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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 IC_{50}.
Results:
Melphalan, used as a positive control, showed the expected effects of a DNA alkylating agent by causing significant G2/M phase arrest and increased apoptosis, confirming DNA damage checkpoint activation. Among the plant extracts, the bark methanol and leaf water extracts of S. rhemanniana demonstrated the strongest cytotoxic activity, inducing apoptosis rates above 28% and 43%, respectively, without prior cell cycle arrest. In contrast, the bark and root dichloromethane (DCM) extracts caused notable accumulation of cells in early mitosis along with measurable apoptosis, indicating a distinct mechanism of action. Meanwhile, the aerial methanol extract exhibited a mechanistic profile like Melphalan, with marked G2 arrest and apoptosis, suggesting it may induce DNA damage or replication stress.
Conclusion:
The findings of the present study indicate 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.

Keywords: Searsia rhemanniana, cell cycle, prostate cancer, DU145

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 DNA damage and mitotic checkpoint defects, rendering cell-cycle–targeted strategies attractive (2). DU145 cells an androgen-independent, 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 G1/S/G2/M is regulated by cyclin-CDK complexes and surveillance checkpoints that delay division when damage or spindle errors are detected (7). The DNA damage response (DDR) engages ATM/ATR–CHK1/CHK2 signalling to enforce a G2/M block until lesions are repaired, 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 agent (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). BCL-2 family proteins integrate stress signals to determine MOMP, while 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 cytotoxic (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-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 prostate-specific antigen (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 toward diagnosis at earlier disease stages (31). The lifetime risk for men being diagnosed with PCa is approximately 17% (one in six), yet only about 3–4% (one in thirty) succumb to the disease (32). This indicates that most men with PCa do not develop clinically significant forms that impact 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). Prostate-Specific Antigen (PSA) is a naturally occurring enzyme secreted almost exclusively by the epithelial cells of the prostate and serves as a key serum biomarker for prostate cancer (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 due 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 (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 effectively estimates prostate volume and guides biopsy needle placement, 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 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 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 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 disorganized, 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 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.

MATERIALS AND METHODS

Plant material

The plant material was authenticated as Searsia rhemanniana by botanists from the botanical garden in Pietermatzburg, 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.

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 (H_2O) 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.

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 Ca^{2+} and Mg^{2+} were purchased from Cytiva (Marlborough, MA, USA). Foetal Bovine Serum (FBS) and penicillin/streptomycin were purchased from Biowest (Nuaillè, France).

Sample preparation
Extracts were solubilized using DMSO to yield a stock concentration of 100 mg/mL and were stored at 4°C until used.

Cell line maintenance
The human prostate cancer cell line, DU145 was used for cytotoxicity screening. Complete growth medium consisted of 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.

Phosphatidylserine (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 IC_{50} values as indicated in the table 1 below.

Table 1: IC_{50} values of extracts used against DU145 cancer cells.

Sample	N=1	N=2	N=3	N=4	Average	Stdev
Barks water	8493	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 µM) was used a positive control as indicated on table 2. Cells were stained according to Annexin V-FITC/PI Kit protocol (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 was diluted in binding buffer to a final concentration of 2 µg/mL and 5 µg/mL, respectively. Thereafter, treatments were aspirated from all wells and 100 µL of the prepared dyes were added to each well and incubated for 20 minutes at room temperature. Propidium iodide (PI) was prepared in binding buffer to a final concentration of 100 µg/mL and 10 µL was added to each well. Cells were imaged using the ImageXpress Micro XLS Widefield Microscope (Molecular Devices).

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.

Percentage of cells in each phase
T1, T2 and T3: Three different transfer numbers for the 3 experiments

Table 2: Melphalan (control) used in the study

	CONTROL				
Phase	**T1**	**T2**	**T3**	**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: Melphalan reaction with phases

	MELPHALAN					INTERPRETATION	T-TEST	
Phase	**T1**	**T2**	**T3**	**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				

Melphalan (Positive Control – DNA Alkylating Agent) showed significant increase in G2 phase ($p<0.05$) indicative of G2/M arrest, consistent with DNA crosslinking-induced checkpoint activation as indicated in table3.

Table 4: Barks water extract reaction with phases

	Barks water				
Phase	T1	T2	T3	**AVG**	SD
G0/G1	Extract interfered - images could not be analysed				
S					
G2					
Early M					
Late M					
Apoptosis					

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 perse (Table 4).

Table 5: Barks MeoH extract reaction with phases

	Barks MeoH					INTERPRETATION	T-TEST	
Phase	T1	T2	T3	**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				

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).

Table 6: Barks DCM extract reaction with phases

	Barks DCM						T-TEST	
Phase	T1	T2	T3	**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				

Likely disrupts mitotic progression (e.g., microtubule interference or spindle poison-like effect). Leaves MeoH extract showed minor decrease in G0/G1 (p<0.05), but no change in apoptosis or arrest (Table 7).

Table 7: Leaves MeoH extract reaction with phases

	Leaves MeoH						**T-TEST**	
Phase	**T1**	**T2**	**T3**	**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				

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.

Table 8: Leaves water extract reaction with phases

	Leaves water								T-TEST	
Phase	**T1**	**T2**	**T3**	**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						

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 toward G2 accumulation, with moderate apoptosis ($p<0.01$) (table 10)

Table 9: Leaves DCM extract reaction with phases

	leaves DCM								T-TEST	
Phase	**T1**	**T2**	**T3**	**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: Roots MeoH extract reaction with phases

	Roots MeoH								T-TEST	
Phase	**T1**	**T2**	**T3**	**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	0<0.01
	100,0	100,0	100,0	100,0						

Table 11: Roots DCM extract reaction with phases

	Roots DCM								**T-TEST**	
Phase	**T1**	**T2**	**T3**	**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						

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).

Table 12: Arterial extract reaction with phases

	Arterial MeoH								**T-TEST**	
Phase	T1	T2	T3	**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						

Mechanistically like Melphalan, potential DNA-damaging or replication stress-inducing compound. The tested extracts exhibited distinct mechanisms of growth inhibition. 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 extracts and leaves DCM extracts showed no meaningful activity, while barks water extract interfered with staining and requires protocol optimization. Collectively, these findings highlight barks MeoH extracts, leaves water extracts, barks DCM extracts, roots DCM extracts and arterial MeoH extracts as priority candidates for further mechanistic and molecular validation.

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 behaviors 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 signaling 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 extracts 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 extracts 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 fiber assembly or kinetochore attachment, analogous to the mechanism of taxanes or vinca alkaloids. Arrest at mitotic prometaphase leads to sustained activation of the spindle assembly checkpoint (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 polypharmacological 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.

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 **f**or further mechanistic dissection, metabolite profiling, and potency optimization. Future studies should include Western blot validation of checkpoint markers (e.g., Cyclin B1, p21, p53, γ-H2AX) and caspase cleavage assays to confirm terminal death pathways. Additionally, LC-MS/MS-guided fractionation will be essential to identify the responsible bioactive constituents and assess structure-activity relationships.

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