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Original Research Article

Selective Cytotoxicity and G_2/M Cell Cycle Arrest Induced by *Taxus sumatran*a (Miq.) de Laub. Leaf Extract in HeLa Cervical Cancer Cells: A Comparative Study with Paclitaxel

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ABSTRACT

Cervical cancer remains a significant health challenge, particularly in regions with limited access to targeted therapies. *Taxus sumatrana*, traditionally used in Indonesian herbal medicine, has limited scientific evidence supporting its anticancer activity. This study aimed to evaluate the selective cytotoxicity of *T. sumatrana* leaf extract against cervical cancer cells. The extract was tested in HeLa cells, normal Vero epithelial cells, and RAW 264.7 cells. Cytotoxicity was assessed using the MTT assay, while flow cytometry was employed to analyse cell cycle distribution and Cyclin D1 protein expression. The extract exhibited preferential cytotoxicity toward HeLa cells, with normal cells showing markedly higher tolerance, resulting in a favourable selectivity index. Cell cycle analysis revealed G_2/M phase arrest accompanied by Sub-G_1 accumulation, suggesting apoptotic induction. Cyclin D1 protein modulation showed a similar trend to that of paclitaxel treatment, though to a lesser extent. These findings suggest that *T. sumatrana* leaf extract may exert antiproliferative activity by disrupting mitotic progression while sparing non-cancerous cells. The selective action observed highlights its potential as a safer phytopharmaceutical candidate or adjunct anticancer therapy, warranting further *in vivo* validation.

Keywords: Tropical medicinal plant, *Taxus sumatrana*, Natural anticancer, Cyclin D1, Flow cytometry, Mitotic.

Introduction

Cervical cancer is one of the leading causes of cancer-related deaths among women worldwide, with the highest burden observed in developing countries.[1] HeLa cells, derived from human cervical carcinoma, are frequently used as an *in vitro* model for evaluating the efficacy of anticancer agents.[2,3] Although drugs such as paclitaxel, doxorubicin, and 5-fluorouracil are widely used, their clinical applications are often limited by severe side effects and poor selectivity toward cancer cells.[4] *Taxus sumatrana* (Miq.) de Laub., a species of Taxaceae native to the highland forests of Sumatra, Indonesia, has traditionally been used to treat ailments, including cancer.[5] Most studies on *Taxus* species have focused on bark-derived paclitaxel[6], while only limited attention has been given to the leaves. Preliminary findings suggest that the ethanol leaf extract of *T. sumatrana* exhibits cytotoxicity against cervical cancer cells at concentrations below 20 µg/mL.[7]

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However, data on its effects on normal cells and its selectivity remain limited. Establishing selectivity is crucial for identifying extracts with therapeutic potential and reduced toxicity. Previous molecular studies have demonstrated that this extract can induce apoptosis by upregulating *p53* and *Bax*, while downregulating *Bcl-2*.[8] Apoptosis results in the formation of apoptotic bodies that macrophages should efficiently clear to prevent inflammation,[9] yet the impact of the extract on macrophage function remains poorly understood. Moreover, the ability of this extract to interfere with cell cycle progression, particularly at the G_2/M phase, has not been evaluated. However, this mechanism is central to the antiproliferative action of many chemotherapeutic drugs. Although several pure compounds have been isolated from *T. sumatrana* leaves, this study focuses on the crude ethanol extract. This reflects its traditional use and allows for potential synergistic interactions among phytoconstituents, thereby enhancing overall activity.[10] Crude extracts are also more practical for phytopharmaceutical development, as they require simpler preparation and involve lower production costs compared to purified compounds. [11] This study aimed to evaluate the selective cytotoxicity of *T. sumatrana* leaf extract against cervical cancer cells, normal epithelial cells, and macrophages, and to determine its ability to induce G_2/M phase arrest and modulate Cyclin D1 protein expression in HeLa cells. Paclitaxel was included as a reference standard due to its well-established mechanism of mitotic arrest. The findings are expected to provide deeper insight into the anticancer potential and cellular mechanisms of *T. sumatrana* leaf extract, thereby supporting its development as a selective, plant-based therapeutic candidate.

