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Original Article

INVESTIGATION OF THE APOPTOSIS MECHANISM INDUCED BY *TAXUS SUMATRANA* EXTRACT LEAF IN CERVICAL CANCER CELLS

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ABSTRACT

Objective: This study aims to investigate apoptosis pathways induced by *T. sumatrana* leaf extract, evaluate their impact on the modulation of p53 protein expression, and the regulation of Bax and Bcl-2 gene expression.

Methods: The study included 3-5 experimental groups: untreated cells, *T. sumatrana* extract-treated cells (one or two concentrations), and paclitaxel-treated cells (one or two concentrations) as a positive control. The leaves were macerated in 70% ethanol to obtain the extract. Flow cytometry was employed to determine apoptosis rates and p53 protein expression. Real-time PCR was used to quantify the expression level of the Bax and Bcl-2 genes. ANOVA was utilized for statistical analysis to compare the mean differences among the groups.

Results: Treatment with the *T. sumatrana* extract significantly increased the apoptosis rates in HELA cells ($P<0.0001$) and upregulated p53 protein expression compared to the untreated cells. The extract also increased Bax and decreased Bcl-2 expression, suggesting a shift towards a pro-apoptotic stage. The extract also increased Bax and decreased Bcl-2 expression, suggesting a shift towards a pro-apoptotic stage. The effects on apoptosis and p53 expression were comparable to paclitaxel at equivalent IC_{50} concentrations.

Conclusion: The ethanol extract of *T. sumatrana* leaf promotes apoptosis in HELA cervical cancer cells by promoting p53 protein expression, increasing Bax, and decreasing Bcl-2 expression. These findings suggest its potential as an apoptosis-inducing agent, comparable to paclitaxel, and highlight its promise for further investigation in cervical cancer therapy.

Keywords: Cemara sumatra, Paclitaxel, p53, Bax, Bcl-2, HELA, Natural product

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INTRODUCTION

Taxus sumatrana, commonly known as the Sumatran yew, is recognized by local communities for its medicinal properties, particularly in cancer treatment. Traditionally, *T. sumatrana* is used for various diseases, including cancer, with its bark and leaves harvested extensively. This extensive use contributes to the increasing rarity of the plant. However, the lack of scientific evidence supporting the efficacy and safety of using any parts of *T. sumatrana,* such as the bark or leaves as used by local populations [1]. The only Taxus-derived compound that has been extensively studied is paclitaxel, which was originally isolated from the bark of *T. brevifolia* [2]. The use of *T. sumatrana* as an anticancer agent has been based on the assumption that, since it belongs to the same genus as *T. brevifolia*, it may possess similar medicinal properties to paclitaxel [3]. However, this assumption is not supported by scientific evidence.

Previous studies on *T. sumatrana* have focused on isolating chemical compounds from the leaves, bark, and twigs. Paclitaxel and baccatin were isolated from the bark [4]. Taxumairol, taxuspine, and wallifoliol were isolated from the leaves and twigs [5]. The tasumatrols, Taiwantaxins, and other chemical compounds have also been reported as isolated from the leaves and twigs [6–14]. Following reports that the bark of *T. sumatrana* also contains paclitaxel, its usage has significantly increased, leading to a rise in the harvesting of not only the bark but also the leaves and twigs. This increased demand has contributed to the plant becoming increasingly rare.

Despite the focus on the leaves, no reports are confirming the presence of paclitaxel in them, nor have there been studies demonstrating the anticancer activity of the leaves, either *in vitro* or *in vivo*. However, the leaves have been widely used, while there is a lack of efficacy and safety data regarding their use as an anticancer agent. This highlights the need for further research to validate medical claims related to *T. sumatrana* leaves.

As an early step, we have screened the cytotoxic activity of the parts of T. sumatrana. The result found that *T. sumatrana* extract exhibits potent cytotoxic activity against cervical cancer cells, with an IC_{50} (Inhibitory Concentration 50) value below 20 µg/ml [15]. HELA cells are a widely used cervical cancer cell line derived from adenocarcinoma [16, 17]. This finding emphasizes the need for further investigation into whether the mechanism of cell death is apoptosis or necrosis.

Apoptosis is vital for maintaining tissue homeostasis and eliminating damaged or abnormal cells, including cancer cells [18, 19]. Ideally, cancer cell death should occur via apoptosis, a form of programmed cell death primarily induced by the p53 gene, a key apoptosis regulator, and other pro-and anti-apoptotic genes such as Bax and Bcl-2. Induction of apoptosis by chemotherapeutic agents such as paclitaxel has been thoroughly investigated and established as effective in cancer treatment [20]. Paclitaxel operates by disrupting microtubule dynamics, leading to cell cycle arrest at the G2/M phase and triggering apoptosis via molecular pathways that include the upregulation of pro-apoptotic proteins such as the Bax gene and the downregulation of anti-apoptotic proteins like Bcl-2 [21, 22].

Considering that the leaves of *T. sumatrana* have been used as a medicine believed to cure cancer, this study will explore their potential as an anticancer agent. This study focuses on leaf extracts as traditionally used. This study investigates the mechanisms of cervical cancer cell death and evaluates its effects on the expression of the p53, Bax, and Bcl-2 genes, the key apoptosis regulator. Untreated cells and paclitaxel were used as comparators. The selection of paclitaxel as a comparator is based on its well-understood mechanism of action, particularly its induction of apoptosis and its effects on apoptotic-related genes such as p53, Bax, and Bcl-2, making it a reliable benchmark for assessing the anticancer potential of *T. sumatrana* leaf extracts [23]. In recent years, plant extracts have given rise to significant attention as potential sources of natural anticancer agents [24, 25].

