RESEARCH PAPER

Dioscorea transversa R. Br extracts Elicit ROS Generation and Apoptotic Signalling through p53 and Rb Modulation in Cervical Cancer Models

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Abstract: Cervical cancer poses a significant global health burden, driven primarily by persistent infection with human papillomavirus (HPV) which continuous to be the leading cause of cancer related morbidity and mortality in women. Conventional chemotherapeutic agents are often limited by toxicity and resistance, highlighting the need for novel therapeutic approaches from plants which are safely consumed by human. This study evaluated anticancer potential of *Dioscorea transversa* R. Br extracts in human cervical cancer cell lines with normal mouse fibroblast cells (L929). The tubers of this plant are consumed by local tribes in northern Kerala during scarcity of food and it is also combined with other ingredients in their traditional postnatal oral rejuvenation preparations. However, this plant is rarely studied for its biochemical impacts in humans. The extracts significantly reduced cell viability in cervical cancer cell lines, while showing no cytotoxicity against L929 cells conforming their selective action. The extracts induced significant increase in intracellular reactive oxygens species (ROS). The elevated ROS level was correlated with mitochondrial dysfunction and instigation of intrinsic apoptotic signals as confirmed by AnnexinV and caspase activation assays. Furthermore, Western blot analyses revealed upregulation of p53 and downregulation of Rb phosphorylation suggesting restoration of key cell cycle regulatory pathways. Importantly, the extracts demonstrated differential sensitivity between HPV positive (HeLa, SiHa) and HPV negative (C33A) cells, indicating involvement in HPV oncogene suppression in their mechanism of action. Collectively, these finding demonstrate that bioactive compounds in extracts selectively induced ROS mediated apoptosis and modulate tumour suppression pathways, while sparing normal fibroblast thereby exerting potent anticancer effects in cervical models. This study highlights the therapeutic promise of *Dioscorea transversa* R. Br as adjunct or alternative agent for cervical cancer management and support their further development for cervical cancer management.

Keywords: Cervical cancer, *Dioscorea transversa,* Polyphenol, HeLa, SiHa, C33A, ROS, apoptosis.

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1. Introduction:

Cervical cancer remains a major basis of cancer related morbidity and mortality in women globally, especially in low and middle-income countries with limited access to diagnostic testing and treatment (Derrick B et al., 2024, Zhuang L et al., 2025). Tenuous infection with risky human papillomavirus (HPV) types drives malignant transformation of cervical epithelial cells, and although surgery, radiotherapy, and platinum or anthracycline based chemotherapy improves survival rates, these modalities are often related with significant toxicity and the upsurge of drug resistance (Irene AG et al., 2022). Phytochemicals from medicinal plants have garnered attention as safer alternatives, leveraging their ability to modulate oxidative stress and selectively induce apoptosis in cancer cells (Mohan MC et al., 2025).

In cancer biology, reactive oxygen species (ROS) generate a therapeutic vulnerability, as many tumors function close to the limit of tolerable oxidative stress because of their elevated metabolic activity and rapid proliferation. Consequently, strategies that further elevate intracellular ROS or impair antioxidant defence systems can selectively induce cancer cell death (Iqra A et al., 2025 and Run H et al., 2021) while sparing normal tissues with more robust redox homeostasis. Tumour suppressors such as p53 and retinoblastoma protein (Rb) serve as major checkpoints for apoptosis and cell cycle control (Kurt E, 2022 and Charles JS et al., 2002). Dysregulation of these pathways is a hallmark of cervical cancer. Hence identifying novel, safer anticancer agents including phytochemicals from medicinal plants that selectively eliminate cervical cancer while sparing healthy tissues remain a priority.

Species of the genus *Dioscorea* have long been used in old-style medicine and are rich in Phyto-active constituents such as phenolics, steroidal saponins, and other secondary metabolites with reported antioxidant, anti-inflammatory, metabolic disease and anticancer properties (Kumar S et al., 2017, Zhen W et al., 2023