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Materials and Methods

Plant Material and Extract Preparation

The leaves of *T. sumatrana* (Miq.) de Laub. were collected from Mount Singgalang, West Sumatra, Indonesia, in May 2025. Botanical identification was performed by a taxonomist at the Herbarium ANDA, Universitas Andalas, and a voucher specimen (No. DEP012024) was deposited for reference. The plant material was air-dried, powdered, and macerated with 70% ethanol for 72 hours at room temperature. The extract was filtered and concentrated under reduced pressure using a rotary evaporator at 40°C and then stored at 4°C until use. A total of 1.475 kg of dried, powdered leaves was extracted with ethanol, yielding a crude extract at 45% w/w.

Preparation of test solutions for cytotoxicity, cell cycle, and protein D1 expression analysis

For the cytotoxicity assay, a series of test solutions of *T. sumatrana* leaf extract was prepared at final concentrations of 1000, 100, 10, 1, and 0.1 µg/mL. A corresponding series of paclitaxel (Ferron, Lot No. L0201NA) solutions was also prepared at final concentrations of 0.1, 0.05, 0.025, and 0.013 µg/mL and used as the positive control, as previously reported for HeLa cells.[8] The extract or paclitaxel was dissolved in dimethyl sulfoxide (DMSO), Vivantis, and the final concentration of DMSO in cell cultures did not exceed 0.1%, a level confirmed not to affect cell viability. To eliminate any possible bias, the same concentration of DMSO was also added to the control group. Paclitaxel, used as a reference compound, exhibited marked cytotoxicity against HeLa cells, with an IC_{50} value of 0.05 µg/mL, consistent with a previous study.[8] The IC_{50} values of both the extract and paclitaxel were subsequently used to determine the working concentrations ($1\times IC_{50}$) for cell cycle and cyclin D1 protein expression analysis. In all assays, untreated cells served as the negative control.

Cell Lines and Culture Conditions

The HeLa, RAW 264.7 murine macrophage cells, and Vero (normal kidney epithelial cells from African green monkey) were obtained from the CCRC (Cancer Chemoprevention Research Centre), the Faculty of Pharmacy and Parasitology Laboratory, and the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Indonesia. Cells were cultured in DMEM (Sigma) containing 10% FBS (Gibco) and 1% penicillin–streptomycin (Gibco), and incubated at 37 °C under a 5% CO_2 humidified atmosphere. Cells were seeded in 6-well plates and allowed to reach 70–80% confluency. For cell cycle and protein expression analyses, cells were treated with the extract or paclitaxel for 48 hours. The treated cells were trypsinised, collected, and centrifuged. The supernatant was removed; the cell pellet was washed twice with cold PBS. The detailed procedure described in our previous study adheres to good cell culture practice guidelines.[12–15]

Cytotoxicity Assay

The cytotoxic effect of the leaf extract was evaluated using the MTT assay. Cells were seeded in 96-well plates (1×10^4 cells/well) and treated with various concentrations of the extract (1000, 100, 10, 1, and 0.1 µg/mL) or paclitaxel (0.1, 0.05, 0.025, and 0.013 µg/mL) (Ferron, Lot. No. L0201NA) for 48 hours. After treatment, MTT reagent (5 mg/mL) was added, and the mixture was incubated for 4 hours. To eliminate solvent-related bias, the same concentration of DMSO was also included in the negative control group (medium only). Each concentration of extract and paclitaxel was tested in triplicate to ensure reliability and reproducibility. The formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm using a microplate reader (iMark, Bio-Rad Laboratories, Japan). IC_{50} values were calculated using GraphPad Prism version 9.0.0.