MATERIALS AND METHODS

Materials

The leaves of *T. sumatrana*, ethanol 70% (Brataco), Acridine Orange (Sigma), Propidium Iodide (Sigma), PI/RNase staining buffer (BD Biosciences), Annexin PI apoptosis kit (BD Biosciences), Dulbecco's Modified Eagle Medium (Sigma), Phosphate Buffered Saline (Invitrogen), Trypsin-EDTA (Gibco), Dimethyl sulfoxide (Vivantis), Fetal Bovine Serum (Gibco), Penicillin-Streptomycin (Gibco), anti-p53 antibody (Invitrogen), Genezol (Geneaid), Sensifast cDNA Synthesis Kit (Bioline), cDNASynthesis Reagents (Bioline), SensiFAST™ SYBR (Bioline), primers for Bax (IDT), Bcl-2 genes (IDT) and GAPDH (IDT).

Methods

This study was designed to evaluate the effects of *T. sumatrana* leaf extract on HELA cells under three different treatment conditions. The first group, extract-treated HELA cells, involved exposing HELA cells to 1×IC_{50} and/or ½×IC_{50} concentrations of *T. sumatrana* leaf extract. The second group, untreated control, included HELA cells that received no treatment, serving as a negative control. The third group, paclitaxel-treated cells, consisted of HELA cells treated with paclitaxel at 1×IC_{50} and/or ½×IC_{50} concentrations, serving as a positive control.

Preparation of *T. sumatrana* leaf extract

T. sumatrana's leaves were collected from Mount Singgalang, West Sumatera. The plant was identified by a botanist, and a voucher specimen was deposited at the Herbarium ANDA, Universitas Andalas, Padang, Indonesia (No. DEP012024). The leaves of *T. sumatrana* were air-dried for two weeks. The dried leaves were then ground into a fine powder using a grinder. The powdered simplicia was macerated with 70% ethanol; the solvent was evaporated using a rotary evaporator to obtain a concentrated extract. The concentrated extract was stored in a sealed glass bottle, shielded from light by wrapping it in aluminum foil, and kept in a refrigerator at 2-8 °C [26].

Determination of IC_{50} values of *T. sumatrana* leaf extract and paclitaxel

The IC_{50} value of *T. sumatrana* leaf extract, 5.93 µg/ml, was determined in a previous preliminary study using the MTT assay method [15]. To ensure consistency and comparability, the IC_{50} value for paclitaxel was re-evaluated in this study under identical experimental conditions. HELA cells were plated at a density of 6,000 cells per well in 96-well plates and treated with serial concentrations of paclitaxel (0.1, 0.05, 0.025, and 0.0125 µg/ml) for 48 h. Cell viability was assessed using the MTT assay, and dose-response curves were generated with GraphPad Prism to calculate the IC_{50} value.

The rationale for selecting these IC_{50} values was to establish standard reference concentrations for all experimental treatments, enabling direct comparison of cytotoxic and apoptotic effects. Paclitaxel was chosen as a positive control due to its well-documented efficacy and safety as a chemotherapeutic agent for various cancers [27, 28], including cervical cancer [29, 30], and its established mechanism of action in inducing apoptosis [31, 32]. Moreover, paclitaxel is a known metabolite found in the bark of *T. sumatrana* [33].

Preparation of test solutions for apoptosis and gene expression analysis

A series of test solutions was prepared. *T. sumatrana* leaf extract was diluted to final concentrations of 5.93 µg/ml and 2.97 µg/ml, corresponding to 1xIC_{50} and ½xIC_{50} the extract against HELA cells. Paclitaxel used as a positive control, served as a comparator at the concentration of 0.05 µg/ml and 0.03 µg/ml, equivalent to the 1xIC_{50} and ½xIC_{50} of paclitaxel on HELA cells and untreated-cell as the negative control.

Cell culturing procedure

HELA cells are used as a representative model for studying cervical cancer [34]. The HELA cell lines were obtained from the Tissue Culture Laboratory, Faculty of Medicine, Gadjah Mada University, Jogjakarta, Indonesia. HELA cells were maintained up to passage XX. Cells were maintained in the Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and maintained in a humidified incubator at 37 °C with 5% CO_2. Cells were seeded in 6-well plates and allowed to reach 70–80% confluency. For apoptosis and gene expression, cells were treated with extract solution at 5.93 and 2.97 µg/ml; and paclitaxel solution at 0.05 and 0.03 µg/ml, for 48 h. The treated HELA cells were trypsinized, collected, and centrifuged. The supernatant was removed; the cell pellet was washed twice with cold PBS. The detailed procedure described in our previous study [35–37] and the whole process comply with good cell culture practice guidance [38].

Analysis of cell death by flow cytometry

The cell pellets were resuspended in 100 µl** of binding buffer and stained with 5 µl** of Annexin V-FITC and 5 µl** of propidium iodide (PI). The cells were then gently mixed and incubated at room temperature in the dark for 15 min. After the staining process, 400 µl** of binding buffer was added to each sample. The stained samples were analyzed using a flow cytometer (BD FACS Canto II) configured to detect Annexin V-FITC and PI signals. For each sample, 10,000 events were recorded. Data were collected and analyzed using BD FACS Diva software, which classified the cells into quadrants representing live cells, early apoptotic cells, late apoptotic cells, or necrotic cells. The percentage of cells in each quadrant was calculated to determine the proportion of live, apoptotic, and necrotic cells in response to the treatments. The detailed procedure was described in the previous study [39].