and Li L et a., 2025).However, tuber and leaf extracts of Dioscorea transversa R. Br, an underexplored species, may therefore represent a promising source of novel anti-cervical cancer compounds, yet their cytotoxic potential and underlying mechanisms of action have not been systematically evaluated in relevant cervical cancer cell models. Using a panel of HPV positive (HeLa, SiHa) and HPV negative (C33A) cervical cancer cell lines, alongside non tumorigenic L929 fibroblasts as a comparative normal cell model, allows assessment of both anticancer efficacy and selectivity. At mechanistic level, many chemotherapeutic agents and natural compounds exert their anticancer effects by generating oxidative stress, impairing mitochondrial function, and activating the intrinsic apoptosis pathway. The generation of reactive oxygen species (ROS) can impair cellular macromolecules and can function as a pro-apoptotic signal. This can lead to mitochondrial membrane potential (MMP) disruption, resulting to release of pro-apoptotic factors and activation of executioner caspases such as caspase 3 and caspase 7. Early and late stages of apoptosis can be quantified by Annexin V based assays, while caspase 3/7 activity provides a functional readout of executioner caspase activation. In parallel, modulation of key tumour suppressor pathways, including upregulation of tumour suppressor protein, p53 and downregulation of retinoblastoma (Rb) protein phosphorylation, contributes to cell cycle arrest and apoptotic commitment in cervical cancer cells.

In this study we assessed cytotoxicity of tuber and leaf extracts from *Dioscorea transversa* R. Br, in comparison to doxorubicin as the standard chemotherapeutic against cervical cancer cell lines and L929 cells as a non-malignant control. We further investigated intracellular ROS generation, mitochondrial membrane potential alterations, apoptosis induction by Annexin V assay, caspase-3/7 activation, and changes in expression of p53 and phosphorylation status of Rb induced by these extracts and doxorubicin at their respective IC_{50} doses in cervical cancer cell lines. Together, these integrated endpoints are intended to elucidate both the efficacy and the potential molecular mechanisms by which D. transversa tuber and leaf extracts exert selective anti-cervical cancer effects.

In this study, we evaluated the cytotoxicity of tuber and leaf extracts from *Dioscorea transversa* R. Br.—compared to doxorubicin as the standard chemotherapeutic—against cervical cancer cell lines, using L929 cells as a non-malignant control.

2. Material and Methods:

2.1 Chemicals and Reagents:

Methanol AR and Folin–Ciocalteu (sd fine chemicals), HeLa (CRM-CCL-2, HPV18), SiHa (HTB-35, HPV-16), C33A (C33-A, HPV negative) and L929 (CCL-1) (ATCC), Gallic acid, Dulbecco's Modified eagle medium (DMEM), 10% fetal bovine serum (FBS), 1% Penicillin- Streptomycin , Dimethyl sulfoxide (DMSO) Doxorubicin (Sigma-Aldrich), 2′,7′-dichlorodihydrofluorescein diacetate, MTT, TMRE, SDS and RIPA cell lysis buffer (Thermo Fisher), Annexin kit (Dodinjo), Caspase-3/7 (Invitrogen), and polyvinylidene fluoride immunoblot membrane (Millipore).

2.2 Extract Sample preparation and estimation of total polyphenol content:

Fresh leaves and tubers of *D. transversa R. Br*, as identified from the 8th edition of the Australian rainforest plants database and the edible plants of the globe database, were collected in February and March from Kanayi, northern Kerala, India. *D. transversa* is also known as D. *punctata* or *D. sativa var. elongata*. The herbarium specimen of the plant was submitted to the Department of Botany at Periyar University. The harvested leaves and tubers of the plant were meticulously washed to eliminate soil and muck, subsequently dried in the shade. The desiccated substances were pounded in a blender to form very fine particles. 250 g of finely powdered leaf and tuber samples were subjected to extraction with double the volume of aqueous methanol (1:1-methanol: water) utilizing the indirect ultrasonic assisted extraction method (Bimakr et al., 2013). The ultrasonic water bath was kept at a temperature of 50°C for three hours. Two further cycles of extraction were conducted; both the extracts were individually concentrated and evaporated utilizing a rotary evaporator. The percentage (%) yield of extracts was determined. Total polyphenol content of the tuber (T) and leaf (L) extracts was determined spectrophotometrically using the Folin–Ciocalteu colorimetric method, with gallic acid as the reference standard. Briefly, appropriately diluted extracts (20µl) were mixed with 1:10 diluted Folin–Ciocalteu reagent (100µl) and (300µl) 10% sodium carbonate solution, incubated at room temperature in the dark, and the absorbance was recorded at 760nm. Total polyphenol content was calculated from the gallic acid calibration curve and expressed as milligrams of gallic acid equivalents (GAE) per gram of dry extract (mg GAE/g dry extract).