Selectivity Index (SI) Determination

The Selectivity Index (SI) was calculated as the ratio of IC_{50} in normal cells (Vero or RAW 264.7) to the IC_{50} in cancer cells (HeLa), as follows:[16,17]

$SI = IC_{50}$ (normal cell) / IC_{50} (cancer cell)

An SI > 2 was considered indicative of selective cytotoxicity.[18,19]

Flow Cytometry Analysis: Cell Cycle and Cyclin D1 Protein Expression

HeLa cells (5×10^5 cells/well) were cultured in 6-well plates and incubated at 37 °C with 5% CO_2 for 24 hours to allow for adherence. Cells were then treated with *T. sumatrana* extract or paclitaxel at their respective IC_{50} concentrations and incubated for another 48 hours. To avoid solvent-related bias, the same final DMSO concentration was used in the untreated control cells. For cell cycle analysis, cells were harvested after 48 hours of treatment with 0.025% trypsin, washed three times with cold PBS (centrifuged at 2500 rpm for 5 min), and fixed in 70% cold ethanol at −20 °C for 2 hours. After fixation, cells were washed to remove residual ethanol and stained with propidium iodide (PI) solution containing RNase A before analysis by flow cytometry (FACScan, BD Biosciences, USA).[20]

For Cyclin D1 protein expression, cells were treated for 48 hours, then fixed, permeabilized, and incubated with anti-Cyclin D1 antibody (Invitrogen), followed by fluorochrome-conjugated secondary antibody (Invitrogen). Fluorescence intensity was measured by flow cytometry (FACS Canto II, BD Biosciences, USA) and analysed using BD FACSDiva software to determine relative protein expression levels, using the same detailed procedure as the previous study.[20] Flow cytometry data were processed using sequential gating to exclude debris and doublets before quantification of DNA content or Cyclin D1 protein expression. Representative dot plots and gating strategies are provided in Supplementary Figures S1–S2, with details following a previous study.[21]

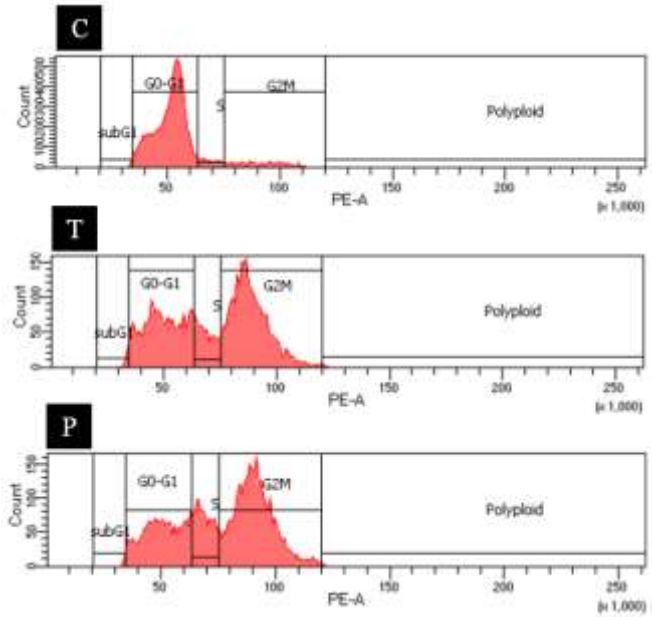

Figure S1: Representative histograms of cell cycle distribution in HeLa cells following treatment with *T. sumatrana* leaf extract (T), paclitaxel (P), and untreated control (C), analysed by flow cytometry. The plots show DNA content (PE-A) versus cell count, illustrating redistribution of cell populations across $subG_1$, G_0/G_1, S, G_2/M, and polyploid phases.

