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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 cervical cancer cells over 24, 48, and 72 hours. Extracts derived from leaf, bark, and root tissues were compared to evaluate how solvent polarity and plant part influence biological activity. 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 = 0.95–0.99) 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 µg/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 selective index (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 toward 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.

KEYWORDS: Searsia rhemaniana, anticancer, cytotoxicity, HeLa Cells.

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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 cervical intraepithelial neoplasia grade 2 (CIN2) and cervical intraepithelial neoplasia grade 3 (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, thermoablation, loop electrosurgical excision (LEEP), and cold knife conization (CKC) (13).

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 tumor suppressor pathways: E6 binds to and degrades p53, while E7 targets the retinoblastoma (Rb) protein, resulting in uncontrolled cell proliferation and tumor formation (17).

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). Within sub-Saharan Africa, Eswatini, Malawi, and Zambia record the highest burden, driven by a combination of high prevalence of high-risk human papillomavirus (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 cervical intraepithelial neoplasia (CIN), which, if left untreated, can progress to invasive carcinoma over a period of 10–20 years (25). Socioeconomic 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.

Risk Factors for Cervical Cancer

Cervical cancer is primarily caused by persistent infection with high-risk types of human papillomavirus (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 behavioral, immunological, and socioeconomic 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 behavioral 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 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/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 socioeconomic status is a significant determinant, as it is often associated with reduced

access to HPV vaccination, limited screening participation, and higher prevalence of risk behaviors (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, behavioral 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 Computed Tomography (CT), Magnetic Resonance Imaging (MRI) or Positron Emission Tomography/Computed 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 Searsia 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 antioxidant and enzyme-inhibiting properties (51). However, further research is needed to fully understand the medicinal properties of this herb.

MATERIALS AND METHODS

Plant material

The plant material was authenticated as Searsia 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.

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 bioassaix (invitro screening for drugs) lab for further processing. At bioassay (invitro screening for drugs) lab powdered plant material was subjected the extraction of compounds 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 IC50 determination of extracts against HeLa Cells Tissue Variability

Sample preparation

All cell lines were subjected to varying doses of the test samples over time. The cytotoxic assessment of the samples was performed using an in vitro PrestoBlue and fluorescent assays. Data disseminating 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 cells; HeLa cell line (Human Cervical Carcinoma) and African green monkey kidney (VERO) cells – American Type Culture Collection, USA); Dulbecco's Modified Eagle Medium (DMEM) - (Sigma Aldrich, South Africa), Foetal bovine serum (FBS), Phosphate-buffered saline (PBS), Pen Strep, and 0.25% Trypsin 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).

Cell culture & 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.

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 was aspirated and 90 µL of the fresh was 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 essay, 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 (10µmg/ml) Hoechst 33324 in PBS. Culture plates were imaged using Cytation3 imaging reader.

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.

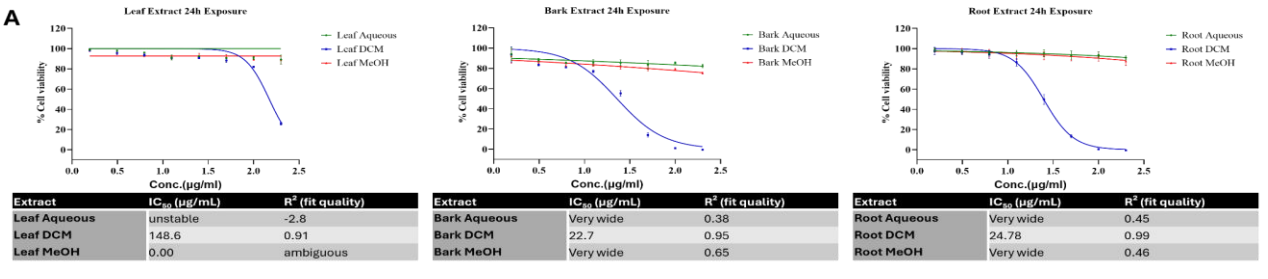
SI = IC50 for normal cell line / IC50 for the relevant malignant cell line.

A good SI value exceeds 1.0, indicating that the tested sample is more effective against tumour cells than it is toxic to normal cells. An SI value of 10 or higher suggests that the sample is a good candidate for more research. An SI value less than one indicates that the sample 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).