2.3 Cytotoxicity assay (MTT):

Cell was plated into a 96 well plates at a density of 5 × 10^3 cells per well in DMEM with 10% FBS and 1% penicillin- streptomycin and incubated for 24 hours at 37°C in a humidified 5% CO_2 incubator. All the four cell lines were treated with different concentrations of the leaf and tuber extracts along with the positive control doxorubicin for 48 hours. The plates were washed with phosphate-buffered saline (PBS) and 10µL of MTT solution (0.5 mg/ml) was added to each well. After 4 hours of incubation at 37°C in 5% CO_2, the formazan crystals formed (Liu Y et al., 1997) were

dissolved in DMSO and the absorbance was read at 570 nm with a FluoStar plate reader. Percentage cytotoxicity was calculated as ((C-T) X100)/C) where C represents the absorbance of untreated cells and T represents the absorbance of cells treated with the test extract samples or positive control doxorubicin. The half-maximal inhibitory concentration (IC_{50}) was calculated by non-linear regression of concentration response curve using GraphPad Prism version 5.

2.4 ROS assay:

Reactive oxygen species (ROS) was evaluated in real time following the method described by Yong et al., (2009), using 2′,7′-dichlorodihydrofluorescein diacetate (H2DCFDA). HeLa, SiHa and C33A cells (4×10^4 per well) were seeded in clear bottom black 96 well plates and incubated for 24hours, followed by treatment with the IC_{50} concentration of both extracts for 2 hours. The cells were then washed with PBS and incubated with 100µL of 20µM H2DCFDA prepared in phenol red free medium. Fluorescence was measured at exi/emi wavelengths of 485/520 nm using a FluoStar microplate reader at 3-minute intervals for 90 minutes. ROS generation was quantified based on fluorescence intensity over time, and the area under the curve (AUC) was calculated using GraphPad Prism and fold increase of ROS in comparison to untreated control cells was calculated as AUC of Test / AUC of control.

2.5 TMRE Mitochondrial Membrane Potential Assay:

HeLa, SiHa, and C33A cells (4×10^4/well) were seeded per well in black clear bottom 96 well plates and incubated overnight at 37°C in 5% CO_2. Treated the cells with IC_{50} doses of test extract samples and doxorubicin for 4 hours. The assay also included untreated cells and cells treated with 5µM FCCP as positive control. Washed the cells with PBS and treated the cells with 100µL of 200nM TMRE solution (Abcam- ab113852 Kit) in phenol red-free medium. Incubated the plates at 37°C, 5% CO_2 for 20 minutes. Plates were washed twice with PBS and 100µL PBS was added per well. Fluorescence reading was measured using a FluoStar microplate reader excitation/emission wavelengths of 549nm/ 573nm. Depolarization percentage with respect to control was calculated as ((C-T) X100) / C, where C represents the fluorescence of untreated cells and T represents the fluorescence of cells treated with the test extract samples or positive control.

2.6 Annexin V Apoptosis Assay:

HeLa, SiHa, and C33A cells (4×10^4/well) were seeded per well in black clear bottom 96 well plates and incubated overnight at 37°C in 5% CO_2. Washed the cells gently with PBS and added 100µL of PBS with 5% FBS and 100µL of IC_{50} doses of test extract samples and doxorubicin in PBS to test wells. Incubated the plate at 37°C in 5% CO_2 for 2 hours. 60µL of working Annexin V solution (Dodinjo, AD12 kit) was added to all wells and incubated the plate at room temperature for 15 minutes. Fluorescence reading was measured using a FluoStar microplate reader with excitation/emission wavelengths of 488 nm/525nm. Fold increase in apoptosis was calculated as T / C where C represents the fluorescence of untreated cells and T represents the fluorescence of cells treated with the test extract samples or positive control.

2.7 Caspase 3/7 activity Assay:

HeLa, SiHa, and C33A cells (4×10^4/well) were plated in clear bottom black 96 well plates and incubated overnight at 37°C in 5% CO_2. Washed the cells gently with PBS and added 100µL of PBS with 5% FBS and 5µM Caspase-3/7 green detection reagent (Invitrogen CellEvent, C10423) to all the wells. Added IC_{50} doses of test extract samples and doxorubicin to wells respectively, Incubated the plate at 37°C in 5% CO_2 for 30 minutes. Fluorescence reading was measured using a FluoStar microplate reader at excitation/emission wavelengths of 502nm/530nm. Fold increase in caspase 3/7 activity was calculated as T / C where C represents the fluorescence of untreated cells and T represents the fluorescence of cells treated with the test extract samples or positive control.