PE-A: Phycoerythrin–area fluorescence intensity (proxy for DNA content); Count: Number of cells detected at each fluorescence intensity; $SubG_1$: DNA fragmentation/apoptotic cells; G_0/G_1: Resting (G_0) and growth (G_1) phases; S: DNA synthesis phase; G_2/M: Growth (G_2) and mitosis phases Polyploid: Cells with >4N DNA content

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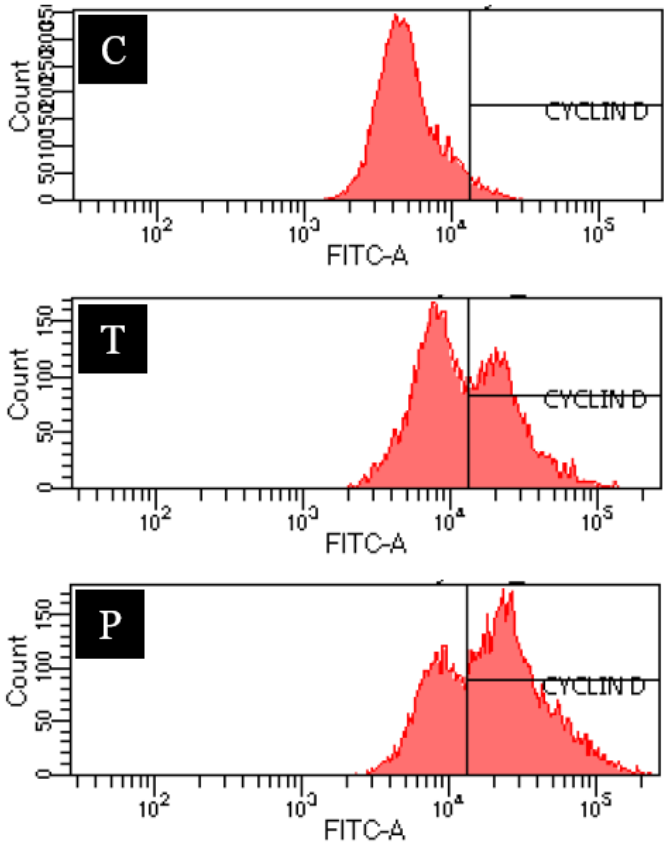

Figure S2: Representative histograms of Cyclin D1 protein expression in HeLa cells following treatment with *T. sumatrana* leaf extract (T), paclitaxel (P), and untreated control (C), analysed by flow cytometry. The plots show fluorescence intensity (FITC-A) corresponding to intracellular Cyclin D1 staining, illustrating the shift in protein expression levels across treatment groups.
FITC-A: Fluorescein isothiocyanate–area fluorescence intensity (proxy for Cyclin D1 protein expression); Count: Number of cells detected at each fluorescence intensity

Statistical Analysis
All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). IC_{50} values and SI were calculated using GraphPad Prism (version 9.0.0). Statistical comparisons between groups were performed using one-way ANOVA followed by Tukey's post-hoc test, with $p<0.05$ considered statistically significant.

Results and Discussion

This study investigates the selective cytotoxic effect of *T. sumatrana* leaf extract on cervical cancer cells. The extract shows promising potential as a plant-derived anticancer agent. Its activities include inducing cell death, modulating cell cycle progression, and regulating key cellular proteins. The effects were evaluated in comparison with paclitaxel, a well-established chemotherapeutic agent. The ethanolic extract of *T. sumatrana* leaves demonstrated selective cytotoxicity toward HeLa cervical cancer cells. When tested on Vero epithelial cells and RAW 264.7 cells, the extract also reduced cell viability in a dose-dependent manner (Figure 1A). However, the IC_{50} value for HeLa cells was substantially lower (5.84 µg/mL) than that observed in Vero (50.85 µg/mL) and RAW 264.7 cells (88.02 µg/mL), as summarised in Table 1, and as did paclitaxel (Figure 1B). In this study, paclitaxel cytotoxicity was assessed only in HeLa cells, as its selectivity profile in normal cells has already been extensively documented in the literature. Paclitaxel was therefore included primarily as a mechanistic comparator for apoptosis, cell cycle arrest, and modulation of Cyclin D1 protein expression, rather than for re-evaluation of selectivity. This approach highlights the novelty of the present study, which provides the first selectivity index data for *T. sumatrana* leaf extract. These differences yielded selectivity index (SI) values of 8.71 (HeLa vs. Vero) and 15.07 (HeLa vs. RAW 264.7), exceeding the threshold of 2.0 commonly used to indicate selective cytotoxicity.[18] Low selectivity remains a key challenge in conventional chemotherapy. Drugs such as doxorubicin and paclitaxel often damage healthy tissues due to their non-specific action.[4,22] Vero cells are commonly used to assess such non-selective toxicity.[23,24] The significantly higher IC_{50} in Vero cells compared to HeLa cells in this study confirms the extract's safety margin.