Analysis of p53 protein expression by flow cytometry

The cell pellets were resuspended in PBS and fixed with fixation buffer for 10-15 min at room temperature, followed by washing with PBS. The cells were then permeabilized with permeabilization buffer for 10-15 min and incubated with blocking buffer for 10-15 min. Next, the cells were stained with 5 µl** of fluorochrome-conjugated anti-p53 antibody and incubated in the dark for 30-60 min. After washing with PBS to remove unbound antibodies, the samples were analyzed using a flow cytometer (BD FACS Canto II) configured to detect the p53 signal. For each sample, 10,000 events were recorded, and data were analyzed using BD FACSDiva software to quantify p53 expression levels.

Analysis of Bax and Bcl-2 gene expression by rt-PCR

The expression of Bax and Bcl-2 genes was analyzed by rt-PCR. RNA was extracted from the cell pellet through cell lysis, RNA phase isolation, and RNA precipitation and washing, followed by dissolution in RNase-free water. Total RNA concentration was measured with a NanoDrop. The RNA was then reverse-transcribed into cDNA using the cDNA Synthesis Kit and reagents. For gene expression analysis, real-time PCR was conducted with SYBR Green qPCR Master Mix and specific primers for Bax, Bcl-2, and GAPDH. GAPDH served as a housekeeping gene, and fold changes in gene expression were calculated using the ΔΔCt method as in the previous study [40].

Data analysis

Apoptosis and genes/protein expression data were derived from three replicates and presented as means±SD. Significant differences of proportion cells in live, apoptosis, and necrosis stages in extract-treated cells, paclitaxel-treated cells, and untreated cells were determined using two-way ANOVA, and total apoptosis, expression of p53, Bax, and Bcl-2 protein/genes were determined using one-way ANOVA. p-values less than 0.05 are considered statistically significant.

RESULTS

The following results present the findings of our study on the investigation of cell death in HELA cervical cancer cells induced by *T. sumatrana* leaf extract. This section is divided into two main topics, induction of apoptosis and impact on apoptosis-related genes (p53, Bax, and Bcl-2) expression.

IC_{50} value of paclitaxel

The effect of paclitaxel on HELA cell viability was assessed in the range of 0.1-0.0125 µg/ml concentrations. The data showed a dose-dependent decrease in cell viability with increasing paclitaxel concentration. At the highest concentration of 0.1 µg/ml, cell viability was reduced by approximately 80%, while at lower concentrations, viability was much higher (fig. 1). Analysis of viability data at various concentrations with GraphPad Prism obtained an IC_{50} value of paclitaxel of 0.05 µg/ml (95% CI: 0.04, 0.06 µg/ml), indicating the concentration required to reduce cell viability by 50%.

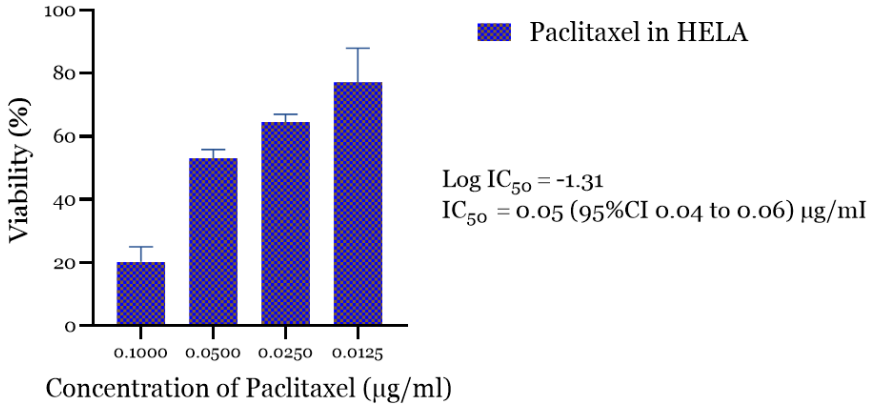

Fig. 1: Mean percentage viability of HELA cells treated with paclitaxel at different concentrations

This paclitaxel IC_{50} value, along with the IC_{50} value for *T. sumatrana* leaf extract obtained from previous studies using the same methodology and procedures were utilized for further testing in apoptosis analysis and gene expression associated with apoptosis.

Detection of cell death by double staining

The mechanism of cervical cancer cell death due to treat of *T. sumatrana* leaf extract was investigated using fluorescence microscopy. Qualitative analysis of fluorescence micrographs provides insight into the mechanism of cell death induced by the leaf extract compared to untreated controls. Fluorescence micrographs show the differences in the mode of cell death between the extract-treated ($T_{L.1}$) and untreated cells (C_0). In the extract-treated cells ($T_{L.1}$), the dominant cell population exhibits yellow fluorescence, indicating that apoptosis is the dominant mode of cell death induced by *T. sumatrana* leaf extract (fig. 2 panel $T_{L.1}$). A small number of necrotic cells (red) can also be observed. In contrast, image C_0 exhibits predominantly green fluorescence, indicating that most of the untreated control cells remain alive (fig. 2 panel C_0).

Analysis of cell death by flow cytometry annexin V/PI staining

Flow cytometric imaging displays characteristic differences in the distribution of HELA cells across the various exposures. Analysis of cell death showed a significant shift in the proportions of live, apoptotic, and necrotic cells in the extract-or paclitaxel-treated cells compared to untreated cells (fig. 3). In untreated cells (C_0), most of the cells were live, with only a lower proportion undergoing early and late apoptosis. After exposure to the extract or paclitaxel, the shift in cell population proportions was observed. There was a decrease in the number of live cells and an increase in cells undergoing necrosis, as indicated by the intensity of colors in each quadrant (fig. 3).

