International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 16, Special Issue 1, 2024

Original Article

CYTOTOXIC ACTIVITY OF *TAXUS SUMATRANA* (MIQ.) DE LAUB. BARK, LEAVES, AND SHOOTS ON HELA, T47D, AND MCF-7/HER2 CELL LINES

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Received: 27 Sep 2023, Revised and Accepted: 23 Nov 2023

ABSTRACT

Objective: *Taxus sumatrana* (Miq.) de Laub. (cemara Sumatra) is one of the plants found in Indonesia and other countries known as a medicine plant. Taxus's bark, leaves, and shoots are used traditionally and massively for some diseases (cancer, etc.), so recently it has become a rare plant. The chemical constituents of *T. sumatrana* are alkaloids, steroids, tannins, and flavonoids. This study aimed to investigate the potential anticancer properties of *T. sumatrana* bark, leaves, and shoot extracts.

Methods: The cytotoxic activity against the HELA, T47D, and MCF-7/HER2 cell lines was determined using the MTT assay. Each cell was cultured on 96 well plates treated with extract of *T. sumatrana* with concentrations of 100, 10, 1, and 0,1 µg/ml. Cells were incubated for 48 h at 37 °C, 5% CO_2 and then given 100 µl MTT solution 0.5 mg/ml in PBS (Phosphate Buffer Saline) for 4 h. The results of the measurements were processed with the GraphPad Prism Program.

Results: The bark, leaves, and shoots extracts have strong cytotoxic activity based on IC_{50} parameters. The mean IC_{50} of bark, leaves, and shoots on the HELA cell line consecutively 8.94; 5.93; and 4.08 µg/ml; on the T47D cell line 5.80, 4.86, and 4,11 µg/ml; and on MCF-7/HER2 cell line 7.46, 10.60, and 13.74 µg/ml).

Conclusion: *T. sumatrana* bark, leaves, and shoots have potential anti-cancer properties.

Keywords: Cemara Sumatra, Breast cancer, Cervix cancer, MTT assay, Natural product

DOI: https://dx.doi.org/10.22159/ijap.2024.v16s1.23 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Taxus sumatrana is one of the popular medicinal plants in Indonesia and is also in other countries including China, Japan, and Taiwan. It is idenfied as *Taxus sumatrana* (Miq.) de Laub. It is one of 24 species in the family Taxaceae [1]. *T. sumatrana* is found growing naturally at Sumatera Island Mount Kerinci in Jambi, Mount Tujuh in Jambi, Dolok Sibuaton Conservation Forest Area in North Sumatera, Mount Dempo in South Sumatera, and Mount Singgalang in West Sumatera [2]. Its local name varies, known as "Cemara Sumatra" in West Sumatera, "Tampinur Batu" in Karo, or "Kayu Taji" in Gunung Dempo [3].

The popularity began when paclitaxel was isolated from the Yew tree (*Taxus brevifolia*) in the Pacific Northwest forests) in 1971 by NCI (National Cancer Institute) [4]. Paclitaxel is a cancer chemotherapy agent indicated for the treatment of various cancers, including breast cancer, ovarian cancer, Non-Small Cell Lung Carcinoma (NSCLC), pancreas adenocarcinoma, or Kaposi's Sarcoma [5]. WHO listed paclitaxel as one of the essential medicines considered to be effective for cancer therapy [6]. Paclitaxel is also isolated from *T. sumatrana* and other Taxus. Furthermore, the plants of the Taxus genus are the most wanted by many people for the treatment of various diseases, not only cancer. Other species, *Taxus canadensis* or *Taxus baccata* are found in North America, Europe, and Western Asia [1].