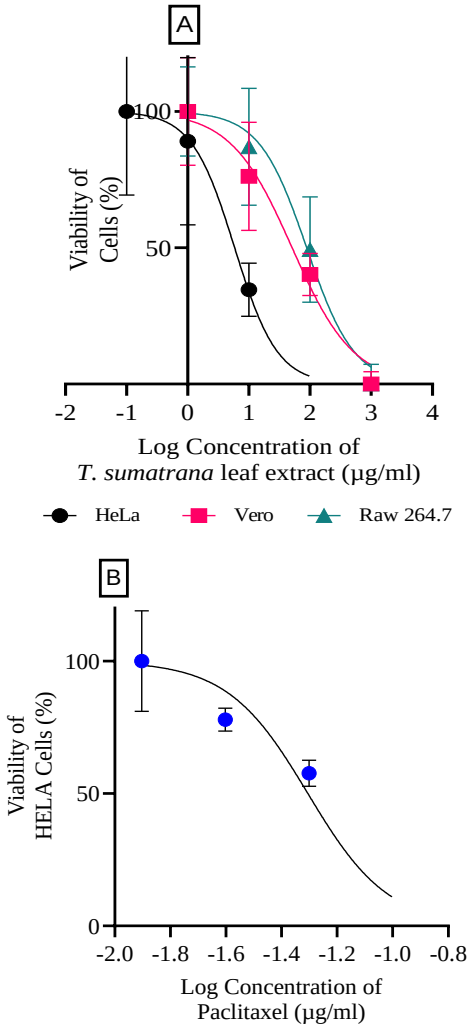

Figure 1: Dose-dependent cytotoxic effect of *T. sumatrana* leaf extracts on HeLa, Vero, and RAW 264.7 cell (A) and paclitaxel in HeLa cells (B). Cell viability (%) was plotted against the logarithm of treatment concentration (µg/ml). IC_{50} values were calculated using nonlinear regression (GraphPad Prism 9.0). Data represent mean ± SD of three independent experiments performed in triplicate.

The IC_{50} value obtained is comparable to those reported for other *Taxus* species, such as *T. baccata* and *T. brevifolia.*[25,26] Cytotoxicity studies of plant-derived substances have shown a wide range of IC_{50} values depending on species, compounds, and cell lines tested. For example, *Grewia velutina* extract had an IC_{50} of 88.76 µg/ml against HeLa cells.[27] The IC_{50} values for the leaf extract and bark extract of *Rhamnus frangula* on the MCF-7 breast cancer cell line were found to be 0.43 mg/ml and 5 mg/ml, respectively.[28] The anticancer activity results of African plants showed that RPA extract reduced MCF-7 cell

proliferation by 18%, while PS, JCP1, and JCP2 induced cell death at 10 µg/ml with IC_{50} values between 23 and 38 µg/ml.[29,30] Isolated compounds like taxuspine M showed moderate cytotoxicity, with an IC_{50} of 195.5 nM in MCF-7 breast cancer cells,[31], or 7-X-10-DTA from T. × media had low IC_{50} values in HeLa and PC-3 cells (0.32–0.76 µM),[32], but their selectivity remains limited. A serum containing *Taxus chinensis* extract inhibited liver cancer cells at 8–9% cell viability.[33]

