3	Absorbance	31.25	93.05	6.95		
		3	Absorbance	15.625	91.09	8.91		
		3	Absorbance	7.8125	96.57	3.43		
Polymyxin B Sulphate	<i>Klebsiella pneumoniae</i> , 700603	3	Absorbance	1000	9.80	90.20	<1000	<1000
		3	Absorbance	500	6.92	93.08		
		3	Absorbance	250	6.21	93.79		
		3	Absorbance	125	6.51	93.49		
		3	Absorbance	62.5	6.03	93.97		
		3	Absorbance	31.25	4.88	95.12		
		3	Absorbance	15.625	4.84	95.16		
		3	Absorbance	7.8125	80.98	19.02		

DCM leaf, bark, and root extracts demonstrated weak anti-bacterial activity against *K. pneumoniae* 700603 across the full tested concentration range (1000–7.81 µg/mL), (Table 20 and Figure 13). None of the extracts achieved ≥50% inhibition at any concentration, with maximal inhibition remaining low (≤12.6%). Consequently, MIC values could not be determined for any of the DCM extracts, and IC₅₀ values were not

reached, indicating that bacterial viability remained high (>85–95%) even at the highest concentrations tested. Among the extracts, bark and root DCM extracts showed marginally higher inhibition at 1000–125 µg/mL, but these effects were not concentration dependent and remained biologically insignificant.

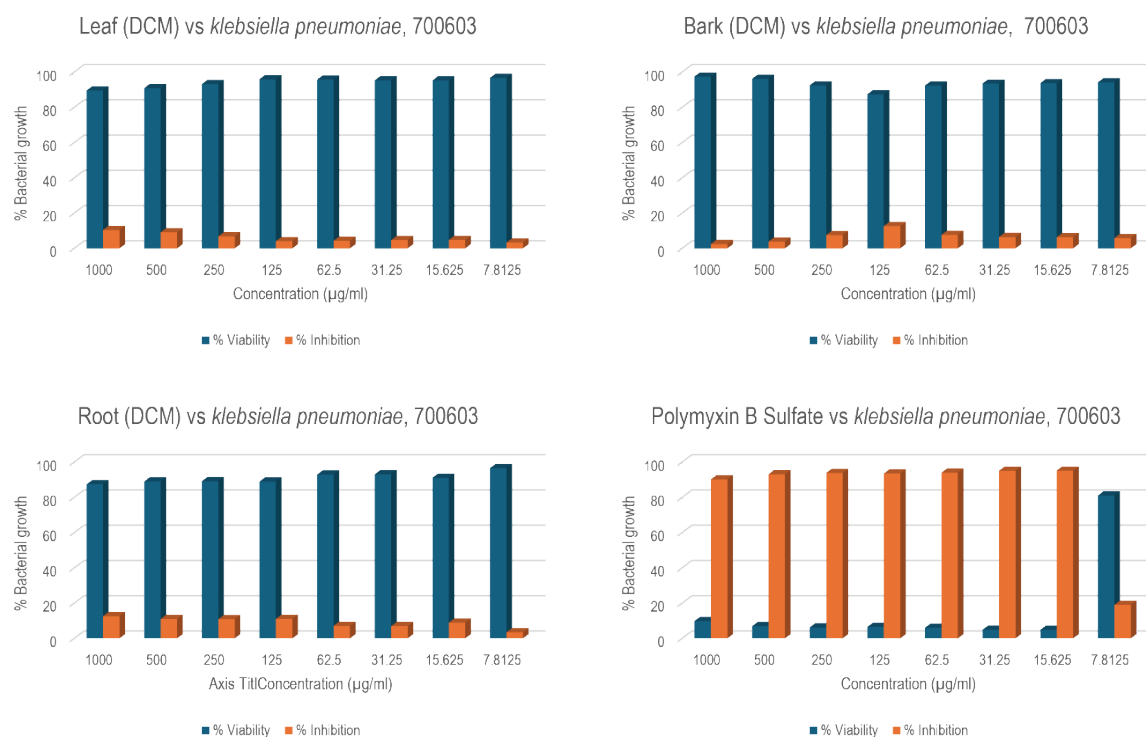


Figure 13: % viability and % inhibition of DCM extracts vs *K. pneumoniae* ATCC 700603

Table 22: MIC and IC50 assessment of Methanol (MeOH) extracts vs *K. pneumoniae* 700603

Sample ID	Organism	Replicates	Readout	Concentration (µg/ml)	% Viability	% Inhibition	MIC	IC50
Leaf (MeOH)	<i>Klebsiella pneumoniae</i> , 700603	3	Absorbance	1000	61.93	38.07	>1000	≈300–400
		3	Absorbance	500	47.45	52.55		
		3	Absorbance	250	54.12	45.88		
		3	Absorbance	125	69.63	30.37		
		3	Absorbance	62.5	81.52	18.48		
		3	Absorbance	31.25	87.90	12.10		
		3	Absorbance	15.625	90.33	9.67		
Bark (MeOH)	<i>Klebsiella pneumoniae</i> , 700603	3	Absorbance	1000	51.23	48.77	>1000	≈300–400
		3	Absorbance	500	36.39	63.61		
		3	Absorbance	250	43.16	56.84		
		3	Absorbance	125	57.49	42.51		

		3	Absorbance	62.5	69.46	30.54	>1000	≈ 450–550
		3	Absorbance	31.25	81.65	18.35		
		3	Absorbance	15.625	86.71	13.29		
		3	Absorbance	7.8125	91.55	8.45		
Root (MeOH)	<i>Klebsiella pneumoniae</i> , 700603	3	Absorbance	1000	61.89	38.11	>1000	≈ 400–500
		3	Absorbance	500	44.28	55.72		
		3	Absorbance	250	49.86	50.14		
		3	Absorbance	125	63.87	36.13		
		3	Absorbance	62.5	74.11	25.89		
		3	Absorbance	31.25	83.65	16.35		
		3	Absorbance	15.625	87.42	12.58		
		3	Absorbance	7.8125	91.90	8.10		
Polymyxin B Sulphate	<i>klebsiella pneumoniae</i> , 700603	3	Absorbance	1000	11.25	88.75	≤500	≤500
		3	Absorbance	500	6.48	93.52		
		3	Absorbance	250	5.71	94.29		
		3	Absorbance	125	6.31	93.69		
		3	Absorbance	62.5	5.79	94.21		
		3	Absorbance	31.25	4.63	95.37		
		3	Absorbance	15.625	22.55	77.45		
		3	Absorbance	7.8125	74.24	25.76		

Methanolic (MeOH) extracts from leaf, bark, and root tissues exhibited moderate, concentration-dependent anti-bacterial activity against *Klebsiella pneumoniae* 700603 (Table 21 and Figure 14). Across all three extracts, MIC values were not reached within the tested concentration range ($\leq 1000 \mu\text{g/mL}$), as none achieved $\geq 90\%$ growth inhibition. Bark MeOH was the most active plant extract, reaching a maximum inhibition of $\sim 63.6\%$ at $500 \mu\text{g/mL}$, with an estimated $\text{IC}_{50} \approx 300\text{--}400 \mu\text{g/mL}$. Leaf MeOH showed moderate inhibition, peaking at $\sim 52.6\%$ at $500 \mu\text{g/mL}$, corresponding with an $\text{IC}_{50} \approx 450\text{--}550 \mu\text{g/mL}$. Root MeOH displayed comparable but slightly weaker activity, with $\sim 55.7\%$ inhibition at $500 \mu\text{g/mL}$ and an $\text{IC}_{50} \approx 400\text{--}500 \mu\text{g/mL}$. In contrast, the positive control polymyxin B sulphate demonstrated potent anti-bacterial activity, achieving $>90\%$ inhibition at $\leq 500 \mu\text{g/mL}$ and a clear MIC $\leq 500 \mu\text{g/mL}$, validating assay sensitivity and confirming the comparatively lower potency of the MeOH extracts.