RESULTS AND DISCUSSION

24-hour anticancer activity of plant extracts assessment against HeLa cell Leaf, bark, and root extracts prepared using three solvents (aqueous, dichloromethane [DCM], and methanol [MeOH]) were evaluated for their cytotoxic activity against HeLa cells following 24 h exposure (Figure 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 (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.

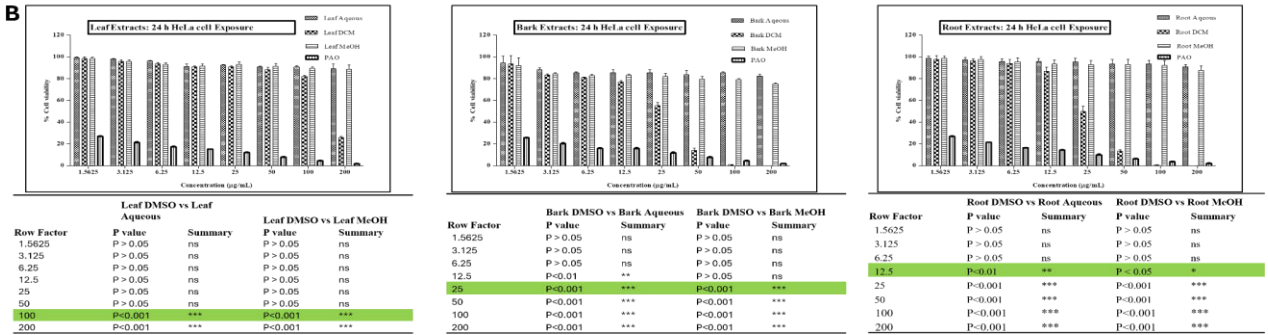
Two-way ANOVA confirmed that both extract type and concentration significantly affected cell viability ($P < 0.001$), particularly at 100, 25, and 12.5 µg/mL, as shown in the grouped bar plots (Figure 1B). The pattern of reduced viability observed exclusively in DCM-treated cells supports the presence 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 anticancer 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 anticancer compounds from this species.



Extract	IC_{50} (µg/mL)	R^2 (fit quality)
Leaf Aqueous	unstable	-2.8
Leaf DCM	148.6	0.91
Leaf MeOH	0.00	ambiguous

Extract	IC_{50} (µg/mL)	R^2 (fit quality)
Bark Aqueous	Very wide	0.38
Bark DCM	22.7	0.95
Bark MeOH	Very wide	0.65

Extract	IC_{50} (µg/mL)	R^2 (fit quality)
Root Aqueous	Very wide	0.45
Root DCM	24.78	0.99
Root MeOH	Very wide	0.46



Row Factor	Leaf DMSO vs Leaf Aqueous P value	Summary	Leaf DMSO vs Leaf MeOH P value	Summary
1.5625	P > 0.05	ns	P > 0.05	ns
3.125	P > 0.05	ns	P > 0.05	ns
6.25	P > 0.05	ns	P > 0.05	ns
12.5	P > 0.05	ns	P > 0.05	ns
25	P > 0.05	ns	P > 0.05	ns
50	P > 0.05	ns	P > 0.05	ns
100	P<0.001	***	P<0.001	***
200	P<0.001	***	P<0.001	***

Row Factor	Bark DMSO vs Bark Aqueous P value	Summary	Bark DMSO vs Bark MeOH P value	Summary
1.5625	P > 0.05	ns	P > 0.05	ns
3.125	P > 0.05	ns	P > 0.05	ns
6.25	P > 0.05	ns	P > 0.05	ns
12.5	P<0.01	**	P > 0.05	ns
25	P<0.001	***	P<0.001	***
50	P<0.001	***	P<0.001	***
100	P<0.001	***	P<0.001	***
200	P<0.001	***	P<0.001	***

Row Factor	Root DMSO vs Root Aqueous P value	Summary	Root DMSO vs Root MeOH P value	Summary
1.5625	P > 0.05	ns	P > 0.05	ns
3.125	P > 0.05	ns	P > 0.05	ns
6.25	P > 0.05	ns	P > 0.05	ns
12.5	P<0.01	**	P < 0.05	*
25	P<0.001	***	P<0.001	***
50	P<0.001	***	P<0.001	***
100	P<0.001	***	P<0.001	***
200	P<0.001	***	P<0.001	***

Figure 1: Anticancer activity of leaf, bark, and root extracts from three solvent systems against HeLa cells (24 h 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 IC_{50} values (µg/mL) and curve fit quality (R^2) are summarized in the tables beneath each graph. Extracts displaying "unstable" or "very wide" IC_{50} 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.