2.8 p53 and Rb expression assays by Western blot:

HeLa, SiHa, and C33A cells (4×10^4/well) were seeded on a 12 well plate and incubated for 48 in DMEM supplement with 10% FBS and 1% penicillin-streptomycin at 37°C in a 5% CO2 incubator. Treated the cells with IC_{50} doses of test extract samples along with positive control doxorubicin (p53) in growth media for 16 hours. Washed the cells with PBS and then 1X trypsin was used to detach the cells quickly and added chilled 2.5% FBS to neutralize the trypsin treated cells. The suspension was centrifuged at 1000 rpm at 4°C for 5 minutes. The cell pellets were washed in chilled PBS and centrifuged at 1000 rpm. Western blot analysis was done as described by Ja W Y et al., 2013 and Jeong W C 2005. The cell extracts were obtained by lysis in a RIPA cell lysis buffer supplemented with complete protease inhibitor tablets (Roche Applied Science). Quantified the protein by BCA assay and loaded with 50 µg protein per lane on 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene fluoride immunoblot membrane (Millipore, Billerica, MA, USA). The probe with anti-p53 antibody (DO-1 clone, 1:1000) and anti-phospho-RbSer807/811 (Thermo-Fischer Scientific). Rb antibody and anti-β-actin (Cell Signalling Technology, Inc.) detected via HRP secondary antibody the

resulting signal was detected using enhanced chemiluminescence (Intron Biotechnology). Band intensities corresponding to p53, phospho-Rb Ser807/811, total Rb, and β-actin are quantified using densitometry software. p53 readings were normalized with β-actin and phospho-Rb with total Rb. Relative changes in the gene expressions of p53 and phospho-Rb was calculated with respective to the untreated cells.

2.9 Quantitative examination and statistical analysis:

Cytotoxicity data and all other quantitative measurements were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test, with positive control. For mechanistic endpoints, intracellular ROS levels, apoptosis induction (Annexin V assay), caspase-3/7 activity, and expression of p53 and phosphorylated Rb at the IC_{50} concentration were considered with respect to corresponding untreated controls. A p value < 0.05was considered statistically significant. All experiments were performed in six independent replicates (n = 6).

3. Results:

3.1 Phytochemical analysis revealed high polyphenolic content:

Aqueous methanolic extraction of *Dioscorea transversa* yielded 86.4% from the leaves and 67.4% from the tubers. The total polyphenol content of the leaf and tuber extracts was 250.6 mg and 67.4 mg gallic acid equivalents (GAE)/g extract, respectively. The extraction yields and the total polyphenolic contents of leaf were significantly higher than the tuber extract which is as shown in figure 1.

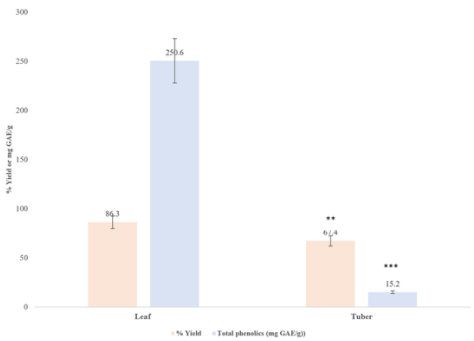

Figure 1. Total yield and polyphenol content of leaf and tuber extracts of Dioscorea transversa. **p < 0.01, ***p < 0.001

3.2 *Dioscorea* extracts showed preferential cytotoxicity toward tumour cells:

Both the extracts and the positive control doxorubicin reduced the viability of HeLa, SiHa, and C33A cells in a dose-dependent manner (Figure 2). The IC_{50} values for all test samples were markedly lower in the cervical cancer cell lines than in the L929 fibroblast line, indicating preferential cytotoxicity toward tumour cells. The IC_{50} values of the leaf (L) and tuber (T) extracts, together with doxorubicin (D), are summarized in Table 1, along with the microscopic images in figure 3.