Based on the changes in cell percentages, it was shown that *T. sumatrana* extract, at concentrations of $1xIC_{50}$ and $½xIC_{50}$, induced an increase in the proportion of cells undergoing apoptosis in both early and late stages compared to the untreated cells. T. sumatrana extract at concentrations of $1xIC_{50}$ and $½xIC_{50}$, increased the proportion of cells undergoing early apoptosis was about 2.9 to 4.1-fold. For late-stage apoptosis ranged from 26.5 to 29.1-fold compared to untreated cells. Similar to extract, paclitaxel also induces apoptosis in cervical cancer cells (fig. 4).

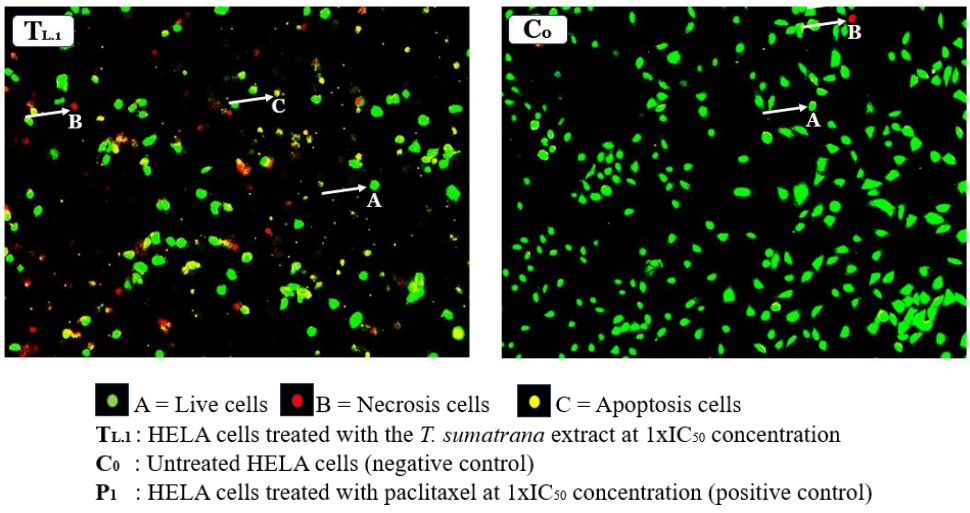

Fig. 2: Fluorescent micrographs of HELA cells double-stained with acridine orange and propidium iodide treated with extract of *T. sumatrana* leaf at $1xIC_{50}$ concentration ($T_{L.1}$) compared to negative control or untreated cells (C_0). A, the green dots represent healthy (live) cells. B, the red dots represent necrotic cells. C, the yellow dots represent the apoptosis cells