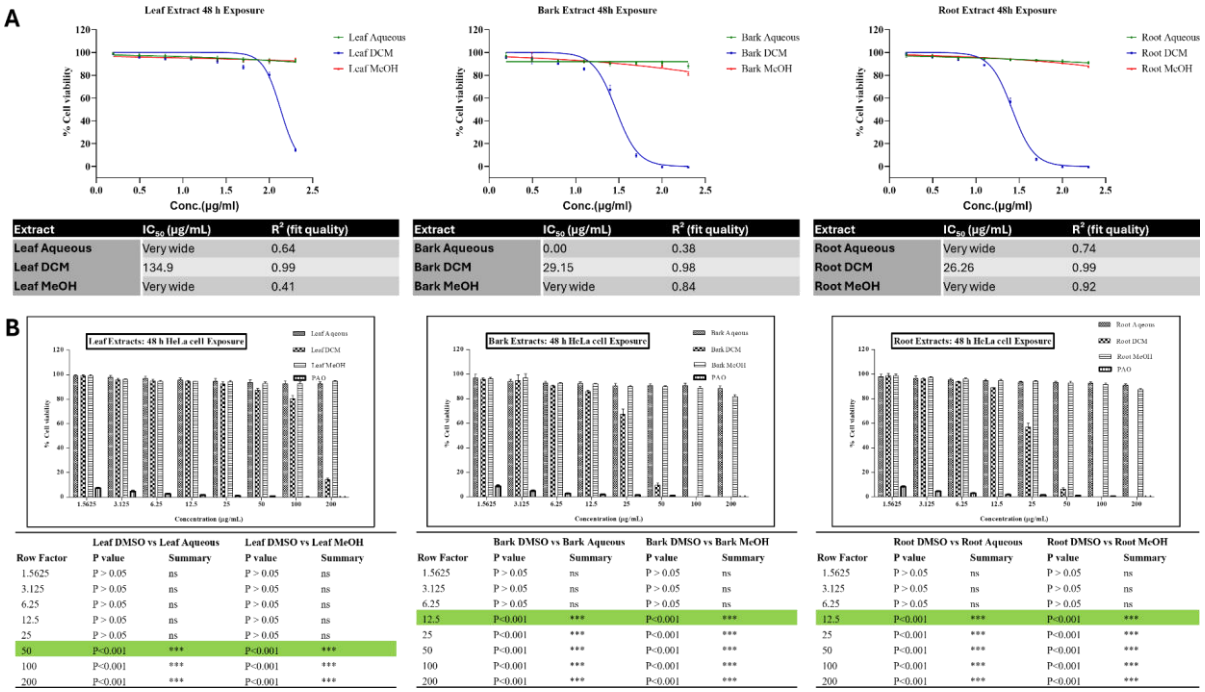
48-hour anticancer activity of plant extracts assessment against HeLa cell

The cytotoxic activity of S.rhemanniana leaf, bark, and root extracts obtained using aqueous, dichloromethane (DCM), and methanol (MeOH) solvents was evaluated against HeLa cells following a 48- hour exposure (Figure 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 µg/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 anticancer 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 µg/mL ($R^2 = 0.98$), while the aqueous extract showed near-complete inhibition at all tested concentrations (apparent $IC_{50} = 0.00$ µg/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 µg/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.



Extract	IC_{50} (µg/mL)	R^2 (fit quality)
Leaf Aqueous	Very wide	0.64
Leaf DCM	134.9	0.99
Leaf MeOH	Very wide	0.41

Extract	IC_{50} (µg/mL)	R^2 (fit quality)
Bark Aqueous	0.00	0.38
Bark DCM	29.15	0.98
Bark MeOH	Very wide	0.84

Extract	IC_{50} (µg/mL)	R^2 (fit quality)
Root Aqueous	Very wide	0.74
Root DCM	26.26	0.99
Root MeOH	Very wide	0.92