Because it is known that Taxus spp contains anticancer compounds (paclitaxel) [4, 7], currently, many people use Taxus as a medicinal herbal. The bark and leaves of Taxus are used traditionally and massively for some diseases (cancer, etc.), so recently, it has become a rare plant. In Indonesia, *T. sumatrana* is currently listed as a protected plant by the Minister of Environment and Forestry Regulation No. P.106/Menlhk/Setjen/Kum.1/12/2018 about the Second Amendment to the Regulation of the Minister of Environment and Forestry No. P.20/Menlhk/Setjen/Kum.1/6/2018 about Protected Plant and Animal Species [8].

The phytochemical study of *T. sumatrana* needles shows that they positively contain alkaloids, flavonoids, and terpenoids. Previous phytochemical investigations of *T. sumatrana* from bark, leaves (needles), or twigs resulted in the isolation of some chemical compounds. Paclitaxel, baccatin III, cephalomannine, 19-hydroxybaccatin III, 19-hydroxy-13-oxobaccatin III, 7-epi-10-deacetyl taxol, 1-epi-10-deacetylcephalomannine, and 10-deacetyl-13-oxobaccatin III isolated from the bark [7]. Wallifoliol, taxuspine F, 13-O-acetyl wallifoliol, and taxumairol Q were from the leaves and twigs [9]. The tasumatrols A-Z were reported isolated from leaves and twigs [10-15]. The leaves and twigs also isolated Taiwantaxins A-D isolated [16] and other chemical compounds [17, 18].

Some of the isolated compounds reported have cytotoxic activities. Among them are wallifoliol (KB and Hepa 59 T/VGH), taxuspine F, and 3, 6, 7, 10-deacetylbaccatin III (Hepa cells), tasumatrol F (A-498, NCI-H226, A549, and PC-3 tumor cells), Tasumatrols I and K (Hepa59T/VGH, NCI, HELA, DLD-1, and Med cell lines), tasumatrol P (HELA and Daoy tumor cells), tasumatrol Z (Hep2 cell line), and Taiwantaxin B (PC-3 tumor cells) [9-17]. The results of clinical studies also indicated that paclitaxel-containing regimens on breast cancer patients provided additional benefits [19]. Despite its long history of traditional use, there have been no reports regarding the anticancer activity of the plant extract itself. So far, the traditional use of this plant for cancer treatment has only been based on assumptions about the efficacy of paclitaxel, a chemical compound isolated from the Taxus spp. The study will use two breast cancer cell lines and one cervix cancer cell line based on approval indication for paclitaxel [20]. In continuation of our study on Sumatran plans, in this research, we are going to explore *T. sumatrana,* another endemic plant from Sumatera [21–24].

MATERIALS AND METHODS

Material

T. sumatrana (bark, leaves, and shoots), ethanol 70%.

Methods

Extraction

The bark, leaves, and shoots of *T. sumatrana* were collected in Mount Singgalang, West Sumatra. The shoots are the part of the

plant that grows above the soil, consisting of bark, leaves, and twigs. The plant materials were air-dried in the greenhouse for 72 h at ambient temperature before being oven-dried at 40 °C for 24 h. The materials were blended in a lab mixer until they were in the form of powder. The sample in powder form was kept in a sealed container until it was required. Each powdered sample of *T. sumatrana* weighed about 1 kg, and it was macerated in 7.5 L of 70% ethanol for 72 h. This process was repeated 3 times. The ethanol extract was evaporated and concentrated using a rotary evaporator at 40 °C [25, 26]. The concentrated was put in a closed glass bottle, protected from light (coated with aluminum foil), and stored in the refrigerator (2-8 °C) [27].

Cell culturing procedure

The cell lines (HELA, T47D, and MCF-7/HER2) were obtained from Cancer Chemoprevention Research Center (CCRC), Faculty of Pharmacy and Parasitology Laboratory, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Indonesia. Cells were maintained in supplemented Dulbecco's Modified Eagle Medium (DMEM)-high glucose. The next procedure described in our previous study [28–30] and the whole process complies with good cell culture practice guidance [31].