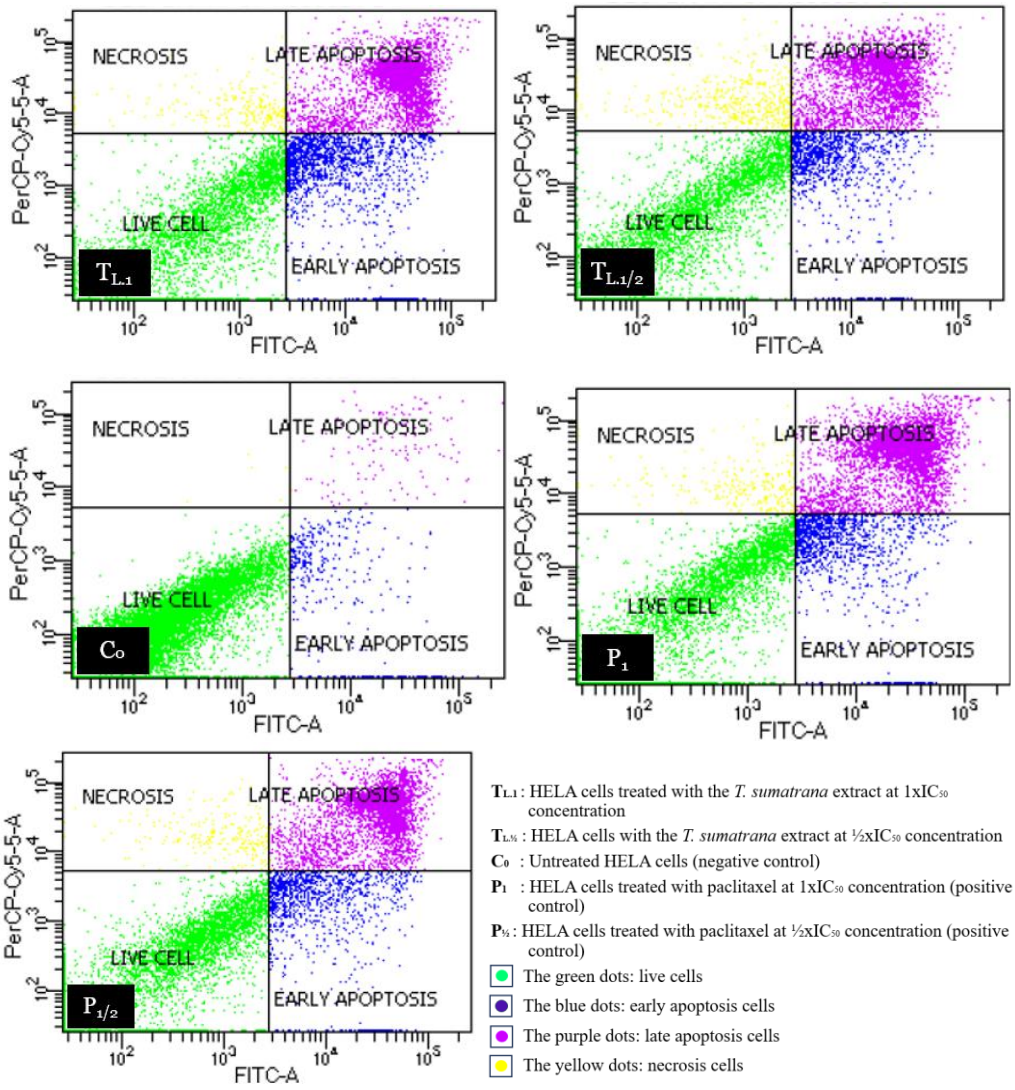

Fig. 3: The impact of *T. sumatrana* leaf extract on HELA cell death at 1xIC$_{50}$ concentration ($T_{L.1}$) and ½xIC$_{50}$ concentration ($T_{L.½}$) compared to the negative control, untreated (C_0) and positive control, paclitaxel at 1xIC$_{50}$ concentration (P_1) and ½xIC$_{50}$ concentration ($P_{½}$). After staining, the cells were analyzed by flow cytometry. The early apoptotic events (Annexin+/PI) were displayed in the lower right quadrant of each panel (blue). The upper right quadrant represents cells in the late stage of apoptosis (purple). In the lower-left panel are live cells (green); the upper-left is necrosis cells (yellow)

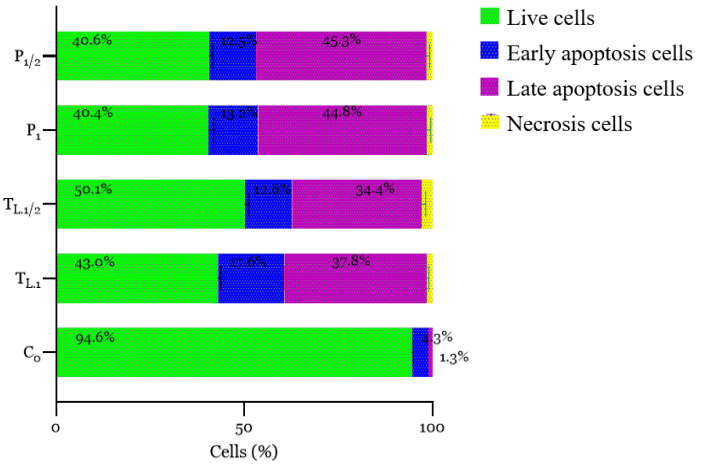

Fig. 4: Changes in the percentage of cells in the live, early apoptosis, late apoptosis and necrosis stages after treatment with the *T. sumatrana* leaf extract at 1xIC$_{50}$ concentration ($T_{L.1}$) and ½xIC$_{50}$ concentration ($T_{L.½}$) compared to the negative control, untreated (C_0) and positive control, paclitaxel at 1xIC$_{50}$ concentration (P_1) and ½xIC$_{50}$ concentration ($P_{½}$)

Analysis of two-way ANOVA of the proportion of cells that changed at the live stage, early apoptosis, late apoptosis, and necrosis after being treated with *T. sumatrana* leaf extract showed statistically significant changes in the decrease in the number of early apoptosis, late apoptosis, and necrosis compared to untreated cells. The paclitaxel as positive control also showed a similar trend and changed to the extract (fig. 5).

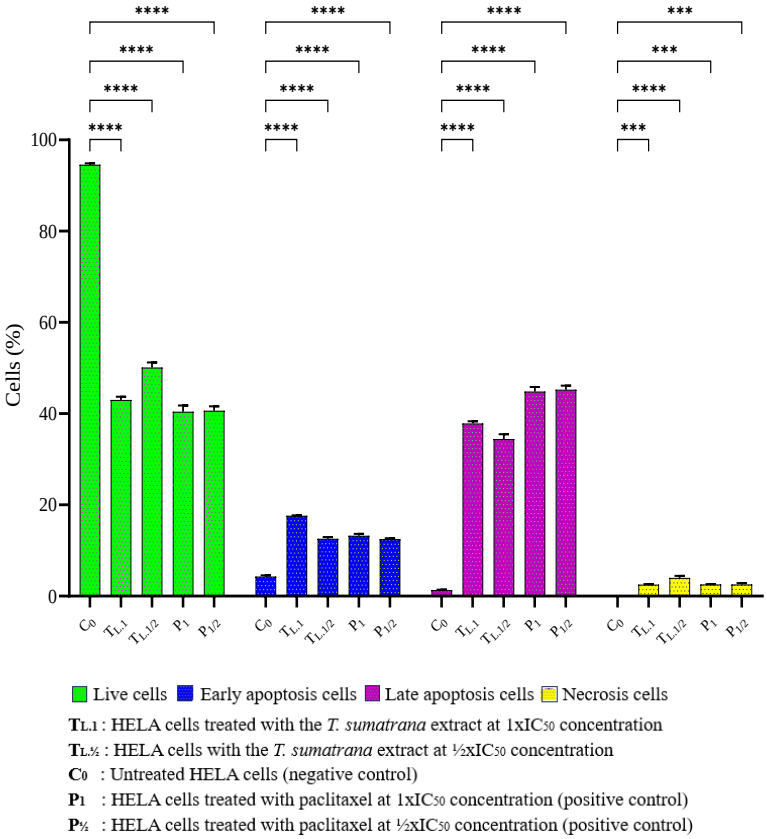

Fig. 5: Analysis proportions of HELA cells on live, apoptotic, and necrotic stages after being treated with *T. sumatrana* leaf extracts at 1xIC$_{50}$ concentration ($T_{L.1}$) and ½xIC$_{50}$ concentration ($T_{L.½}$) compared to untreated and paclitaxel-treated at 1xIC$_{50}$ concentration (P_1) and ½xIC$_{50}$ concentration ($P_{½}$). *** p<0.001 **** p<0.0001