	Leaf DMSO vs Leaf Aqueous		Leaf DMSO vs Leaf MeOH	
Row Factor	P value	Summary	P value	Summary
1.5625	P > 0.05	ns	P > 0.05	ns
3.125	P > 0.05	ns	P > 0.05	ns
6.25	P > 0.05	ns	P > 0.05	ns
12.5	P > 0.05	ns	P > 0.05	ns
25	P > 0.05	ns	P > 0.05	ns
50	P<0.001	***	P<0.001	***
100	P<0.001	***	P<0.001	***
200	P<0.001	***	P<0.001	***

	Bark DMSO vs Bark Aqueous		Bark DMSO vs Bark MeOH	
Row Factor	P value	Summary	P value	Summary
1.5625	P > 0.05	ns	P > 0.05	ns
3.125	P > 0.05	ns	P > 0.05	ns
6.25	P > 0.05	ns	P > 0.05	ns
12.5	P<0.001	***	P<0.001	***
25	P<0.001	***	P<0.001	***
50	P<0.001	***	P<0.001	***
100	P<0.001	***	P<0.001	***
200	P<0.001	***	P<0.001	***

	Root DMSO vs Root Aqueous		Root DMSO vs Root MeOH	
Row Factor	P value	Summary	P value	Summary
1.5625	P > 0.05	ns	P > 0.05	ns
3.125	P > 0.05	ns	P > 0.05	ns
6.25	P > 0.05	ns	P > 0.05	ns
12.5	P<0.001	***	P<0.001	***
25	P<0.001	***	P<0.001	***
50	P<0.001	***	P<0.001	***
100	P<0.001	***	P<0.001	***
200	P<0.001	***	P<0.001	***

Figure 2: Dose–response curves, IC_{50} values, and statistical analyses of leaf, bark, and root extracts of [Plant species] 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 ± SD of three replicates. IC_{50} values and curve fit (R^2) are shown for each extract. Group comparisons and two-way ANOVA indicate significant effects of solvent type and plant part ($p < 0.001$).

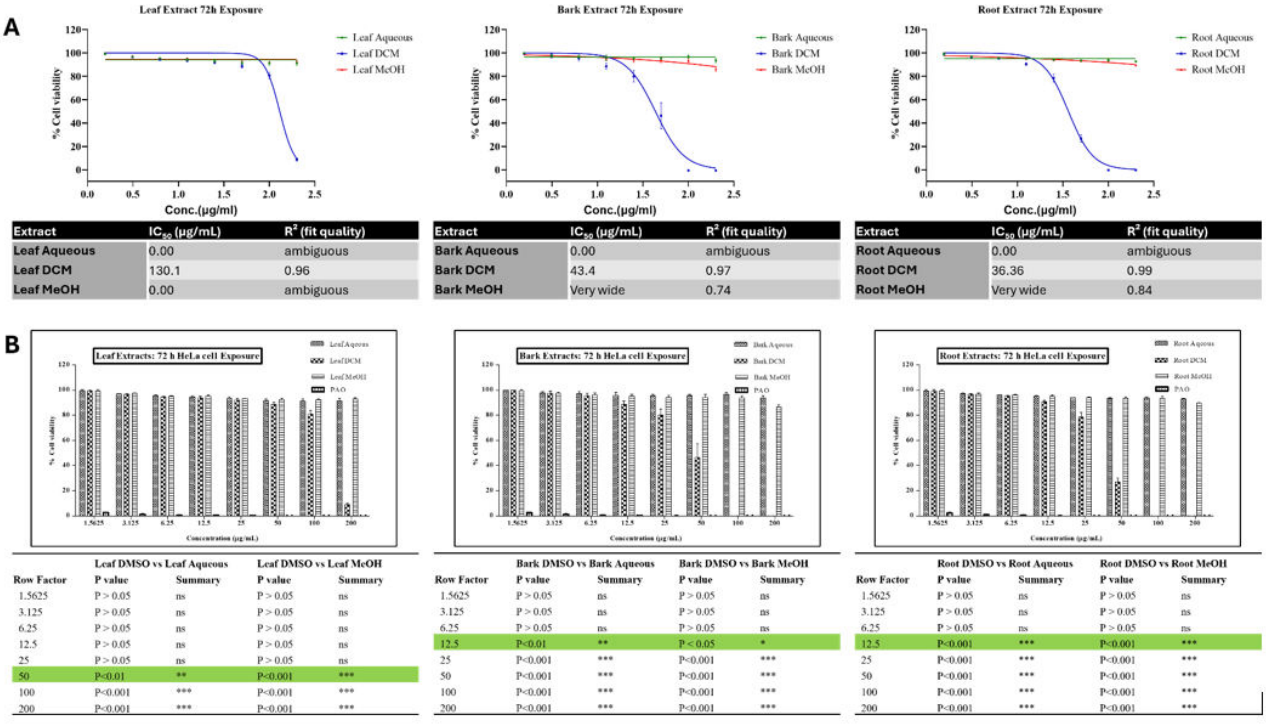
78-hour anticancer activity of plant extracts assessment against HeLa cell