Cytotoxicity IC_{50}(µg/ml)	L929-D	L929-L	L929-T	HeLa-D	HeLa-L	HeLa-T
	125.7	4892	5053	2.54	33.05	96.19
	SiHa-D	**SiHa-L**	**SiHa-L**	**C33A-D**	**C33A-L**	**C33A-T**
	2.78	38.48	122.2	3.26	121.3	378.3

Table 1: IC_{50} values of doxorubicin (D), *Dioscorea transversa* leaf (L) and tuber (T) extracts against cervical cancer cell lines, $p < 0.001$ versus normal mouse fibroblast cells (L929)

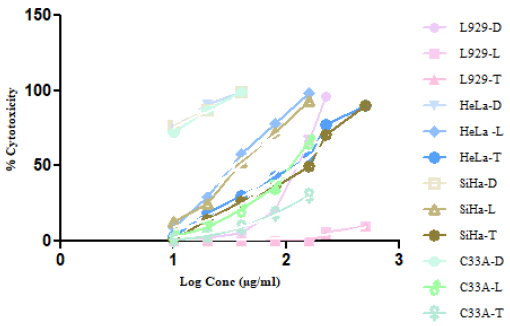

Figure 2. Comparative dose-dependent cytotoxicity of doxorubicin (D) and Dioscorea transversa leaf (L) and tuber (T) extracts in cervical cancer cell lines versus normal mouse fibroblast cells (L929)

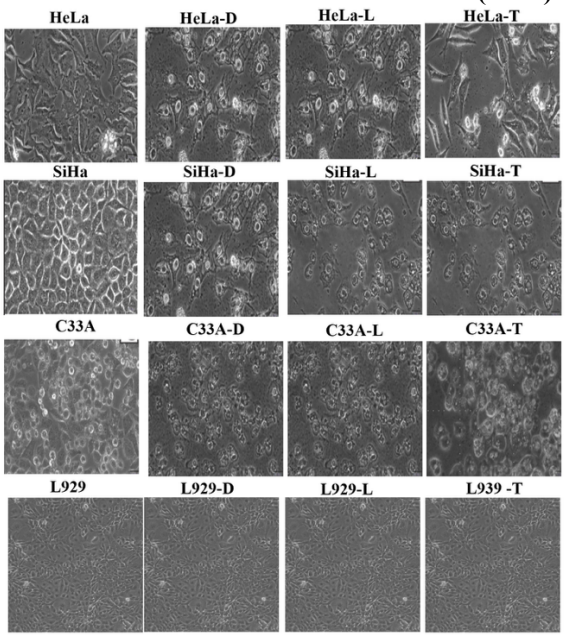

Figure 3. Microscopic images of cervical cancer cell lines and normal mouse fibroblast cells treated with doxorubicin (D) and Dioscorea transversa leaf (L) and tuber (T) extracts

3.3 ROS generations were differentially modulated by the extracts:

ROS generation in cervical cancer cells was differentially modulated by the extracts and the positive control at their IC_{50} doses. Both extracts and doxorubicin increased intracellular ROS in HeLa and SiHa (HPV positive) cells, whereas in C33A (HPV negative) cells a marked ROS increase was observed only with the positive control, and the extracts produced little enhancement only. The relative fold increases in ROS to untreated cells for each treatment and cell line are presented in Figure 4.

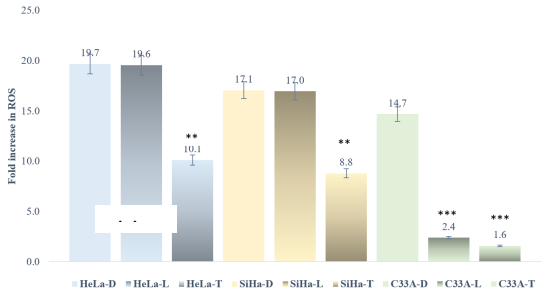

Figure 4. Fold increase in reactive oxygen species (ROS) in cervical cancer cell lines following treatment with doxorubicin (D) and Dioscorea transversa leaf (L) and tuber (T) extracts. **p < 0.01, ***p < 0.001 versus doxorubicin treated cells

3.4 *Dioscorea* extracts restored the alteration in mitochondrial membrane potential:

Depolarization of the mitochondrial membrane was observed at the IC_{50} doses of both extracts and the positive control in HeLa and SiHa (HPV positive) cells. In contrast, in C33A (HPV negative) cells a pronounced depolarization was detected only in response to theIC_{50} dose of positive control, whereas the extracts induced only minimal loss of membrane potential. The relative percentage depolarization to untreated cells for each treatment and cell line is shown in Figure 5.