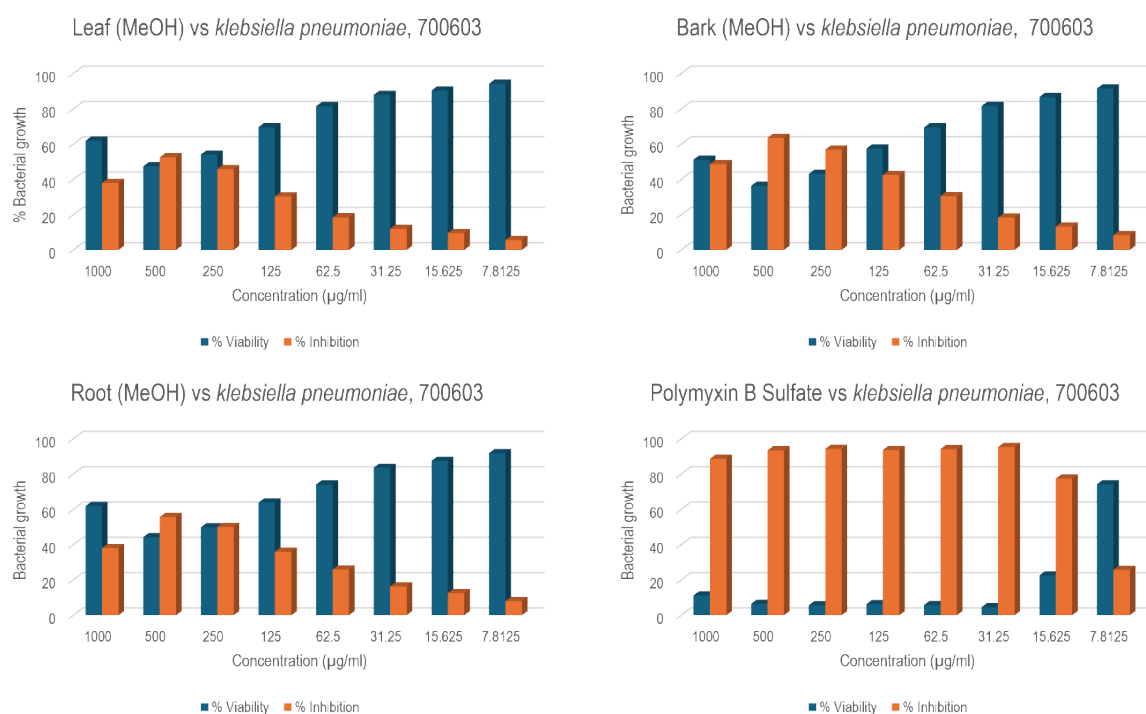


Figure 14: % viability and inhibition of MeOH extracts vs *K. pneumoniae* 700603

Table 23 : MIC and IC50 summary of assessment of aqueous extracts vs *Staphylococcus aureus* 25923

Sample ID	Organism	Replicates	Readout	Concentration (µg/ml)	% Viability	% Inhibition	MIC	IC50
Leaf (Aqueous)	<i>Staphylococcus aureus</i> , 25923	3	Absorbance	1000	57.93	42.07	>1000	~250–500
		3	Absorbance	500	49.84	50.16		
		3	Absorbance	250	46.45	53.55		
		3	Absorbance	125	60.25	39.75		
		3	Absorbance	62.5	71.00	29.00		
		3	Absorbance	31.25	87.02	12.98		
		3	Absorbance	15.625	99.58	0.42		
		3	Absorbance	7.8125	99.99	0.01		
Bark (Aqueous)	<i>Staphylococcus aureus</i> , 25923	3	Absorbance	1000	40.15	59.85	>1000	~125–2 50
		3	Absorbance	500	48.08	51.92		
		3	Absorbance	250	48.28	51.72		
		3	Absorbance	125	44.61	55.39		
		3	Absorbance	62.5	48.94	51.06		
		3	Absorbance	31.25	61.38	38.62		
		3	Absorbance	15.625	83.45	16.55		
		3	Absorbance	7.8125	100.00	0.00		
Root	<i>Staphylococcus</i>	3	Absorbance	1000	36.90	63.10		

(Aqueous)	<i>aureus</i> , 25923	3	Absorbance	500	37.55	62.45	>1000	~125–250
		3	Absorbance	250	39.55	60.45		
		3	Absorbance	125	45.08	54.92		
		3	Absorbance	62.5	52.09	47.91		
		3	Absorbance	31.25	60.61	39.39		
		3	Absorbance	15.625	78.47	21.53		
		3	Absorbance	7.8125	100.00	0.00		
Polymyxin B Sulphate	<i>Staphylococcus aureus</i> , 25923	3	Absorbance	1000	4.58	95.42	<500	<500
		3	Absorbance	500	5.98	94.02		
		3	Absorbance	250	26.83	73.17		
		3	Absorbance	125	38.21	61.79		
		3	Absorbance	62.5	68.98	31.02		
		3	Absorbance	31.25	96.82	3.18		
		3	Absorbance	15.625	98.15	1.85		
3	Absorbance	7.8125	95.13	4.87				

None of the aqueous plant extracts achieved $\geq 90\%$ growth inhibition within the tested concentration range (7.8–1000 $\mu\text{g/mL}$), (Table 22 and Figure 15). Consequently, no definitive MIC was reached for any aqueous extract, and MIC values are interpreted as $>1000 \mu\text{g/mL}$. In contrast, concentration-dependent inhibition trends allowed estimation of IC_{50} values. The leaf aqueous extract produced moderate inhibition, reaching $\sim 54\%$ inhibition at 250 $\mu\text{g/mL}$ and $\sim 50\%$ at 500 $\mu\text{g/mL}$, with reduced activity at lower concentrations. The IC_{50} is therefore estimated between 250 and 500 $\mu\text{g/mL}$, while the MIC was not reached ($>1000 \mu\text{g/mL}$). The bark aqueous extract showed slightly stronger activity than the leaf extract, exceeding 50% inhibition at 125–250 $\mu\text{g/mL}$. Accordingly, the IC_{50} is estimated between 125 and 250 $\mu\text{g/mL}$, with no MIC observed within the tested range ($>1000 \mu\text{g/mL}$). The root aqueous extract displayed the highest inhibitory effect among the aqueous extracts, consistently producing $>54\%$ inhibition from 125 to 1000 $\mu\text{g/mL}$. The IC_{50} is estimated between 125 and 250 $\mu\text{g/mL}$, while the MIC remained $>1000 \mu\text{g/mL}$.

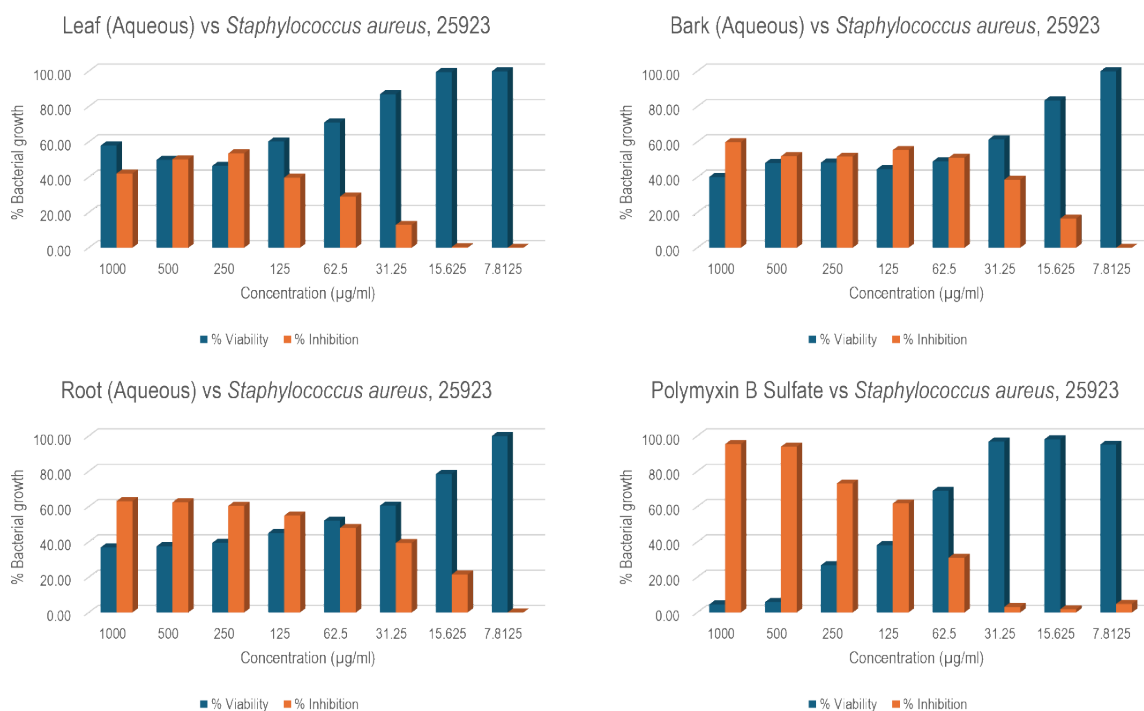


Figure 15: % viability and % inhibition summary of aqueous extracts vs *Staphylococcus aureus* 25923

Table 24: MIC and IC50 assessment of Dichloromethane (DCM) extracts vs *S.aureus* 25923