Selective cytotoxicity data for *T. sumatrana* remain limited. However, other *Taxus* species have shown promising results. For example, *T. baccata* from Spain demonstrated a high selectivity index (SI) of 157.3 ± 110.6 against A549 lung cancer cells.[34] In contrast, natural extracts from other plant sources have shown lower selectivity. A study reported diterpenoid compounds from *Caesalpinia pulcherrima*, structurally similar to paclitaxel, with SI values ranging from 0.3 to 1.5 against HeLa cells.[35] A novel synthetic compound, 4-methyl-3-benzoyl allylthiourea, also showed moderate selectivity, with SI values of 2 for T47D cells and 4 for MCF-7/HER2 cells.[36] Although potent, isolated compounds often lack adequate selectivity. For example, paclitaxel has demonstrated very low IC_{50} values in previous studies, reported at 0.05 µg/mL[8] and 5–10 nM (≈ 0.0043–0.0086 µg/mL)[37], with an SI of only 0.94 against HeLa cells. Doxorubicin showed an IC_{50} of 3.57 µM with an SI of 1.31, while cisplatin had an IC_{50} of 2.91 µM and an SI of 2.11 in HeLa cells.[38] Compared with other cancer types, doxorubicin and 5-fluorouracil (5-FU) showed SI values of 22.53 and 1.93 for MCF-7 and HT-29 cells, respectively.[39]

Table 1: IC_{50} and SI of the leaf extract of *T. sumatrana* on HeLa, Vero, and RAW 264.7 cells, and IC_{50} of paclitaxel on HeLa cell lines

Samples	Parameters	Type of cells		
		HeLa	**Vero**	**RAW 264.7**
Extract of *T. sumatrana* leaf	IC_{50} (95% CI) (µg/ml)	5.84 (2.03 to 14.90)	50.85 (23.67 to 105.60)	88.02 (39.72 to 175.80)
	SI	-	8.71	15.07
Paclitaxel	IC_{50} (95% CI) (µg/ml)	0.05 (0.04 to 0.06)	-	-

Values are Mean (95% CI), n = 3.
SI, Selectivity index; IC_{50}, Inhibitory Concentration 50%

This study also examined the effect of *T. sumatrana* leaf extract on the viability of RAW 264.7 cells, as shown in Figure 1A and Table 1. Macrophages play an essential role in removing apoptotic cells and maintaining tissue homeostasis.[40,41] Failure to clear apoptotic bodies efficiently can lead to secondary necrosis and inflammation.[42,43] In this study, the IC_{50} value in RAW 264.7 cells was 88.02 µg/ml, which is considerably higher than in HeLa cells (5.84 µg/ml). This suggests that the extract is significantly less toxic to macrophages than to HeLa cells. This is important because many anticancer agents work by inducing apoptosis, such as paclitaxel or doxorubicin, which trigger programmed cell death in cancer cells.[44,45] A previous study showed that activated RAW 264.7 cells successfully phagocytosed apoptotic A549 lung cancer cells after cisplatin treatment.[46] Furthermore, general reviews have reported that numerous plant-based extracts can trigger cancer cell death while keeping macrophages functional.[47] These insights highlight the potential safety of *T. sumatrana* extract when it comes to immune cell compatibility.