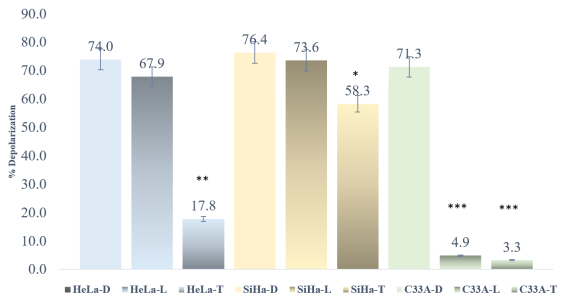

Figure 5. Percentage increase in mitochondrial membrane depolarization in cervical cancer cell lines following treatment with doxorubicin (D) and Dioscorea transversa leaf (L) and tuber (T) extracts. *p < 0.05, **p < 0.01, ***p < 0.001 versus doxorubicin treated cells

3.5 *Dioscorea* extracts enhanced apoptosis in HeLa and SiHa cells:

Both extracts and the positive control increased apoptosis in HeLa and SiHa (HPV positive) cells. In C33A (HPV negative) cells, however, a clear enhancement of apoptosis was evident only with the positive control. The relative fold increase in apoptosis compared with untreated cells for each cell line is shown in Figure 6.

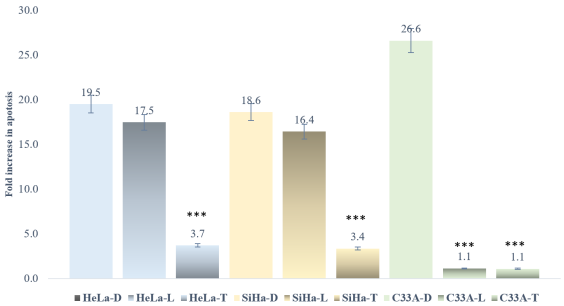

Figure 6. Fold increase in apoptosis in cervical cancer cell lines following treatment with doxorubicin (D) and Dioscorea transversa leaf (L) and tuber (T) extracts. **p < 0.01, ***p < 0.001 versus doxorubicin treated cells

3.6 *Dioscorea* extracts activates Caspase-3/7 in HPV positive (HeLa, SiHa) cells

Positive control activated Caspase-3/7 in all the studied three cervical cancer cell lines. However, both the extracts activated Caspase-3/7 in HPV positive (HeLa, SiHa) only. The relative fold increase in Caspase-3/7 activation to untreated cells for each cell line is shown in Figure 7.

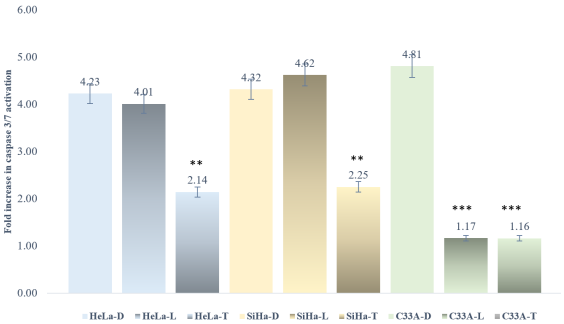

Figure 7. Fold increase in Caspase-3/7 activity in cervical cancer cell lines following treatment with doxorubicin (D) and Dioscorea transversa leaf (L) and tuber (T) extracts. **p < 0.01, ***p < 0.001 versus doxorubicin-treated cells

3.7 *Dioscorea* extracts modulates p53 and Rb phosphorylation:

p53 upregulation and decreased phospho-Rb was observed at IC_{50}dose of positive control in HeLa, SiHa, C33A cells. Both the extracts showed p53

upregulation and decreased phospho-Rb at their IC_{50} dose in HPV positive (HeLa and SiHa) cell only. The relative western-blot analysis and the upregulation of p53 and down regulation of phospho-Rb at IC_{50} dose treated and untreated cells for each cell line are shown in Figure 8.

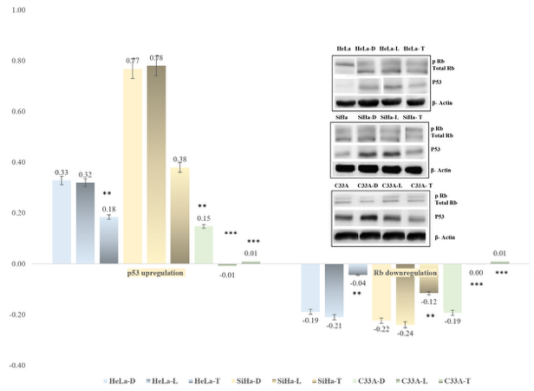

Figure 8. Relative western-blot analysis along with upregulation of p53 and downregulation of Rb in cervical cancer cell lines following treatment with doxorubicin (D) and *Dioscorea transversa* leaf (L) and tuber (T) extracts. **p < 0.01, ***p < 0.001 versus doxorubicin treated cells