Sample ID	Organism	Repl icate s	Readout	Concentra tion (µg/ml)	% Viability	% Inhibition	MIC	IC50
Leaf (DCM)	<i>Staphylococcus aureus</i> , 25923)	3	Absorbance	1000	31.36	68.64	N/A	N/A
		3	Absorbance	500	99.16	0.84		
		3	Absorbance	250	98.24	1.76		
		3	Absorbance	125	96.10	3.90		
		3	Absorbance	62.5	89.55	10.45		
		3	Absorbance	31.25	87.20	12.80		
		3	Absorbance	15.625	84.07	15.93		
		3	Absorbance	7.8125	79.85	20.15		
Bark (DCM)	<i>Staphylococcus aureus</i> , 25923)	3	Absorbance	1000	100.00	0.00	N/A	N/A
		3	Absorbance	500	90.04	9.96		
		3	Absorbance	250	90.30	9.70		
		3	Absorbance	125	86.14	13.86		
		3	Absorbance	62.5	91.02	8.98		
		3	Absorbance	31.25	92.88	7.12		
		3	Absorbance	15.625	88.87	11.13		
		3	Absorbance	7.8125	81.00	19.00		
		3	Absorbance	1000	98.54	1.46		
		3	Absorbance	500	95.16	4.84		
		3	Absorbance	250	92.91	7.09		
		3	Absorbance	125	82.57	17.43		

Root (DCM)	<i>Staphylococcus aureus</i> , 25923)	3	Absorbance	62.5	90.71	9.29	N/A	N/A
		3	Absorbance	31.25	89.98	10.02		
		3	Absorbance	15.625	83.03	16.97		
		3	Absorbance	7.8125	73.29	26.71		
Polymyxin B Sulphate	<i>Staphylococcus aureus</i> , 25923)	3	Absorbance	1000	3.46	96.54	< 250	< 250
		3	Absorbance	500	3.60	96.40		
		3	Absorbance	250	19.83	80.17		
		3	Absorbance	125	35.24	64.76		
		3	Absorbance	62.5	87.84	12.16		
		3	Absorbance	31.25	94.98	5.02		
		3	Absorbance	15.625	100.00	0.00		
		3	Absorbance	7.8125	95.42	4.58		

Root (DCM)	Staphylococcus aureus, 25923)	3	Absorbance	62.5	90.71	9.29	N/A	N/A
		3	Absorbance	31.25	89.98	10.02		
		3	Absorbance	15.625	83.03	16.97		
		3	Absorbance	7.8125	73.29	26.71		
Polymyxin B Sulphate	Staphylococcus aureus, 25923)	3	Absorbance	1000	3.46	96.54	< 250	< 250
		3	Absorbance	500	3.60	96.40		
		3	Absorbance	250	19.83	80.17		
		3	Absorbance	125	35.24	64.76		
		3	Absorbance	62.5	87.84	12.16		
		3	Absorbance	31.25	94.98	5.02		
		3	Absorbance	15.625	100.00	0.00		
		3	Absorbance	7.8125	95.42	4.58		

Across all DCM extracts (leaf, bark, and root), no MIC or IC₅₀ could be determined within the tested concentration range (7.8–1000 µg/mL), (Table 23 and Figure 16). The leaf DCM extract demonstrated an unprecedented highest activity among the plant-derived samples, with a maximum inhibition of ~68.6% observed only at the highest concentration (1000 µg/mL), while inhibition at lower concentrations remained ≤20%. Bark and root DCM extracts exhibited consistently weak anti-bacterial effects, generally producing ≤27% inhibition even at the lowest tested concentrations, and no clear dose-response sufficient to define MIC or IC₅₀ values.

Table 25: MIC and IC50 summary of the assessment of Methanol (MeOH) extracts vs Staphylococcus aureus ATCC 25923

Sample ID	Organism	Replicas	Readout	Concentration (µg/ml)	% Viability	% Inhibition	MIC	IC50
		3	Absorbance	1000	41.51	58.49		
		3	Absorbance	500	44.17	55.83		

Root (DCM)	Staphylococcus aureus, 25923)	3	Absorbance	62.5	90.71	9.29	N/A	N/A
		3	Absorbance	31.25	89.98	10.02		
		3	Absorbance	15.625	83.03	16.97		
		3	Absorbance	7.8125	73.29	26.71		
Polymyxin B Sulphate	Staphylococcus aureus, 25923)	3	Absorbance	1000	3.46	96.54		
		3	Absorbance	500	3.60	96.40		
		3	Absorbance	250	19.83	80.17		
		3	Absorbance	125	35.24	64.76		
		3	Absorbance	62.5	87.84	12.16		

		3	Absorbance	31.25	94.98	5.02	< 250	< 250
		3	Absorbance	15.625	100.00	0.00		
		3	Absorbance	7.8125	95.42	4.58		

		3	Absorbance	250	45.09	54.91		
		3	Absorbance	125	49.82	50.18		
Leaf (MeOH)	<i>Staphylococcus aureus, 25923</i>	3	Absorbance	62.5	77.21	22.79	>1000	~125
		3	Absorbance	31.25	96.28	3.72		
		3	Absorbance	15.625	100.00	0.00		
		3	Absorbance	7.8125	98.08	1.92		
		3	Absorbance	1000	36.21	63.79		
		3	Absorbance	500	28.53	71.47		
		3	Absorbance	250	31.11	68.89		
		3	Absorbance	125	35.51	64.49		
Bark (MeOH)	<i>Staphylococcus aureus, 25923</i>	3	Absorbance	62.5	41.81	58.19	>1000	~60–125
		3	Absorbance	31.25	57.47	42.53		
		3	Absorbance	15.625	83.50	16.50		
		3	Absorbance	7.8125	100.00	0.00		
		3	Absorbance	1000	45.67	54.33		
		3	Absorbance	500	26.93	73.07		
		3	Absorbance	250	35.53	64.47		
		3	Absorbance	125	39.02	60.98		
Root (MeOH)	<i>Staphylococcus aureus, 25923</i>	3	Absorbance	62.5	45.65	54.35	>1000	~60–125
		3	Absorbance	31.25	61.37	38.63		
		3	Absorbance	15.625	87.95	12.05		
		3	Absorbance	7.8125	100.00	0.00		
		3	Absorbance	1000	3.91	96.09		
		3	Absorbance	500	5.72	94.28		
		3	Absorbance	250	20.04	79.96		
		3	Absorbance	125	30.55	69.45		
Polymyxin B Sulphate	<i>Staphylococcus aureus, 25923</i>	3	Absorbance	62.5	86.12	13.88	<250	<250
		3	Absorbance	31.25	98.65	1.35		
		3	Absorbance	15.625	100.00	0.00		
		3	Absorbance	7.8125	96.35	3.65		

As reported in Table 24 and Figure 10, none of the MeOH plant extracts achieved $\geq 90\%$ growth inhibition within the tested concentration range (7.8–1000 $\mu\text{g/mL}$), indicating MIC values $>1000 \mu\text{g/mL}$ for all three extracts. The leaf MeOH extract

showed moderate activity, reaching ~50% inhibition at 125 µg/mL, corresponding to an $IC_{50} \approx 125$ µg/mL. The bark MeOH extract exhibited stronger inhibition at higher concentrations (~64–71% at 125–500 µg/mL), with an estimated IC_{50} between ~60–125 µg/mL. The root MeOH extract demonstrated comparable moderate potency, achieving ~55–73% inhibition at 62.5–500 µg/mL, yielding an IC_{50} in the ~60–125 µg/mL range. As expected, the positive control polymyxin B sulphate displayed potent activity with ≥90% inhibition at low concentrations (MIC within the tested range), validating assay performance.