The cytotoxic activity of the leaf, bark, and root extracts prepared using aqueous, dichloromethane (DCM), and methanol (MeOH) solvents was evaluated against HeLa cells after a 72-hour exposure period (Figure 3). The results demonstrated marked differences in anticancer 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 µg/mL (leaf), 43.4 µg/mL (bark), and 36.36 µg/mL (root). The dose– response curves for these extracts showed steep sigmoidal patterns with high coefficients of determination ($R^2 = 0.96–0.99$), 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 ($IC_{50} = 36.36$ µg/mL), followed closely by the bark DCM extract (43.4 µg/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 ($p < 0.01–0.001$). 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.



Extract	IC_{50} (µg/mL)	R^2 (fit quality)
Leaf Aqueous	0.00	ambiguous
Leaf DCM	130.1	0.96
Leaf MeOH	0.00	ambiguous

Extract	IC_{50} (µg/mL)	R^2 (fit quality)
Bark Aqueous	0.00	ambiguous
Bark DCM	43.4	0.97
Bark MeOH	Very wide	0.74

Extract	IC_{50} (µg/mL)	R^2 (fit quality)
Root Aqueous	0.00	ambiguous
Root DCM	36.36	0.99
Root MeOH	Very wide	0.84

Row Factor	Leaf DMSO vs Leaf Aqueous P value	Summary	Leaf DMSO vs Leaf MeOH P value	Summary
1.5625	P > 0.05	ns	P > 0.05	ns
3.125	P > 0.05	ns	P > 0.05	ns
6.25	P > 0.05	ns	P > 0.05	ns
12.5	P > 0.05	ns	P > 0.05	ns
25	P > 0.05	ns	P > 0.05	ns
50	P<0.01	**	P<0.001	***
100	P<0.001	***	P<0.001	***
200	P<0.001	***	P<0.001	***

Row Factor	Bark DMSO vs Bark Aqueous P value	Summary	Bark DMSO vs Bark MeOH P value	Summary
1.5625	P > 0.05	ns	P > 0.05	ns
3.125	P > 0.05	ns	P > 0.05	ns
6.25	P > 0.05	ns	P > 0.05	ns
12.5	P<0.01	**	P < 0.05	*
25	P<0.001	***	P<0.001	***
50	P<0.001	***	P<0.001	***
100	P<0.001	***	P<0.001	***
200	P<0.001	***	P<0.001	***

Row Factor	Root DMSO vs Root Aqueous P value	Summary	Root DMSO vs Root MeOH P value	Summary
1.5625	P > 0.05	ns	P > 0.05	ns
3.125	P > 0.05	ns	P > 0.05	ns
6.25	P > 0.05	ns	P > 0.05	ns
12.5	P<0.001	***	P<0.001	***
25	P<0.001	***	P<0.001	***
50	P<0.001	***	P<0.001	***
100	P<0.001	***	P<0.001	***
200	P<0.001	***	P<0.001	***

Figure 3: Dose–response and IC_{50} and group chart and statistical (Two-way ANOVA) Analysis for plant extracts against HeLa cells 72 h.