4. Discussion:

The aqueous methanolic extracts of *Dioscorea transversa* demonstrated remarkable selective cytotoxicity toward cervical cancer cell lines while sparing normal fibroblasts, The IC_{50} results showed that the extracts had a prominent differential selectivity index (SI=L929 IC_{50}/cervical cancer cell line IC_{50}) between the HPV positive cells and HPV negative cells. HeLa cells were highly sensitive to the leaf extract (IC_{50}=33.05 µg/ml, SI=148) followed by SiHa cells (IC_{50}=38.48 µg/ml, SI=127) and C33A (IC_{50}=121.3 µg/ml), while showing a very low cytotoxicity against L929 fibroblast cells (IC_{50}=4992 µg/ml). This distinct selectivity in cytotoxicity shows that bioactive compounds in Dioscorea *transversa* extracts specifically target transformed cells while upholding an extensive safety margin in normal tissues which was similar to finding reported for other phytopharmaceuticals (Jose MCM et al., 2021). The tuber extract was less potent than the leaf extract, still demonstrated selective activity (HeLa IC_{50}= 96.19 µg/ml, SI =53; SiHa IC_{50}=122.2 µg/ml SI=41) relating directly with its lower total polyphenol content. The dose response relationship confirms that phenolic compounds particularly gallic acid equivalents quantified in the Folin-Ciocalteu assay are the contributors to the selective cytotoxicity. Doxorubicin, the positive control was equipotent across HPV positive (HeLa and SiHa) and HPV negative cells (C33A) and IC_{50} values ranged between 2.54-3.26 µg/ml. However, its cytotoxic concentration was higher in L929 fibroblast cells (IC_{50} =125.7 µg/ml; SI=45-50). This suggests that *Dioscorea transversa* extracts may provide a broader range of selective therapeutic window compared to doxorubicin, avoiding the cardiotoxicity and other organ damage typical of anthracycline therapy (Vasvi B et al., 2025). This HPV selective cytotoxicity is mechanistically profound. Studies showed that HPV positive cells depend heavily on inactivation of p53 and Rb tumor suppressors via viral oncoproteins E6 and E7 (DeFilippis RA et al., 2003). Research demonstrates that HPV positive cervical carcinomas exhibit an enhanced dependence on ATM/CHK2 signalling and display elevated antioxidant enzyme levels, making them paradoxically more sensitive to ROS inducing agents.

HPV negative C33A cells carry a wild type p53 mutation (R273C), resulting in a non-functional p53 protein that cannot be further inactivated by viral oncoproteins (Walason A et al., 2022). The superior potency of the leaf extract directly correlates with its higher total polyphenol content (250.6 mg GAE/g vs. tuber: 67.4 mg GAE/g). This ~3.7-fold difference in phenolic abundance mirrors the ~2.9-fold difference in IC_{50} potency between leaf and tuber extracts in HeLa cells (33.05 vs. 96.19 µg/ml). Studies have shown that phenolic compounds including flavonoids, stilbenes, and phenolic acids are well characterized as pro-oxidant agents capable of generating intracellular ROS through redox cycling with cellular iron and NADPH oxidase activation (Li L et al.2025). Moreover, this correlation validates the utility of phytochemical standardization for reproducible screening and future clinical translation (Li L et al.2025, Mohan et al., 2019).

Increased level of intracellular ROS, mitochondrial membrane depolarization, Annexin V positive apoptosis, caspase-3/7 activation, p53 upregulation, and Rb dephosphorylation collectively outline a coherent cascade of ROS driven intrinsic apoptosis with restoration of tumor suppressor function. This mechanistic manner is distinct from doxorubicin's primarily genotoxic pathway and highlights how phytochemical combinations can engage multiple apoptotic nodes simultaneously. Increased reactive oxygen species is driven by polyphenolic pro-oxidants and metabolic stress. Elevated ROS disturbs mitochondrial membrane potential through activation of pro-apoptotic Bcl-2 family proteins (e.g., Bax/Bak oligomerization), triggering cytochrome c release and apoptosome formation. This mitochondrial permeabilization activates the canonical caspase cascade (caspase-9 → caspase-3/7), culminating in PARP cleavage and DNA fragmentation (Annexin V binding). Critically, the higher intracellular ROS environment is incompatible with sustained HPV oncoprotein function. HPV E6 and E7 are known to upregulate antioxidant responses and suppress ROS triggered apoptosis (Maria F et al., 2014). Thus, the

ROS rigorous attack by the extracts may effectively overwhelm HPV infected cells antioxidant defences, explaining the HPV dependent selectivity observed. The downregulation of Rb phosphorylation reflecting restoration of hypo phosphorylated, transcriptionally active Rb indicates release of E2F transcription factors from Rb mediated suppression. This initiates a pro-apoptotic transcriptional program. This Rb re-activation is particularly notable in C33A cells, where p53 is mutated. Restoration of Rb function may partially compensate for p53 loss (Zsuzsanna S et al., 2024) explaining why even HPV negative C33A cells showed some sensitivity to the extracts at higher concentrations.