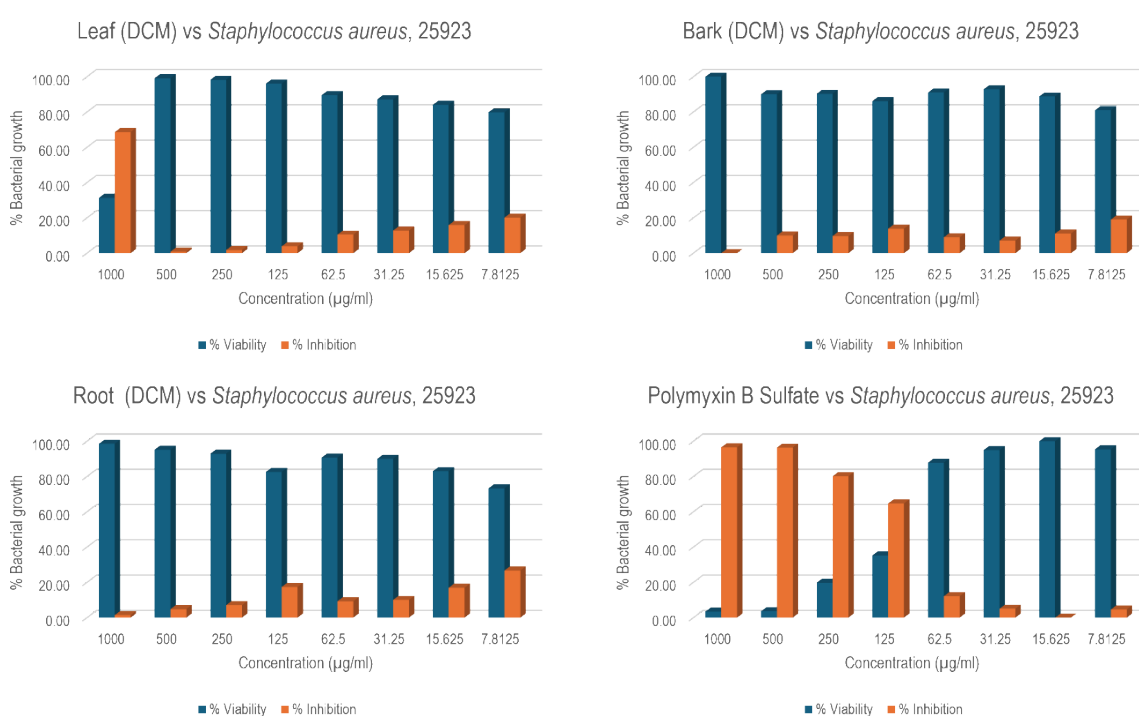


Figure 16: % viability and % inhibition summary of DCM extracts vs *Staphylococcus aureus* 25923

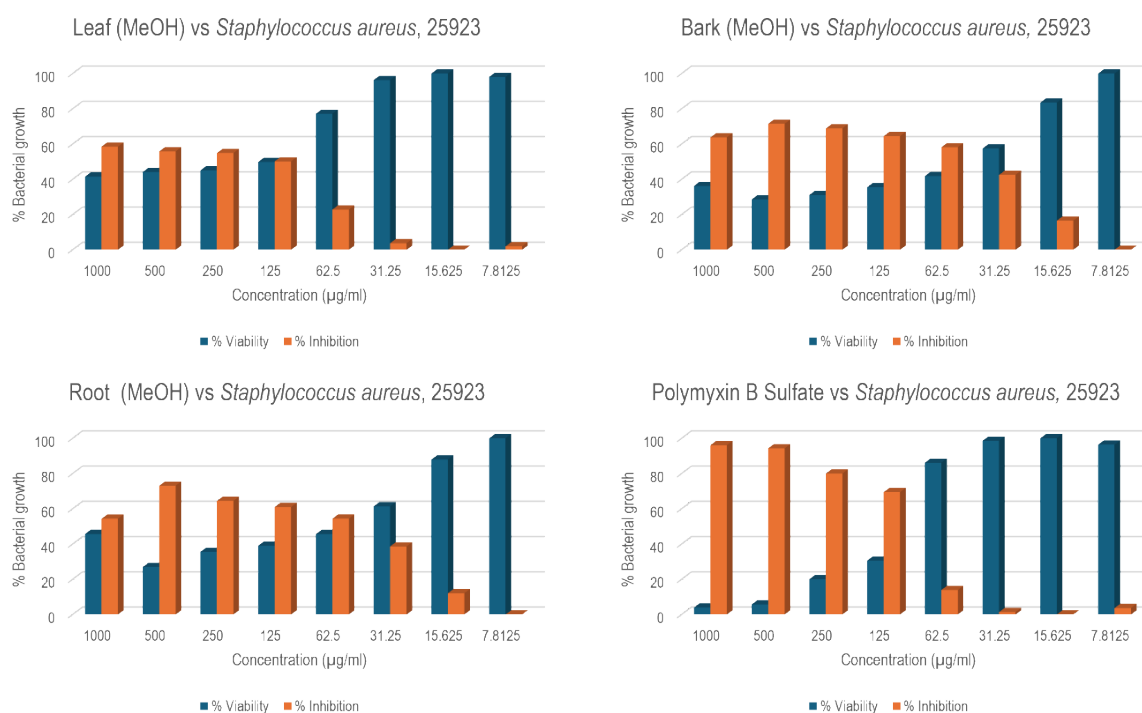


Figure 17: % viability and % inhibition summary of MeOH extracts vs *Staphylococcus aureus* 25923

Across the anti-bacterial screening Figures 8 to 17, clear solvent and strain dependent susceptibility patterns observed among *S.aureus*, *E. coli*, and *K.pneumoniae*. Overall, methanolic MeOH extracts exhibited the strongest anti-bacterial activity, with *S.aureus* remaining the most susceptible strain, showing the highest levels of growth inhibition. Notably, *K.pneumoniae* displayed a more pronounced inhibitory response to the solvent extracts than *E. coli*, particularly for the MeOH extracts, indicating greater sensitivity of *K.pneumoniae* to the extracted bioactive constituents. Aqueous extracts demonstrated moderate anti-bacterial activity, again with *S.aureus* as the most susceptible strain, while *K.pneumoniae* showed consistently greater inhibition than *E. coli*, albeit at lower levels than those observed for MeOH extracts. In contrast, DCM extracts exhibited the weakest anti-bacterial effects overall, with low inhibition across all strains; however, even under these conditions, *K.pneumoniae* tended to be slightly more susceptible than *E. coli*, reinforcing the relative resistance of *E.coli* across solvent systems. Importantly, polymyxin, included as a positive control, consistently induced near-complete inhibition across all tested strains, validating assay robustness and providing a reliable benchmark for extract performance. Collectively, these results highlight the strong influence of extraction solvent on anti-bacterial efficacy and demonstrate that, across solvent systems, *K.pneumoniae* is more susceptible than

E.coli, while MeOH extracts show the greatest overall anti-bacterial potential, particularly against *S.aureus*.

6.5 CONCLUSION

This study demonstrates that methanolic plant extracts possess the greatest anti-bacterial potential, particularly against *S.aureus* ATCC 25923, with moderate activity also evident against *K.pneumoniae* ATCC 700603. In contrast, *Escherichia coli* ATCC 25922 exhibited pronounced resistance to all tested extracts. The anti-bacterial screening of *S.rhemaniana* extracts (aqueous, DCM, and MeOH extracts from leaf, bark, and root tissues) revealed a clear solvent- and strain-dependent activity profile. Overall, MeOH extracts demonstrated the greatest anti-bacterial potency, followed by aqueous extracts with moderate activity, while DCM extracts were largely inactive. Among the tested organisms, *S.aureus* ATCC 25923 was consistently the most susceptible, *K.pneumoniae* ATCC 700603 showed intermediate susceptibility, and *E.coli* ATCC 25922 was the most resistant across all solvent systems. The DCM extracts (leaf, bark, and root) were predominantly inactive across all three bacterial strains, failing to reach either MIC or IC₅₀ values within the tested concentration range (7.8–1000 µg/mL). Similarly, all extract types against *E.coli* ATCC 25922 (aqueous, DCM, and MeOH) exhibited weak inhibition (<25%), indicating intrinsic resistance of this strain and a lack of meaningful anti-bacterial efficacy under the experimental conditions.

The most promising anti-bacterial activity was observed for MeOH extracts against *S.aureus* ATCC 25923, particularly bark and root MeOH extracts, which achieved IC₅₀ values in the ~60–125 µg/mL range. Aqueous bark and root extracts also showed moderate, concentration-dependent inhibition against *S.aureus* and *K.pneumoniae*, with IC₅₀ values generally between ~125–500 µg/mL. Against *K.pneumoniae*, MeOH extracts, especially bark, displayed consistent mid-range inhibitory activity, identifying this strain as a secondary susceptible target, particularly for polar solvent extracts. The inclusion of polymyxin B sulphate, alongside doxycycline and kanamycin, confirmed excellent assay performance and sensitivity. Polymyxin B consistently produced near complete (>90%) growth inhibition across all strains at low concentrations, establishing a robust reference benchmark. The clear separation between growth,

vehicle, and positive controls validated assay robustness, reproducibility, and suitability for MIC and IC₅₀ estimation. Key findings include that extraction solvent critically influenced anti-bacterial activity, with MeOH, aqueous finally by DCM. Gram-positive *S.aureus* was the most susceptible organism, showing the strongest and most consistent inhibition. *K.pneumoniae* was more susceptible than *E.coli*, particularly to MeOH and aqueous extracts. No plant extract achieved bactericidal MIC thresholds ($\geq 90\%$ inhibition) within the tested range, indicating predominantly bacteriostatic or moderate inhibitory effects. The observed activity patterns were dose-dependent and reproducible, supporting biological relevance rather than assay artefact. Although none of the extracts reached definitive MIC values under the current conditions, several MeOH and aqueous extracts displayed IC₅₀ values within biologically relevant ranges, justifying further bioassay-guided fractionation, phytochemical characterization, and expanded anti-microbial profiling. Overall, the work provides a robust, well-controlled foundation for prioritizing polar extracts in downstream anti-bacterial discovery efforts.