MTT assay

The growth inhibition ability of the extract on a cell line was determined using an MTT assay. This assay measures the activity of mitochondrial dehydrogenase in living cells, which can convert pale yellow soluble MTT to insoluble purple formazan product. It is a colorimetric assay that determines the mitochondrial dehydrogenase in living cells [32].

All procedures were described in our previous study [28–30]. The viability was determined using the formula:

$$\text{Viability (\%)} = \frac{\text{Average absorbance of duplicate extract wells}}{\text{Average absorbance of duplicate control wells}} \text{ x } 100$$

Analysis

The cytotoxic activity was calculated as the IC_{50} parameter by using the GraphPad Prism Program.

RESULTS

The MTT assay was used to evaluate the cytotoxic effect of *T. sumatrana* extract on HELA, T47D, and MCF-7/HER2 cell lines. The effective concentration was determined from the concentration-response curve. The mean percentages of viability are shown in table 1-3 and fig. 1-3.

The relationship concentration-viability response on each cell line is shown in fig. 4-6.

Table 1: Mean percentage viability of HELA cell line

Concentration	0.1	1	10	100
Bark	99.36%±8.10%	85.77%±4.44%	67.62%±1.04%	32.16%±2.39%
Leaves	89.73%±9.01%	85.84%±9.08%	66.24%±2.87%	53.80%±6.38%
Shoot	96.72%±7.63%	86.24%±4.92%	57.45%±6.68%	42.88%±1.59%

Table 2: Mean percentage viability of T47D cell line

Concentration	0.1	1	10	100
Bark	97.61%±19.22%	83.68%±17.54%	57.07%±6.93%	29.11%±1.74%
Leaves	86.32%±7.25%	75.75%±12.51%	55.56%±7.88%	39.13%±2.79%
Shoot	97.12%±25.88%	79.45%±15.16%	53.10%±5.11%	45.66%±7.62%

Table 3: Mean percentage viability of MCF-7/HER2 cell line

Concentration	0.1	1	10	100
Bark	92.78%±12.51%	81.37%±18.32%	56.86%±8.94%	23.59%±2.65%
Leaves	98.23%±11.41%	90.06%±15.44%	75.25%±75.25%	35.13%±8.08%
Shoot	90.09%±6.48%	85.44%±5.43%	68.11%±5.41%	23.56%±2.24%

Concentration in µg/ml

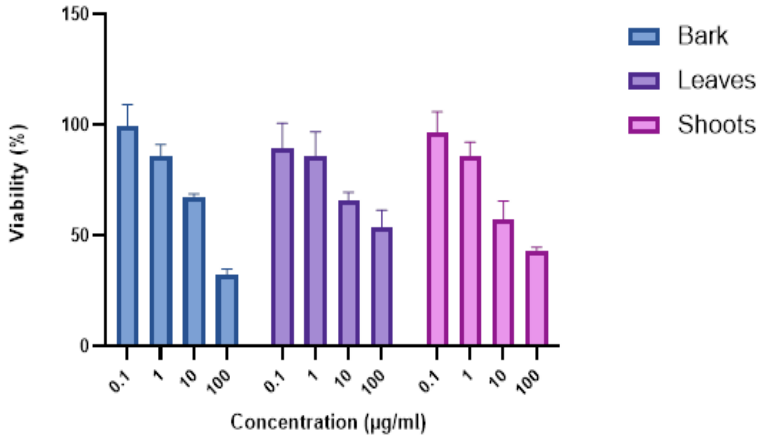

Fig. 1: Mean percentage viability of bark, leaves, and shoots extracts on HELA cell line