Subsequently, to focus more specifically on apoptosis, fig. 6 illustrates the percentage of total apoptotic cells in each treatment and control group. Statistical analysis using one-way ANOVA, the proportion of cells undergoing apoptosis was higher in groups treated with *T. sumatrana* leaf extract (both $T_{L.1}$ and $T_{L.½}$) and paclitaxel (P_1 and $P_{½}$) compared to the control (C_0), with highly significant differences observed in all treated groups (T_1, $T_1/_2$, and P_1, p<0.0001). When comparing the extract-treated cells with the paclitaxel-treated cells, the percentage of apoptotic cells was similar in the T_1 cells to the P_1 cells, the difference did not reach statistical significance (p=0.541) (fig. 6).

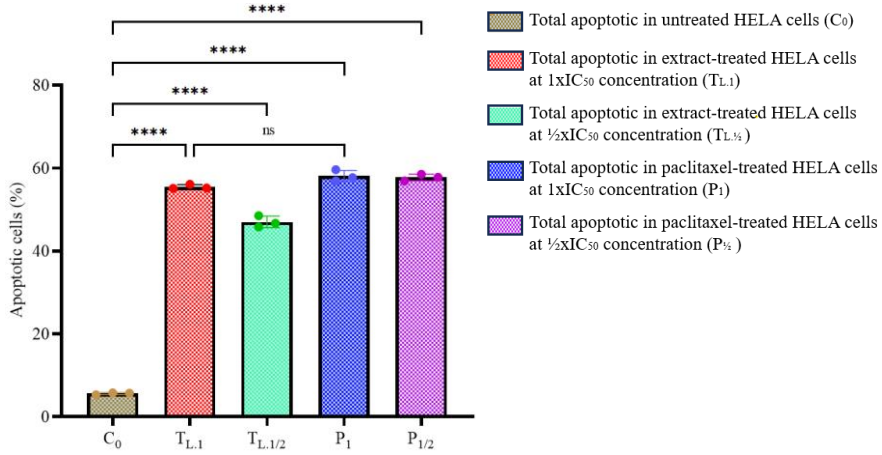

Fig. 6: The total proportion of cells in the apoptotic stage after treatment with the *T. sumatrana* leaf extract at 1xIC$_{50}$ concentration ($T_{L.1}$) and ½xIC$_{50}$ concentration ($T_{L.½}$) compared to negative control, untreated (C_0) and positive control, paclitaxel at 1xIC$_{50}$ concentration (P_1) and ½xIC$_{50}$ concentration ($P_{½}$). Statistical significance is expressed as p<0.05, **** p<0.0001; ns p>0.05

Expression of p53 protein

Based on the p53 protein expression, there was a significant increase in gene expression in extract-treated cells and paclitaxel-treated cells compared to the untreated cells (fig. 7). In the control group, the relative expression was minimal. In $T_{L.1}$, p53 expression increased to more than 30%, reaching a high level of statistical significance ($p<0.0001$). Similarly, P_1 also exhibited high p53 expression, with the group P_1 reaching 34.8%, statistically significant compared to the control ($p<0.0001$). In the comparison of extract and paclitaxel groups at the same concentration level, the expression of the p53 protein of $T_{L.1}$ was lower than in P_1 although the difference was not considered highly significant ($p=0.0253$).

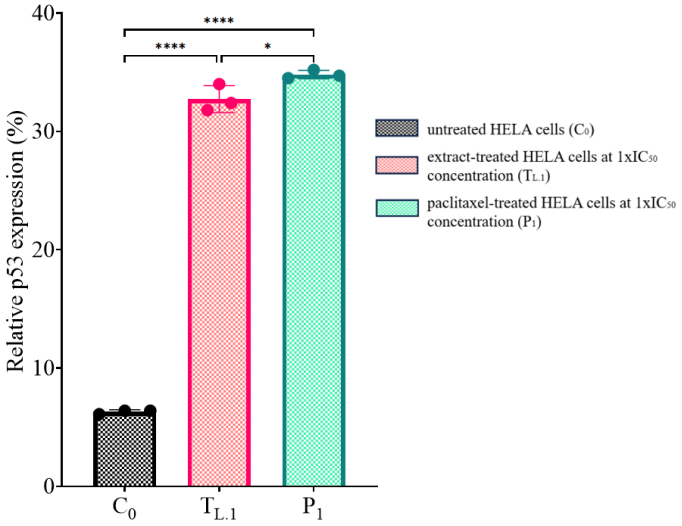

Fig. 7: Relative expression of p53 protein in HELA cells after treatment with the *T. sumatrana* leaf extract at 1xIC$_{50}$ concentration ($T_{L.1}$) compared to negative control, untreated (C_0) and positive control, paclitaxel at 1xIC$_{50}$ concentration (P_1)

Expression of bax and Bcl-2 genes

The study revealed that extract-treated (T_{L1}) and paclitaxel-treated (P_1) HELA cells increased the levels of expression of the Bax gene compared to untreated cells, as shown in fig. 8A. The T_L resulted in approximately a 4.95-fold increase in Bax expression, while P_1 showed an elevation at around 4.73-fold. Analysis of the increase in Bax expression in both the extract-treated and paclitaxel-treated groups compared to the untreated group, using one-way ANOVA, indicated that the levels of Bax expression were statistically significant ($p<0.0001$). Although the increase in Bax expression in T_L was greater than in P_1, the difference was not statistically significant ($p=0.6892$).