While doxorubicin demonstrated lower IC_{50} values (2.54–3.26 µg/ml across all cervical cell lines), its non-selective, equipotent cytotoxicity across HPV positive and HPV negative cells differences sharply with the extract's HPV selective profile. Doxorubicin's mechanism intercalation into DNA and generation of reactive oxygen species via topoisomerase poisoning and is effective but indiscriminate, leading to broad toxicity and dose limiting cardiotoxicity in clinical practice. In contrast, *D. transversa* extracts (L&T) selective enhancement of ROS mediated apoptosis in HPV positive cells (HeLa, SiHa) with reduced potency in HPV negative C33A suggests a molecularly targeted attack on transformed cells (Maria F et al., 2014). Furthermore, the therapeutic index favours the extracts. Leaf extract SI 148 in HeLa exceeds doxorubicin SI 45–50, implying a 3-fold wider safety margin. This elevated selectivity index combined with HPV dependent preferential targeting, positions *D. transversa* extracts as promising candidates for adjuvant therapy or combination regimens with conventional chemotherapy. High dose doxorubicin induces cardiotoxicity (Efentakis P et al., 2020) through mitochondrial dysfunction and cardiomyocyte apoptosis; phytochemicals from *D. transversa*, if administered at non-systemic toxic doses, may augment HPV positive tumor apoptosis while minimizing collateral damage to normal tissues.

The dramatic selectivity indices underscore the potential for *D. transversa* extracts to be developed as low toxicity, plant derived anticancer agents. Selectivity Index >10 is widely considered a threshold for promising in vitro activity; leaf extract achieves SI = 148, dramatically exceeding this benchmark. Plant derived compounds with high selectivity indices e.g., *Tetraclinis articulata* leaf extract, SI = 378.3 against lung cancer (Jose MCM et al., 2021) have been identified as robust leads for further development. By analogy, *D. transversa* leaf extract warrants progression to additional in vitro and in vivo (xenograft, syngeneic) models to assess anticancer efficacy, bioavailability, and systemic toxicity (Jose MCM et al., 2021).The traditional use of *D. transversa* tubers by northern Kerala tribes for "bone and muscle strength" and postnatal rejuvenation, combined with the demonstrated selective anticancer potency and favourable selectivity indices, suggests a reasonable safety profile in humans, though formal toxicology and pharmacokinetics studies remain essential. The aqueous methanolic extraction method employed in this study preserves heat labile and polar bioactives (phenolics, saponins) while avoiding lipophilic contaminants, making the extract suitable for standardization and formulation into pharmaceutical delivery systems.

5. Conclusion

The aqueous methanolic extracts of *D. transversa* leaves and tubers demonstrate selective, HPV dependent anti-cervical cancer activity characterized by ROS mediated apoptosis, mitochondrial dysfunction, and restoration of p53 and Rb tumor suppressor pathways. The leaf extract's superior potency, high selectivity index (SI=148), and superior targeting of HPV positive cervical cancer cells over HPV negative cancer cells and normal fibroblasts, position it as a promising therapeutic lead. The observed differential sensitivity between HPV positive (HeLa, SiHa) and HPV negative (C33A) cells in contrast to equipotent doxorubicin suggests novel, HPV targeted mechanisms of action worthy of further mechanistic and translational investigation. These findings support the traditional use of *D. transversa* and highlight its potential as an adjunct or alternative agent for HPV positive cervical cancer management. Future work needed to be conducted in identifying the Phyto-actives, in vivo efficacy, pharmacokinetics, and clinical development to understand this plant's therapeutic potential.

Conflict of Interest: The authors declare no conflict of interest.

Author contributions: K G proposed a hypothesis and collaborated with V to examine the literature to identify the absence of wet analysis on D. transversa. K G formulated the study plan and executed the wet analysis. MCM was involved in editing and reviewing the manuscript. The analysis of results and the review of the article were conducted collaboratively by both the authors prior to submission.

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