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CHAPTER 7: SUMMARY AND CONCLUSIONS

Medicinal plants have long served as an important source of therapeutic agents for the management of human diseases. A significant proportion of currently available pharmaceutical drugs originate either directly from plant sources or from chemical derivatives of plant-derived compounds. In recent decades, the search for novel bioactive compounds from medicinal plants has intensified due to the growing prevalence of chronic diseases such as cancer, the emergence of antimicrobial resistance, and the limitations associated with conventional therapies. Natural products provide structurally diverse chemical scaffolds that may exhibit potent biological activities while potentially offering improved safety profiles compared with synthetic drugs. The genus *Searsia*, belonging to the *Anacardiaceae* family, has attracted increasing attention in pharmacological research due to its rich phytochemical composition and its extensive use in traditional medicine. Previous phytochemical investigations of species within this genus have revealed the presence of flavonoids, phenolic acids, tannins, terpenoids, and other secondary metabolites that possess anti-oxidant, anti-inflammatory, anti-microbial, and anti-cancer activities. Despite the recognised medicinal potential of this genus, many *Searsia* species remain underexplored with respect to their pharmacological properties. The present study investigated the biological activities of crude extracts derived from *S.rhemanniana*, with particular emphasis on their cytotoxic, genotoxic, cell cycle, anticancer, and antibacterial properties. The research focused on evaluating the effects of extracts obtained from bark, leaves, and roots of the plant using solvents of varying polarity, including DCM, MeOH, and water. Biological evaluation was conducted using established *in vitro* models including the DU145 prostate cancer cell line, the HeLa cervical cancer cell line, and Vero cells as a model of non-cancerous mammalian cells.

In this study the *Searsia* genus, belonging to the *Anacardiaceae* family, was used due to richness in bioactive compounds such as flavonoids, phenolic acids, and tannins, which exhibit potent anti-inflammatory, antibacterial, antioxidant, and anticancer properties. There is a scientific record regarding its possible use in the treatment of cancer globally. This has drawn scientific interest, especially regarding their potential in cancer treatment. This study examined in its medicinal properties with a particular

focus on their anticancer activities. The anti-inflammatory properties of this plant, coupled with their antioxidant activities, suggest a promising role in cancer prevention and treatment, although further research is necessary to confirm these benefits in clinical settings. This study embarked on *in vitro* cell cycle analysis of crude extracts of *S.rhemanniana* extracts against DU145 cancer cells. This study assessed the effects of crude *S.rhemanniana* extracts on cell cycle progression in the human prostate cancer cell line DU145. Selection of this test was informed by understanding that prostate cancer progression is characterized by extensive disruption of normal cell-cycle regulatory mechanisms and the ability of cancer cells to evade programmed cell death. These alterations lead to uncontrolled cellular proliferation, genomic instability, and resistance to therapeutic interventions. The results of study showed that *S.rhemanniana* possesses a diverse array of bioactive constituents that can specifically influence the survival and behaviour of prostate cancer cells. These compounds appear to act through various molecular signalling mechanisms, suggesting their potential to modulate key cellular processes such as proliferation, apoptosis, and differentiation in a selective and targeted manner. These results warranted further testing and this evaluated the safety and effectiveness of the plants by using extracts of human prostate cancer cells. This study evaluated the cytotoxicity activity of the crude extracts against human cell line DU145, due to the importance of the safety around the plants. Prostate cancer is the second-most common cancer among men globally and the leading cancer in African men. Current treatments are often inaccessible or limited by resistance. Natural products, particularly from underexplored plants like *S.rhemanniana*, offer a promising alternative for anticancer drug discovery. Among the nine extracts tested, bark DCM, leaf DCM and roots DCM demonstrated IC_{50} values below 100 $\mu\text{g/mL}$, indicating significant cytotoxicity. The remaining extracts showed moderate to negligible effects. R^2 values from nonlinear regression confirmed reliable curve fitting. *S.rhemanniana* exhibits promising antiproliferative activity, justifying further research into its potential as a source of new anticancer agents.

One of the central objectives of this study was to evaluate the cytotoxic and cell cycle regulatory effects of *S.rhemanniana* extracts on prostate cancer cells. Prostate cancer remains one of the most frequently diagnosed malignancies among men worldwide and represents the leading cancer affecting African men. Despite advances in medical

treatments, therapeutic resistance and limited access to effective therapies remain major challenges in many regions. The DU145 prostate cancer cell line was selected as an experimental model because it represents an androgen independent prostate carcinoma that is commonly associated with advanced and treatment-resistant forms of the disease. Unlike androgen-dependent prostate cancer models, DU145 cells exhibit characteristics associated with aggressive tumour behaviour, including resistance to apoptosis and dysregulation of normal cell cycle control mechanisms. Normal cell cycle progression is tightly regulated through the coordinated action of cyclins, cyclin-dependent kinases (CDKs), and tumour suppressor proteins such as p53 and retinoblastoma protein (Rb). Disruption of these regulatory pathways results in uncontrolled cellular proliferation, genomic instability, and tumour progression. Consequently, compounds capable of interfering with cell-cycle progression represent promising candidates for anticancer drug development.

The results obtained in this study demonstrated that crude extracts of *S.rhemanniana* exerted measurable effects on DU145 cell viability and proliferation. Several extracts, particularly those obtained using dichloromethane, exhibited significant cytotoxic activity as evidenced by reduced cell viability and low IC₅₀ values. These findings suggest that *S.rhemanniana* contains bioactive constituents capable of interfering with cellular processes essential for tumour cell survival. The observed antiproliferative effects may be attributed to the presence of phytochemical compounds such as flavonoids and phenolic compounds, which have been widely reported to induce cell cycle arrest and apoptosis in various cancer-cell models. In addition, phenolic compounds are known to exert antioxidant effects that may modulate oxidative stress within tumour cells. Cancer cells often exhibit elevated levels of reactive oxygen species (ROS), which contribute to DNA damage and genomic instability. Certain phytochemicals can either suppress ROS production or induce oxidative stress beyond tolerable levels within cancer cells, ultimately triggering apoptosis. The cytotoxic activity observed in this study therefore suggests that the bioactive constituents of *S.rhemanniana* may influence multiple molecular targets involved in cancer-cell survival and proliferation. The anticancer potential of *S.rhemanniana* extracts was further investigated using the HeLa cervical cancer cell line.

Cervical cancer remains one of the most common cancers affecting women globally and is particularly prevalent in low- and middle income countries where access to screening and treatment is limited. The results obtained from this study demonstrated that extracts prepared using dichloromethane consistently exhibited the strongest cytotoxic activity against HeLa cells. These extracts produced dose dependent reductions in cell viability and demonstrated relatively low IC_{50} values when compared with methanolic and aqueous extracts. Notably, the bark and root DCM extracts exhibited the most potent antiproliferative effects, with IC_{50} values ranging between approximately 22.7 $\mu\text{g/mL}$ and 43.4 $\mu\text{g/mL}$. These results suggest that bioactive compounds responsible for anticancer activity are likely concentrated within this plant. The differential activity observed between extracts highlights the importance of plant-part-specific phytochemical distribution within medicinal plants.

Previous phytochemical studies have demonstrated that secondary metabolites often accumulate preferentially in specific plant organs as part of their ecological defence mechanisms against herbivores, pathogens, and environmental stress. The strong cytotoxic activity observed in DCM extracts may be explained by the ability of relatively non-polar solvents to extract lipophilic phytochemicals such as terpenoids, triterpenes, and certain alkaloids. These classes of compounds have been widely reported to exhibit potent anticancer properties through mechanisms including mitochondrial dysfunction, apoptosis induction, inhibition of angiogenesis, and suppression of tumour metastasis. Statistical analysis using two-way ANOVA revealed that both solvent polarity and plant tissue type significantly influenced cytotoxic outcomes. This finding underscores the importance of extraction methodology in phytochemical research and highlights the need for systematic solvent selection in the identification of biologically active compounds. An important consideration in anticancer drug development is the ability of therapeutic agents to selectively target malignant cells while minimizing toxicity towards normal cells. To evaluate the selectivity of *S.rhemanniana* extracts, the SI was calculated using IC_{50} values obtained from normal Vero cells. The results indicated that the DCM bark extract exhibited the highest selectivity index ($SI > 1$), suggesting preferential toxicity towards cancer cells relative to non-cancerous cells. This finding is particularly important, because many conventional chemotherapeutic agents lack selectivity and can cause significant toxicity in healthy tissues. The selective cytotoxicity observed in this study may reflect

the ability of certain phytochemicals to target cellular processes that are more active or dysregulated in cancer cells, such as increased metabolic activity, oxidative stress, and altered signalling pathways. These characteristics make tumour cells more vulnerable to specific molecular interventions. The demonstration of selective anticancer activity therefore supports the potential therapeutic relevance of *S.rhemanniana* extracts and provides justification for further investigation into their underlying mechanisms of action.