Furthermore, the antiproliferative mechanism of *T. sumatrana* leaf extract was explored by analysing the cell cycle distribution of HeLa cells after 24-hour treatment. As shown in Table 2, both the extract (T) and paclitaxel (P) caused significant shifts compared to the untreated control (C). Quantitative analysis in Figure 2 confirmed a marked accumulation of cells in the G_2/M phase, rising from 11.6% in the control to 48.6% and 52.0% in the extract- and paclitaxel-treated groups, respectively ($p < 0.0001$). This suggests mitotic arrest, consistent with paclitaxel's mechanism of stabilising microtubules at the spindle checkpoint.[48] This G_2/M arrest was accompanied by a reduction in the G_0/G_1 population (from 81.8% to 36.9% in the extract and 30.7% in the paclitaxel group) and an increase in the Sub-G_1 fraction, indicative of DNA fragmentation and apoptosis. Both treatments showed a similar trend of cell cycle redistribution, suggesting that the extract may partially mimic paclitaxel's mechanism of action. Although the precise mechanism of the extract remains under investigation, its activity is likely mediated by multiple bioactive constituents. Previous phytochemical studies on *Taxus* species have identified taxane-related diterpenoids such as wallifoliol, taxuspine F, taxumairol,[49] tasumatrols,[50–55], and taiwantaxins,[56]; along with other compounds, including flavonoids and phenolics.[57,58] These constituents, some of which share structural similarities with paclitaxel, may contribute synergistically to the observed cytotoxicity and G_2/M arrest. G_2/M arrest is a common outcome of DNA damage and checkpoint activation, often leading to apoptosis.[59] Indeed, plant-derived compounds, including paclitaxel, have shown G_2/M arrest as a major antiproliferative strategy.[60–62] This finding aligns with our previous observation of p53 upregulation, where the extract increased p53 expression to 32.9% (vs. 3.6% in control).[8] Since p53 is a key regulator of both apoptosis and cell cycle arrest, its elevation likely supports checkpoint engagement and contributes to the observed G_2/M checkpoint.[63]

Table 2: Distribution of HeLa cells across different cell cycle phases after treatment with *T. sumatrana* leaf extract (T) or paclitaxel (P), compared with untreated control (C).

Samples	Cell cycle phase (%)				
	SubG_1	**G_0/G_1**	**S**	**G_2/M**	**Polyploid**
C	1.0±0.38	81.8±2.71	5.4±0.58	11.6±1.93	0.0±0.04
T	1.5±0.13	36.9±0.67	12.1±0.29	48.6±0.53	0.1±0.04
P	1.0±0.20	30.7±0.42	15.6±0.18	52.0±0.49	0.1±0.04

SubG_1: DNA fragmentation/apoptotic cells
G_0/G_1: Resting (G_0) and growth (G_1) phases
S: DNA synthesis phase
G_2/M: Growth (G_2) and mitosis phases
Polyploid: Cells with >4N DNA content

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This study evaluated Cyclin D1 protein levels in HeLa cervical cancer cells after 48 hours of treatment with *T. sumatrana* leaf extract and paclitaxel. Both treatments led to a marked increase in Cyclin D1 protein expression, rising to approximately 43.7% in extract-treated cells and 65.5% in paclitaxel-treated cells, compared to 4.9% in untreated controls (Figure 3). However, the interpretation of Cyclin D1 protein upregulation should be made cautiously, as its biological role is context-dependent. While overexpression of Cyclin D1 protein has been associated with cell proliferation in many cancers, previous reports also show that under stress conditions, its accumulation may contribute to mitotic arrest or trigger apoptotic signaling.[64,65] Our results therefore suggest a preliminary involvement of Cyclin D1 dysregulation in *T. sumatrana*–induced cytotoxicity, but further studies are needed to confirm its precise role.