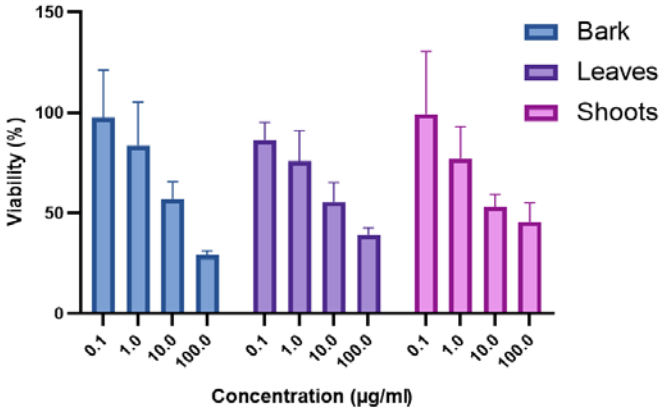

Fig. 2: Mean percentage viability of bark, leaves, and shoots extracts on T47D cell line

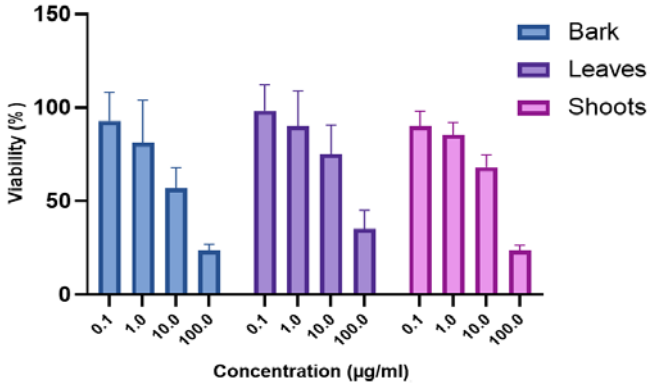

Fig. 3: Mean percentage viability of bark, leaves, and shoots extracts on MCF-7/HER2 cell line

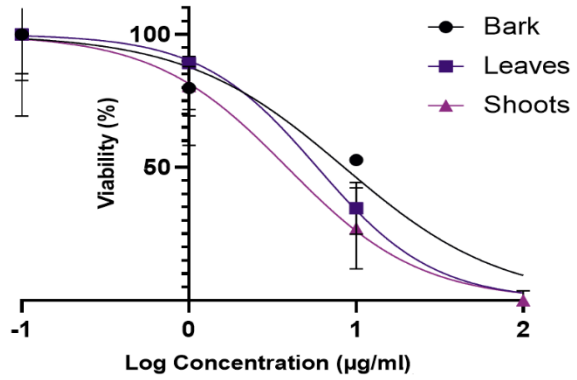

Fig. 4: Concentration-viability response of bark, leaves, and shoots extracts on HELA cell line

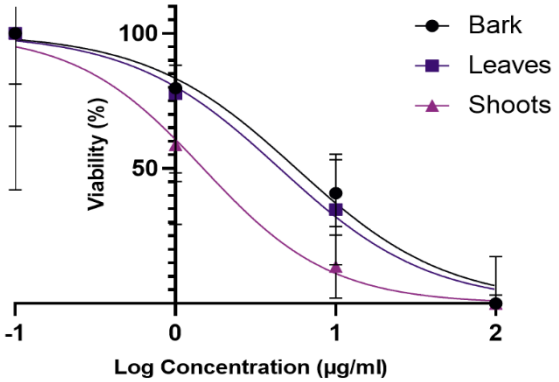

Fig. 5: Concentration-viability response of bark, leaves, and shoots extracts on T47D cell line

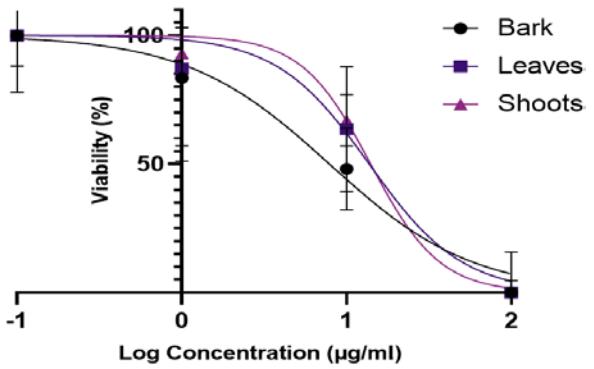

Fig. 6: Concentration-viability response of bark, leaves, and shoots extracts on MCF-7/HER2 cell line