While cytotoxicity studies provide valuable information regarding the anticancer potential of plant extracts, safety evaluation remains an equally critical aspect of medicinal plant research. Herbal preparations may contain compounds capable of inducing DNA damage or chromosomal instability, which could pose significant health risks if used therapeutically. In the present study, the genotoxic potential of *S.rhemanniana* extracts was evaluated using the micronucleus assay in Vero cells. The micronucleus test is widely regarded as a reliable method for detecting chromosomal damage resulting from clastogenic or aneugenic mechanisms. The results indicated that the aqueous bark extract induced a significant increase in micronucleus formation across all tested concentrations. This observation suggests that certain compounds present in the bark may interact with DNA or interfere with mitotic spindle formation during cell division. Similarly, the DCM bark extract and methanolic leaf extract also produced statistically significant increases in the frequency of micro-nucleated cells. These findings indicate that some phytochemicals present in these extracts may possess genotoxic properties under certain conditions. However, it is important to interpret these results cautiously.

Genotoxic responses observed *in vitro* do not necessarily translate directly into genotoxic effects *in vivo*, as metabolic detoxification pathways present in whole organisms may modify the activity of these compounds. Nevertheless, the findings highlight the importance of further toxicological evaluation, including *in vivo* genotoxicity studies and detailed phytochemical characterization. In addition to investigating anticancer activity, this study also evaluated the antibacterial potential of *S.rhemanniana* extracts against clinically important bacterial pathogens. AMR has emerged as one of the most pressing global health challenges, threatening the effectiveness of existing antibiotics. The results demonstrated that methanolic extracts

exhibited the strongest antibacterial activity, particularly against *S.aureus* ATCC 25923. Bark and root extracts displayed moderate inhibitory activity with IC₅₀ values ranging between approximately 60 and 125 µg/mL. The differential susceptibility observed among bacterial strains is consistent with previously reported differences in cell-wall structure between Gram-positive and Gram-negative bacteria. Gram-negative bacteria such as *E.coli* possess an outer membrane that restricts the penetration of many antimicrobial compounds, which may explain the resistance observed in this study. The predominantly bacteriostatic activity observed suggests that the extracts inhibit bacterial growth rather than causing direct bacterial cell death. This type of activity may still be therapeutically valuable, as bacteriostatic agents can limit infection progression while allowing host immune responses to eliminate pathogens.

The identification of antibacterial activity in *S.rhemanniana* further supports the traditional medicinal use of this plant and highlights its potential as a source of novel antimicrobial agents. In conclusion the results of this study demonstrate that *S.rhemanniana* possesses significant biological activity, including cytotoxic, anticancer, genotoxic, and antibacterial properties. Dichloromethane extracts derived from bark and root exhibited particularly strong antiproliferative effects against prostate and cervical cancer cell lines, while methanolic extracts demonstrated notable antibacterial activity against *S.aureus*. The selective cytotoxicity observed in certain extracts suggests that *S.rhemanniana* may represent a promising source of novel anticancer compounds. However, the identification of genotoxic responses in some extracts highlights the importance of comprehensive toxicological evaluation. Overall, the findings of this study contribute to the growing body of scientific evidence supporting the medicinal potential of *Searsia* species and provide a foundation for future investigations aimed at developing plant-derived therapeutic agents.

Furthermore, the study assessed the *in vitro* genotoxicity screening of *S.rhemanniana* extracts against vero cells. Genotoxicity assessment is an essential part of the framework for the development of herbal products and risk assessment. As the use of traditional medicinal products increases as well as regulatory control increases, the integration of genotoxic endpoints with standard toxicology is becoming a key requirement for public health. Bark water extract produced a significant increase in

micronucleus formation across all tested concentrations, indicating that it may contain constituents capable of interacting directly with DNA or interfering with the mitotic spindle apparatus. Also, bark DCM extract induced a significant genotoxic response, despite exhibiting cytotoxicity at higher concentrations. Leaves MeOH also demonstrated a statistically significant increase in the proportion of micro-nucleated cells within the concentration. The micronucleus assay confirmed that bark water, bark DCM, and leaves MeOH, possess genotoxic potential, as evidenced by a significant increase in the frequency of micro-nucleated cells. The study also assessed the anticancer profiles of *S.rhemanniana* extracts and tissue variability in Hela cervical cells. Cervical cancer is the fourth-most common cancer among women globally. Resistance frequently restricts or prevents access to current medicines. Natural products present a possible substitute for anticancer drug discovery, especially those derived from understudied plants like *S.rhemanniana*. Across all exposure periods, DCM extracts consistently produced the most potent and dose-dependent suppression of cell viability, characterized by significantly lower IC_{50} values and high curve fitting reliability ($R^2 = 0.95\text{--}0.99$) compared to aqueous and methanolic extracts.

Notably, the DCM bark and root fractions showed the strongest activity, with IC_{50} values ranging between 22.7 and 43.4 $\mu\text{g/mL}$, while the leaf DCM extract displayed moderate effects. Two-way ANOVA revealed that both solvent type and plant tissue had a significant impact on cytotoxic outcomes ($p < 0.001$). The SI, determined using IC_{50} values from normal Vero cells, showed that the DCM bark extract possessed the highest selectivity ($SI > 1$), indicating preferential toxicity towards cancer cells while sparing non-cancerous cells. The overall potency trend (DCM > MeOH > Aqueous) underscores the importance of solvent polarity in isolating lipophilic bioactive compounds such as terpenoids and alkaloid derivatives. Collectively, these results identify the DCM bark and root extracts as promising candidates for the development of selective, plant-derived anticancer agents, warranting further phytochemical and mechanistic investigations. Also, the assessment of quantitative antibacterial profiling of leaf, bark and root extracts of *S.rhemanniana* uses standardized MIC and IC_{50} determination. AMR is a major global public health concern that threatens the effective management of infectious diseases. The increasing resistance of clinically important pathogens such as *S.aureus*, *E. coli*, and *K.pneumoniae* has intensified the search for alternative antimicrobial agents, particularly from medicinal plants with diverse

bioactive compounds. Results demonstrated a clear solvent- and strain-dependent activity profile. Methanolic extracts exhibited the greatest antibacterial activity, particularly against *S.aureus* ATCC 25923, with bark and root extracts showing IC₅₀ values of approximately 60–125 µg/mL. Aqueous extracts displayed moderate inhibition, while DCM extracts were largely inactive. *K. pneumoniae* showed intermediate susceptibility, whereas *E. coli* exhibited pronounced resistance across all extract types. None of the extracts achieved bactericidal MIC thresholds within the tested concentration range, indicating predominantly bacteriostatic effects. These findings highlight the antibacterial potential of polar extracts of *S.rhemanniana* and support further bioassay guided fractionation and phytochemical investigation for antimicrobial drug discovery.

7.1 CONCLUSION

The results showed that *S.rhemanniana* possesses a diverse array of biologically active phytochemicals capable of exerting cytotoxic, genotoxic, anticancer, and antibacterial effects *in vitro*. The results highlight the significant pharmacological potential of this plant species, particularly the dichloromethane extracts of bark and root tissues, which exhibited strong and selective antiproliferative activity against cancer-cell lines.