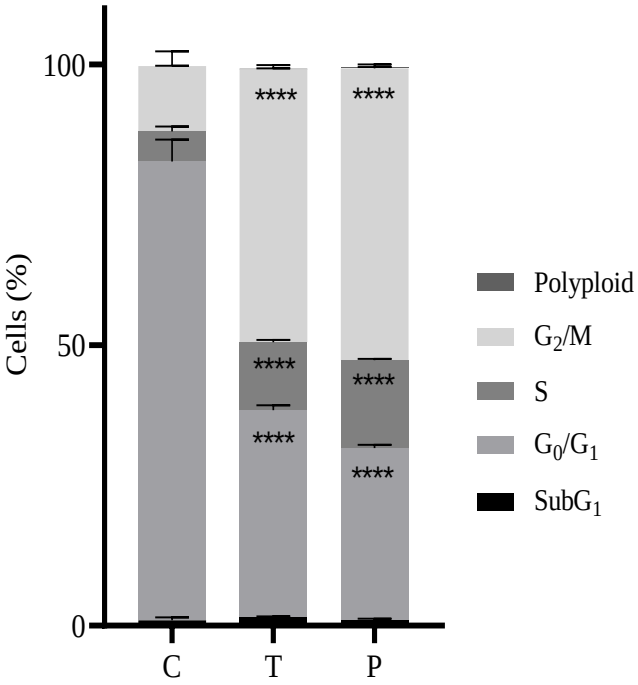

Figure 2: Quantitative comparison of the percentage of HeLa cells in each cell cycle phase after treatment with *T. sumatrana* leaf extract (T) and paclitaxel (P), compared to untreated cells (C). Data were analysed using two-way ANOVA followed by Tukey's multiple comparison tests.
$p < 0.05$ is considered statistically significant. ****$p < 0.0001$ versus control
$SubG_1$: DNA fragmentation/apoptotic cells; G_0/G_1: Resting (G_0) and growth (G_1) phases; S: DNA synthesis phase; G_2/M: Growth (G_2) and mitosis phases; Polyploid: Cells with >4N DNA content

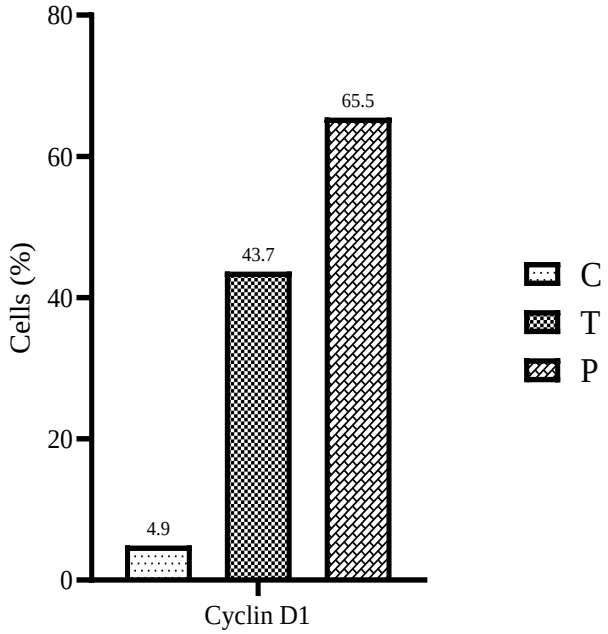

Figure 3: Effect of *T. sumatrana* leaf extract and paclitaxel on protein D1 expression in HeLa cells measured by flow cytometry. Protein expression levels of Cyclin D1 in HeLa cells following treatment with *T. sumatrana* leaf extract (T) and paclitaxel (P), each at their respective $1\times IC_{50}$ concentrations, compared to untreated control cells (C). Protein expression was measured using flow cytometry.

Conclusion

This study shows that *T. sumatrana* leaf extract exerts selective cytotoxicity against cervical cancer cells by inducing G_2/M arrest and modulating Cyclin D1 protein expresion, with mechanistic similarities to paclitaxel, while being less toxic to normal epithelial and macrophage-like cells. However, the chemical composition of extract was not characterized in this study, and the findings are limited to *in vitro* assays in a single cell line. Further research should focus on phytochemical profiling (e.g., HPLC, LC-MS), testing in multiple cancer models, and *in vivo* evaluation. These results provide a preliminary foundation for developing *T. sumatrana* as a potential source of selective, plant-based anticancer agents.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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