Based on the MTT assay, it was found that the extract of *T. sumatrana* had an IC_{50} value of<20 µg/ml for all parts on the HELA, T47D, and MCF-7/HER2 cell lines (table 4 and fig. 7). The criteria of cytotoxicity for the crude extract, as established by the U. S. National Cancer Institute (NCI), are an IC_{50}<20 µg/ml in the preliminary assay [33]. Many studies use this method to screen the anticancer activity from natural products [29, 30].

Table 4: IC_{50} value of *T. sumatrana* extract

Samples type of cell line	HELA	T47D	MCF-7/HER2
Bark	8.94	5.80	7.46
Leaves	5.93	4.86	10.60
Shoots	4.08	4.11	13.74

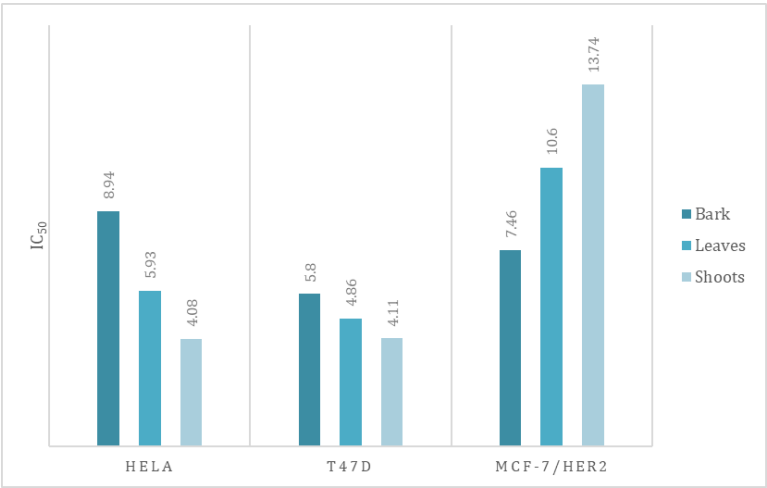

Fig. 7: The IC_{50} value of *T. sumatrana*'s extracts

DISCUSSION

MTT (Microtetrazolium) assay is a colorimetric test to determine the number of living cells. This test is based on changes in a solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. It works by forming yellow to purple formazan crystals through the action of active mitochondria in living cells. MTT is absorbed into living cells and broken down through the oxidation reaction by nicotinamide adenine dinucleotide (NAD+), an enzyme in the mitochondrial respiratory chain, forming a formazan that is not water-soluble. The intensity of the purple color is directly proportional to the amount of active cell metabolism. The darker the color, the higher the absorbance value, indicating the presence of more living cells [32].

Considering the one approval indication of paclitaxel for the treatment of breast cancer and the high incidence of it, also limited choice medicine for cervix cancer, the study focuses on these two cancer types. The cell line used represents two types of cancer. Three cell lines were used to screen the cytotoxic activity. They are the HELA cell line for cervix cancer and the T47D and MCF-7/HER2 cell lines for breast cancer. These cell lines are sensitive to chemotherapeutic agents and have a fast replication capability, making them suitable for cytotoxic testing.

The concentration-response curve was used to determine the effective concentration of extract from *T. sumatrana*. All extracts exhibited the ability to inhibit 50% of cell viability compared to the control at a concentration of 10 µg/ml. Data on cytotoxic activity can be found in table 1-3 and fig. 1-3. Across all cell lines showed that the higher extract concentration correlated with the increased inhibition of cell viability (fig. 4-6).