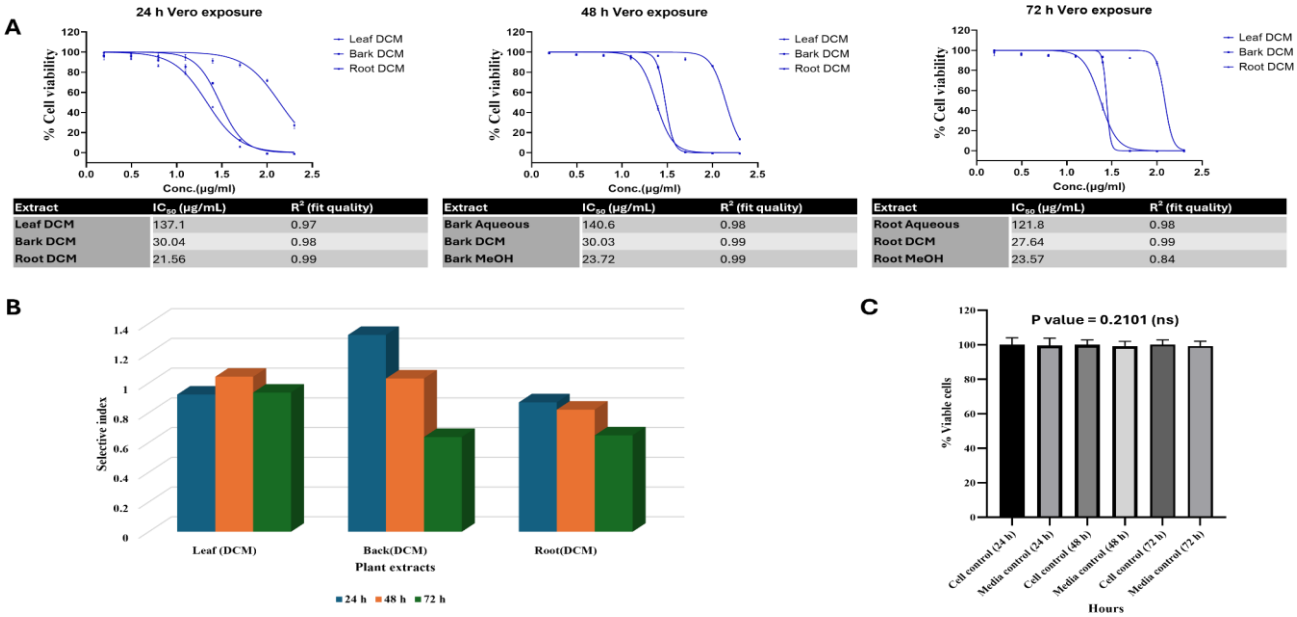
Dose–response curves, IC_{50} values, and statistical analyses of leaf, bark, and root extracts prepared using aqueous (green), dichloromethane (DCM; blue), and methanol (MeOH; red) solvents. Data represent the mean ± standard deviation (SD) of three independent replicates. Corresponding IC_{50} values (µg/mL) and coefficient of determination (R^2) 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 IC_{50} 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 (SI

The selective index (SI) values of leaf, bark, and root extracts obtained using aqueous, dichloromethane (DCM), and methanolic solvents was assessed. The SI was calculated as the ratio of IC_{50} values from normal cell lines (Figure 5A) to those from HeLa cancer cells (Figure 2, 3, and 4). Higher SI values indicate greater selectivity toward 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 selective index (SI) was used to evaluate the preferential cytotoxicity of plant extracts toward cancer cells relative to normal cells. Generally, SI values greater than 2 indicate favourable selectivity for anticancer activity. As shown in Figure X, 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 toward 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—DCM > MeOH > 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 anticancer 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 the final percentage of the DMSO (1%) in the culturing media had no adverse effect on cell proliferation.



Extract	IC_{50} (µg/mL)	R^2 (fit quality)
Leaf DCM	137.1	0.97
Bark DCM	30.04	0.98
Root DCM	21.56	0.99

Extract	IC_{50} (µg/mL)	R^2 (fit quality)
Bark Aqueous	140.6	0.98
Bark DCM	30.03	0.99
Bark MeOH	23.72	0.99

Extract	IC_{50} (µg/mL)	R^2 (fit quality)
Root Aqueous	121.8	0.98
Root DCM	27.64	0.99
Root MeOH	23.57	0.84

Figure 4: Dose–response and IC_{50} for plant extracts against Vero cells 24, 48 and 72 h, 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 IC_{50} values from normal cell lines (Vero) to those from HeLa cancer cells. Higher SI values indicate greater selectivity toward 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.

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 dichloromethane (DCM) extracts consistently showed the strongest and most selective anticancer 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 anticancer 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 anticancer agents.

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