Contrarily, the expression of the Bcl-2 gene decreased in both treated HELA cells compared to untreated HELA cells (fig. 8B). The T_L reduced Bcl-2 expression to 0.50-fold, and P_1 also exhibited a lower expression level, falling below the limit of detection (0≈ not detected). Analysis of the decrease in Bcl-2 expression in both the extract-treated and paclitaxel-treated groups compared to the untreated group, using one-way ANOVA, revealed statistically significant results ($p<0.0001$).

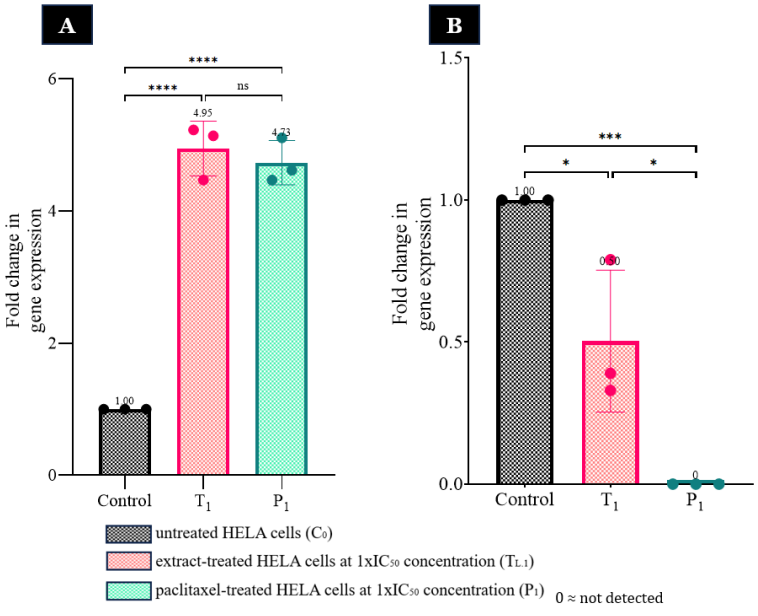

Fig. 8: The expression levels of the Bax (A) and Bcl-2 genes (B) in HELA cells after treatment with the T. *sumatrana* leaf extract at 1xIC$_{50}$ concentration ($T_{L.1}$) compared to negative control, untreated (C_0) and positive control, paclitaxel at 1xIC$_{50}$ concentration (P_1)

DISCUSSION

The results of this study demonstrate that the ethanol extract of *T. sumatrana* leaf has a significant ability to induce cancer cell death through apoptosis. This finding aligns with King *et al.* (2021) reported on paclitaxel [41] and Xu *et al.* (2024), reported that other taxane chemotherapy agents, such as docetaxel and cabazitaxel, induce apoptosis by binding to and stabilizing microtubules [22]. This stabilization triggers caspase-mediated destruction of cellular components, finally leading to cell death [42]. Our study confirms that *T. sumatrana* leaf extract can induce apoptosis at each of its IC_{50} concentrations, comparable to paclitaxel. Other chemotherapy agents, such as doxorubicin [43] and cisplatin [44], have also been reported to induce cancer cell death through apoptosis.

The ability of a drug to induce apoptosis can be seen as a sign of anticancer activity. In this study, the induction of apoptosis exceeded 55%, representing a ten-fold increase compared to untreated cells. It is important to highlight that apoptosis is the preferred pathway for cancer therapy due to its nature as programmed cell death [45]. This process allows for the orderly elimination of damaged or abnormal cells while minimizing harm to surrounding healthy tissues [46]. Additionally, apoptosis prevents inflammatory responses related to necrosis, making it a more favorable mechanism in therapeutic contexts. A promising aspect of this study is that the increase in necrosis was minimal, at less than 4%. In necrosis, cells die via unregulated processes that lead to inflammation and damage to surrounding tissues [47]. This suggests that side effects in the subsequent studies involving animals or humans are expected to be minimal. Targeting cancer cells for elimination through apoptosis can effectively reduce tumor growth and metastasis, thereby improving patient outcomes [31]. When drugs effectively induce apoptosis, they can inhibit cancer cell growth and spread; systematically destroying cancer cells reduces the likelihood of tumor development or metastasis.

The extract of *T. sumatrana* leaf demonstrated apoptotic activity comparable to paclitaxel at equivalent concentrations corresponding to their respective IC_{50} values. 0.05 µg/ml of paclitaxel is equivalent to 5.93 µg/ml of leaf extract. The previous study reported that the extraction yield of paclitaxel is low [48]. For example, the bark of *T. brevifolia* yields approximately 0.015% of dry weight. To produce 1 kg of paclitaxel, approximately 7,000 kg of raw material is needed, which comes from around 2,000 to 2,500 yew trees [49]. The limited yields and extensive harvesting practices have significantly contributed to the rarity of these plants [50].

If the activity of this extract is validated in clinical trials, it could suggest a new alternative to chemotherapy and may help reduce or prevent the scarcity of Taxus species. For example, the dosage of paclitaxel is 175 mg/m^2 for one injection [51]. An individual weighing 70 kg would require about 320 mg of paclitaxel for a dose. This amount is approximately 2.24 kg of dry bark, equivalent to about 0.72 Taxus trees. When converted to leaf extract, this corresponds to around 38.08 g of *T. sumatrana* leaf extract. Harvesting the leaves does not kill the plant; the leaves can quickly regrow, allowing for sustainable approaches.