The cytotoxic activity is determined as the IC_{50} parameter by using the GraphPad Prism Program. The extract of the bark, leaves, and shoots of *T. sumatrana* exhibited significant activity against the HELA cell lines with IC_{50} consecutively 8.90, 5.93, and 4.08 µg/ml; T47D cell lines with IC_{50} 5.80, 4.86, and 4.11 µg/ml; and MCF-7/HER2 cell line with IC_{50} 7.46, 10.60, and 13.74 µg/ml (table 5 and fig. 7). The results show that all parts of *T. sumatrana* showed high cytotoxic activity, indicated by an IC_{50} value of less than 20 µg/ml.

Based on NCI (*National Cancer Institute*), the p-value of extract<20 µg/ml is categorized as having strong cytotoxic activity [34].

The study results provide initial evidence that Taxus extracts exhibit cytotoxic properties, which means they can kill cancer cells. Low concentrations ranging from 4.08 to 13.74 µg/ml can eliminate 50% of breast and cervical cancer cells. This may represent the ability to inhibit the cancer cell as paclitaxel. Liebmann *et al.*'s study shows that the IC_{50} of paclitaxel on cancer cell lines, including MCF-7 and HELA, are 2.5 and 2.6 nM [35]. The IC_{50} of paclitaxel in HELA cells is 2.5 nM, equivalent to 2.14 µg/ml. Compared to the shoot extracts, which have a 4.08 µg/ml concentration, we can observe that the cytotoxicity of paclitaxel is only twice as much as that of the extract. It is significant because the yield of paclitaxel is low. Considering the very low rendering of paclitaxel, around 0.0001–0.0008% [36], the IC_{50} of extract less than 20 µg/ml, developing this plant as cancer medicine is very promising.

Based on clinical studies, the use of taxane-containing adjuvant chemotherapy regimens provides an additional benefit for women with operable early breast cancer based on overall survival and disease-free survival [19]. However, on the other hand, the use of taxanes has significant side effects, including neuropathy and neutropenia. The incidence of neutropenia is very high, observed in more than 90% of patients [37].

However, this data is still limited, and further studies are necessary to demonstrate the selectivity of cytotoxicity on normal cells. Suppose it can be proven that this extract works selectively against normal cells. In that case, Taxus will be a new hope because using the paclitaxel or taxane-containing regimens has significant side effects, including neutropenia or peripheral neuropathy. In addition, the mechanism of killing cells needs to be investigated, and *in vivo* studies need to be conducted to confirm the consistency of cytotoxic activity.

Furthermore, it is important to investigate the mechanisms of action underlying cell death to understand cytotoxic activity. In addition, conducting *in vivo* studies is important to validate and establish the consistency of the observed cytotoxic effects. This multifaceted approach, including selectivity, mechanism of action, and *in vivo* studies, will contribute significantly to the robustness and applicability of the findings of this study.

CONCLUSION

The study demonstrated the strong cytotoxic potential of *T. sumatrana* extracts against breast and cervix cancer cell lines. The extracts showed significant inhibition of cell viability, with IC_{50} values below 20 µg/ml, indicating promising anticancer activity. However, further research is necessary to evaluate selectivity against normal cells, investigate cell death mechanisms, and conduct *in vivo* studies for validation. This comprehensive approach will enhance the reliability and applicability of our findings, showing a potential breakthrough in cancer therapeutics.

ACKNOWLEDGMENT

This Research was funded by the Research and Community Service Institute, Universitas Andalas, through the basic research scheme of professor publication research cluster [PDU-KRP1GB-UNAND) Batch 1 2023 with contracts no T/6/UN16.19/KO-PDU-KRP1GB-Unand/2023. The Forestry Office Province of West Sumatera was also acknowledged for providing samples used for this study.

AUTHORS CONTRIBUTIONS

Fatma Sri Wahyuni: methodology, supervision, funding acquisition; Desi Eka Putri: investigation, formal analysis, writing-original draft; Yozarwardi Usama Putra: provide the testing sample (*Taxus Sumatrana*); Dachriyanus: conceptualization, project administration. The published version of the manuscript has been read and approved by all authors.

CONFLICT OF INTERESTS

We have no conflicts of interest to disclose.

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