7.2 LIMITATIONS OF THE STUDY

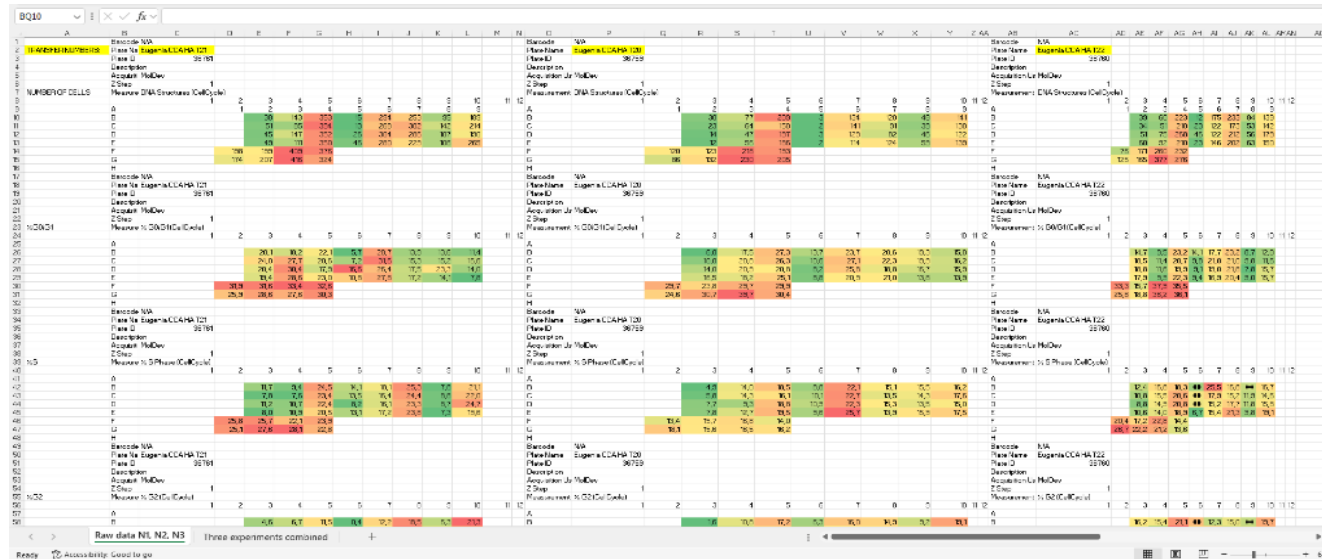
The present study was limited by the number of microbial organisms tested and the absence of advanced compound identification techniques. Although further phytochemical analysis using Gas Chromatography–Mass Spectrometry (GC-MS) could have enabled the identification of bioactive constituents responsible for the observed activity, this was not undertaken due to the scope of the study objectives and financial constraints associated with such analyses.

7.3 RECOMMENDATIONS

Future studies on this plant may investigate bioassay-guided fractionation to isolate and identify the specific phytochemicals responsible for the observed biological activities. Advanced analytical techniques such as GCMS, LC-MS, HPLC, and NMR

spectroscopy may be employed for structural characterization. Studies should also be conducted to determine how these compounds interact with molecular targets involved in cancer-cell survival and proliferation.

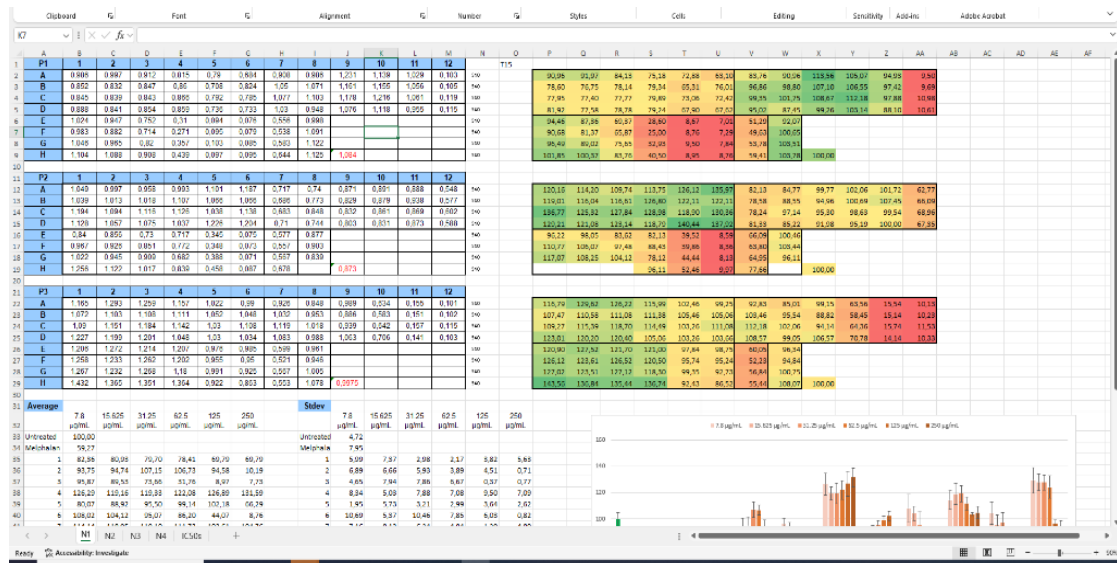
Appendix A: Cell Analysis Combined



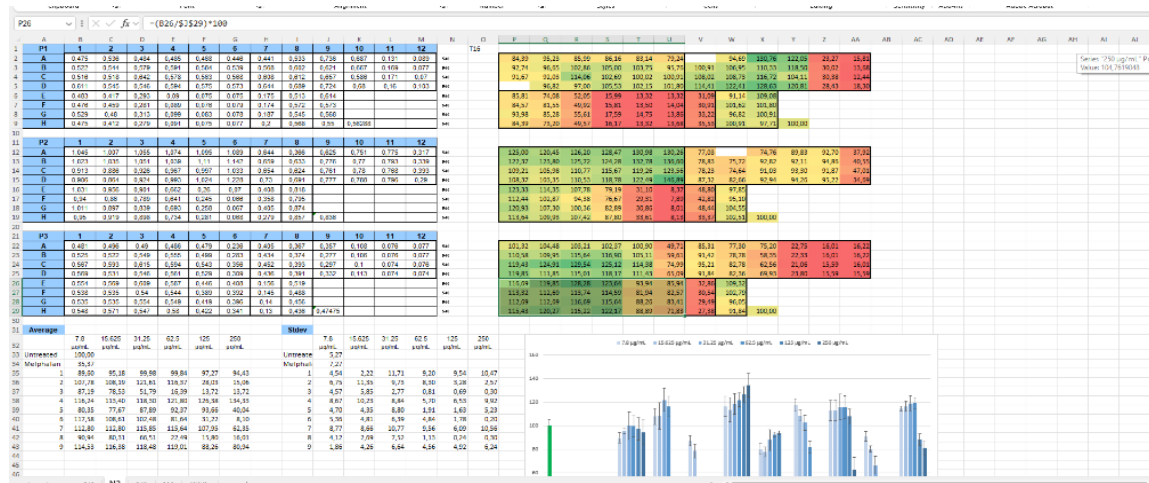
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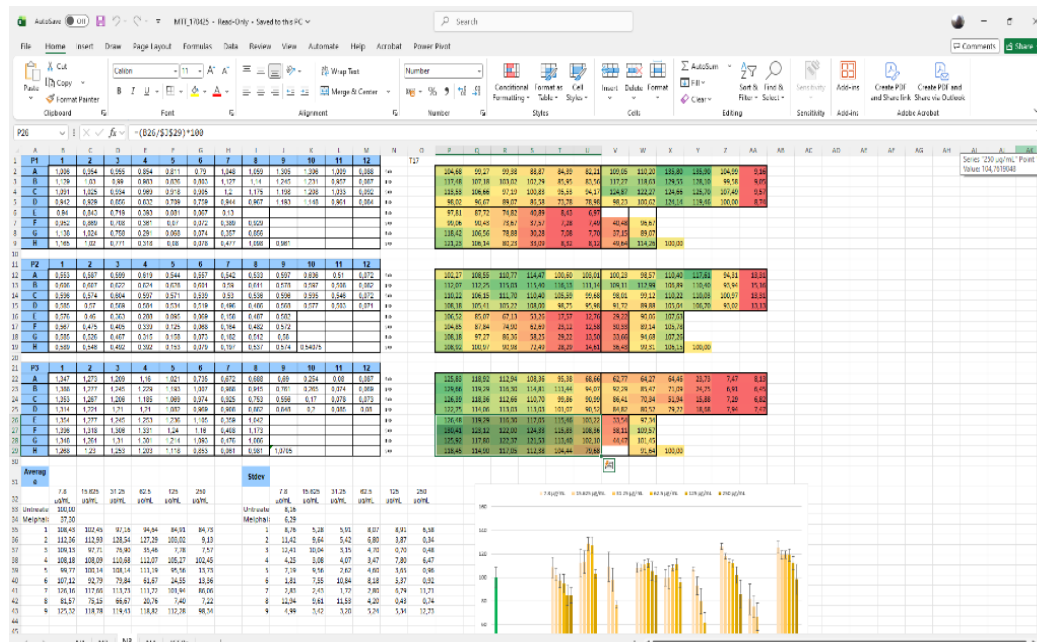
Appendix B: MTT_170425 (N1)