The findings from this study demonstrated a significant increase in p53 protein expression in both the *T. sumatrana* extract-treated and paclitaxel-treated cells compared to the untreated cells. These results suggest that the *T. sumatrana* extract may induce apoptosis by activating the p53 signaling pathway. This aligns with the established role of p53 as a key regulator in the apoptosis process, where it functions to control the cell cycle and promote cell death in response to DNA damage [52]. Similar studies have shown that paclitaxel can induce apoptosis by activating the p53 pathway in various cancer cell lines, including nasopharyngeal carcinoma cells [53], human ovarian cancer cells [54], oral cancer cells [55], and lung cancer cells [56]. Two natural product studies reported p53-induction by the bark fraction of *Cassia fistula L.* in HELA cells [57] and *Herba Patriniae* in HCT116 and SW480 colorectal cancer cells [58].

The further findings of this study highlighted the significant alterations in the expression of Bax and Bcl-2, two critical regulators of the apoptotic pathway. The increase in Bax expression indicates a shift towards pro-apoptotic signaling, promoting cell death in response to stressors [59, 60], such as the *T. sumatrana* extract and paclitaxel treatment. In contrast, the observed decrease in Bcl-2 expression, an anti-apoptotic protein, further supports the pro-apoptotic environment created by these treatments [61]. Several chemotherapy compounds are identified to induce up-regulation of the Bax gene and down-regulation of the Bcl-2 gene [62]. In addition to Paclitaxel [63, 64], other chemotherapeutic agents, such as Doxorubicin and Cisplatin exhibit similar effects [65, 66]. The balance between Bax and Bcl-2 is critical, as the ratio of pro-apoptotic to anti-apoptotic proteins determines the destiny of the cell [62]. An elevated Bax to Bcl-2 ratio is associated with increased apoptosis, reinforcing the potential of the *T. sumatrana* extract as an effective anticancer agent by disrupting this balance and triggering cell death in cancer cells.

In addition to promoting apoptosis and restoring the balance between pro-apoptotic and anti-apoptotic proteins, decreasing Bcl-2 expression can make cancer cells more sensitive to chemotherapeutic agents. These findings support the idea that targeting Bcl-2 could be a strategy for developing new anticancer therapies aimed at reducing resistance and enhancing treatment responses. Currently, several chemotherapies that target Bcl-2 have been developed [61].

The presence of chemical compounds such as taxumairol, taxuspine, wallifoliol, tasumatrols, and Taiwantaxins in *T. sumatrana* leaves [5–14] indicates a promising potential for various anticancer activities. These compounds may work synergistically to improve the overall therapeutic effects of the extract [67]. However, it is important to consider the potential of antagonistic interactions that could be damaging, highlighting the need for comprehensive studies to evaluate both beneficial and harmful effects in the context of cancer treatment [68]. Although these possibilities are intriguing, they remain speculative, highlighting the need for further study to isolate other compounds in the leaves and evaluate the individual activities of each component.

All findings of this study demonstrated the potential of the *T. sumatrana* extract as a source of novel anticancer compounds that can target the p53-mediated apoptotic pathway and modulate Bax and Bcl-2 expression. The comparable effects of the *T. sumatrana* extract and paclitaxel on the expression of p53, Bax, and Bcl-2 suggest that the extract may have a mechanism of action similar to that of paclitaxel. Further studies on cell cycle analysis are necessary to complete the data on the mechanism of action, as paclitaxel is known to induce cell cycle arrest at the G2/M phase.

This study is limited to *in vitro* investigations, and insufficient data are available to support its use as a cancer treatment in humans. Further investigations, including *in vivo* studies and clinical trials, are warranted to fully elucidate the therapeutic potential and safety profile of the *T. sumatrana* extract and its specific bioactive compounds responsible for the observed effects on the p53 signaling pathway.

Another limitation of this study is the potential variability in the concentrations of bioactive compounds within *T. sumatrana* leaf extracts. Geographic location [69], environmental conditions [70], and harvesting time [71] can significantly influence the phytochemical composition. These variabilities become the challenges in maintaining consistent bioactivity across different batches of extracts. Future studies should prioritize standardizing the extraction process and quantifying the bioactive compounds using analytical methods such as high-performance liquid chromatography (HPLC) or liquid chromatography-mass spectrometry (LC-MS). Addressing these issues is essential to enhance the reproducibility and translational potential of *T. sumatrana* leaf extract as a therapeutic candidate.

CONCLUSION

The results of this study suggest that treating HELA cervical cancer cells with *T. sumatrana* leaf extract activates the apoptotic pathway. Significant increases in p53 and Bax expression were demonstrated while simultaneously reducing Bcl-2 expression. This modulation

suggests a pro-apoptotic effect that may enhance the sensitivity of cancer cells to treatment. Furthermore, the comparable effects of the extract and paclitaxel imply a potentially similar mechanism of action, particularly regarding the induction of apoptosis through the p53 signaling pathway. These findings emphasize the promise of the extract of *T. sumatrana* leaf as a candidate for nature-based cancer therapy.

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AUTHORS CONTRIBUTIONS

Fatma Sri Wahyuni: methodology, supervision, and funding acquisition; Desi Eka Putri: sample preparation, methodology, investigation, formal analysis, and writing-original draft; Almahdy: data analysis; Yozarwardi Usama Putra: provide the testing sample (*Taxus Sumatrana*); Dachriyanus: conceptualization and project administration**.**

CONFLICT OF INTERESTS

We have no conflicts of interest to disclose

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