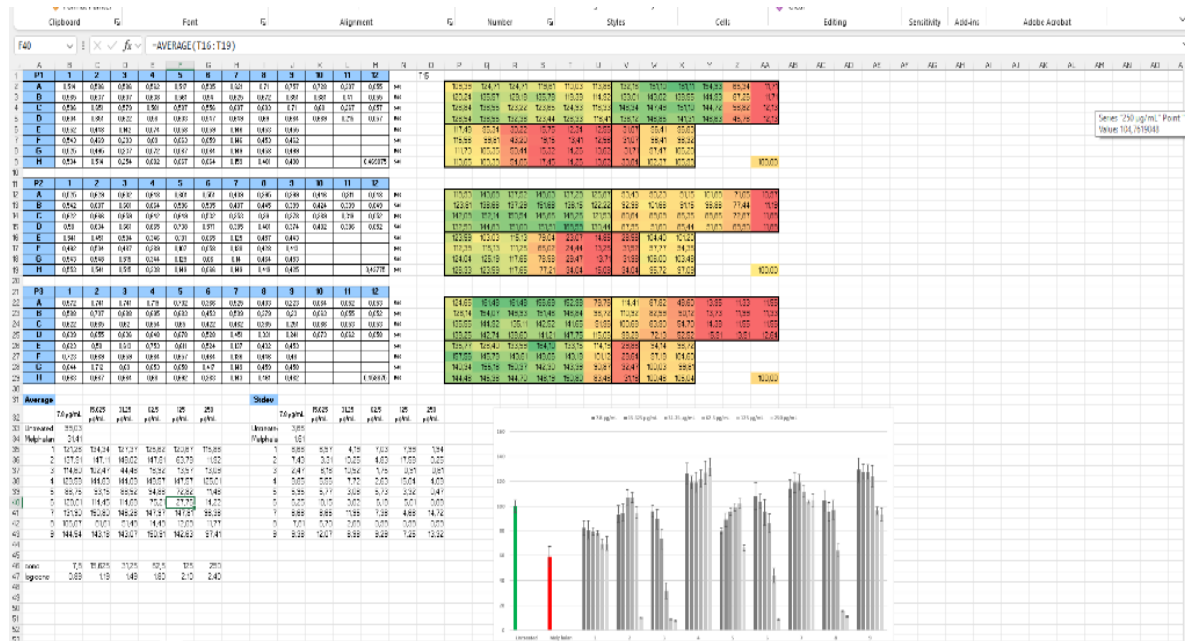
Appendix C: (N2)



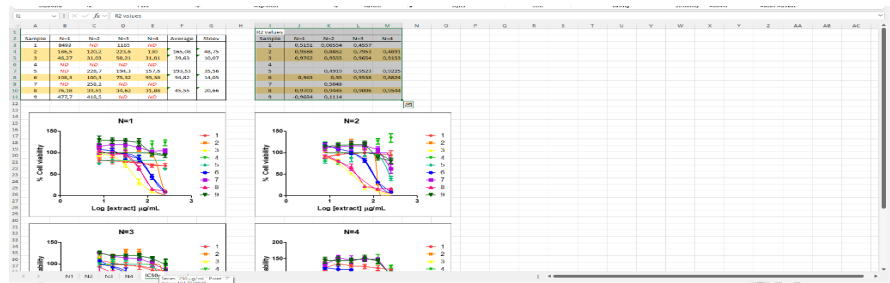
Appendix D: N3



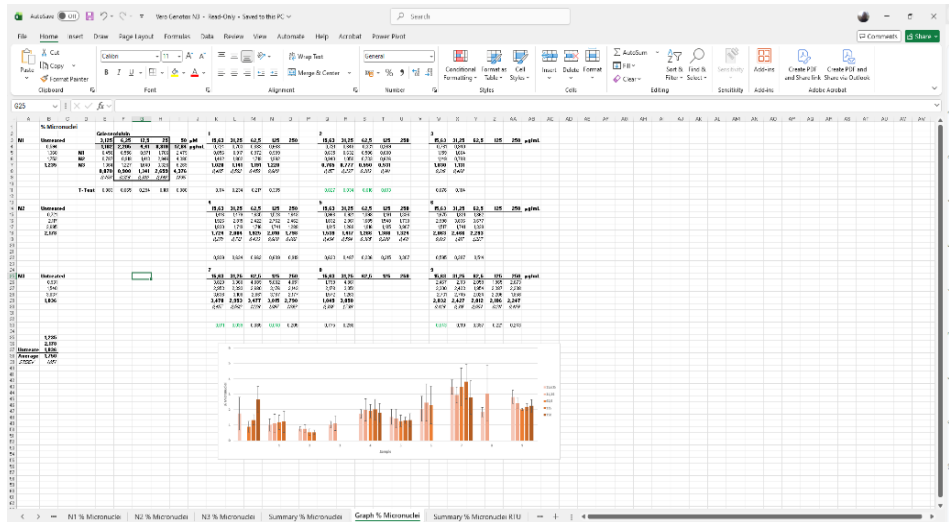
Appendix E: N4



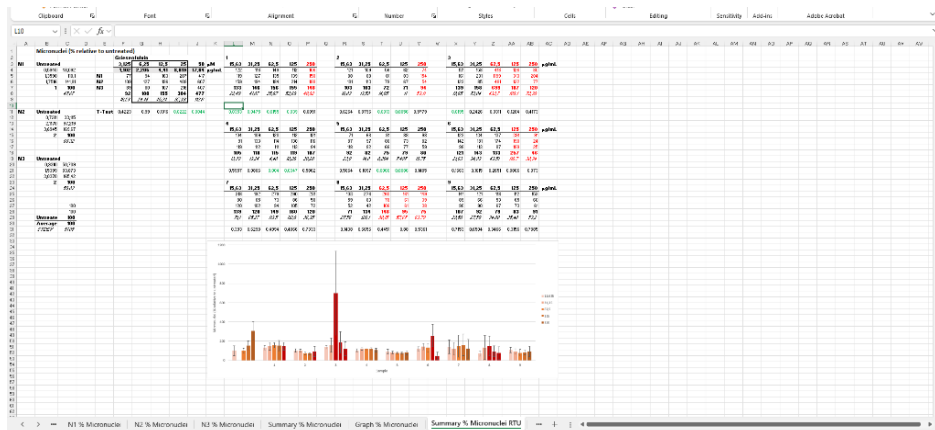
Appendix F: IC50



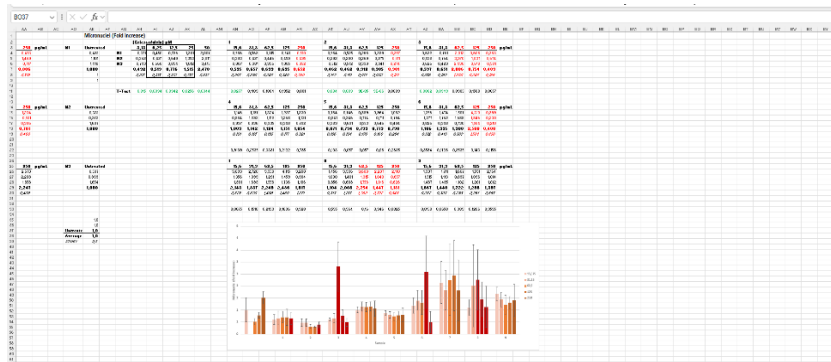
Appendix I: Graph % Micronuclei



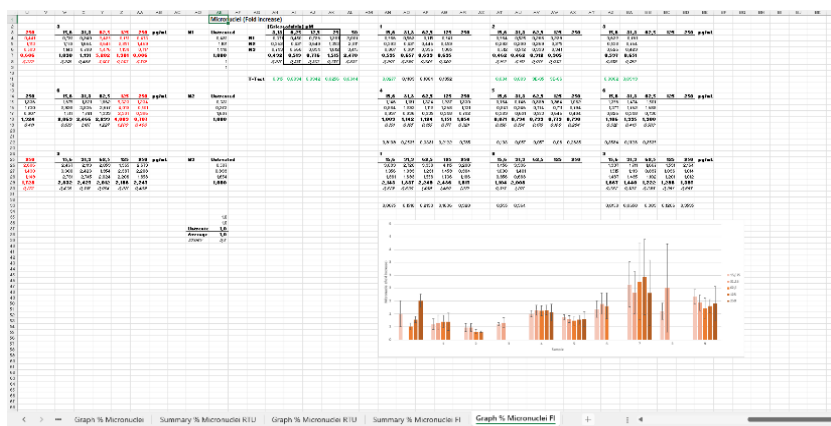
Appendix J: Summary % MiconucleiRTU



Appendix K: Summary % Micronuclei (FI) Fold Increase



Appendix L: Graph% Micronuclei (FI) Fold Increase



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**SEARSIA RHEMANNIANA AS A POTENTIAL ANTICANCER AGENT:
UNVEILING MECHANISMS AND THERAPEUTIC POTENTIAL**

by

Dijeng Euginia Rampana

All changes were indicated by track changes and comments for the author to verify, clarify aspects that are unclear, make the necessary adjustments, check the referencing in particular and finalise. The editor takes no responsibility in the instance of this not being done. The document remains the final responsibility of the author.



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