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 identified as Taxus sumatrana (Miq.) de Laub. (cemara Sumatra) is one of the plants found in Indonesia and other countries 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 shoots 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% CO2, 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 IC50 parameters. The mean IC50 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
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:

\[
\text{Viability (\%)} = \frac{\text{Average absorbance of duplicate extract wells}}{\text{Average absorbance of duplicate control wells}} \times 100
\]

**Analysis**

The cytotoxic activity was calculated as the IC₅₀ 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>
<thead>
<tr>
<th>Table 1: Mean percentage viability of HELA cell line</th>
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<tbody>
<tr>
<td><strong>Concentration</strong></td>
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<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Bark</td>
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<tr>
<td>Leaves</td>
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<tr>
<td>Shoot</td>
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</table>

<table>
<thead>
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<th>Table 2: Mean percentage viability of T47D cell line</th>
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<tr>
<td><strong>Concentration</strong></td>
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<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Bark</td>
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<td>Leaves</td>
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<td>Shoot</td>
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<table>
<thead>
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<th>Table 3: Mean percentage viability of MCF-7/HER2 cell line</th>
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<tbody>
<tr>
<td><strong>Concentration</strong></td>
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<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Bark</td>
</tr>
<tr>
<td>Leaves</td>
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<td>Shoot</td>
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</tbody>
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Concentration in µg/ml

![Fig. 1: Mean percentage viability of bark, leaves, and shoots extracts on HELA cell line](image-url)
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
Based on the MTT assay, it was found that the extract of *T. sumatrana* had an IC₅₀ 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 IC₅₀ ≤ 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: IC₅₀ value of *T. sumatrana* extract**

<table>
<thead>
<tr>
<th>Samples type of cell line</th>
<th>HELA</th>
<th>T47D</th>
<th>MCF-7/HER2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bark</td>
<td>8.94</td>
<td>5.80</td>
<td>7.46</td>
</tr>
<tr>
<td>Leaves</td>
<td>5.93</td>
<td>4.86</td>
<td>10.60</td>
</tr>
<tr>
<td>Shoots</td>
<td>4.08</td>
<td>4.11</td>
<td>13.74</td>
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</tbody>
</table>

**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 IC₅₀ 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 IC₅₀ consecutively 8.90, 5.93, and 4.08 µg/ml; T47D cell lines with IC₅₀ 5.80, 4.86, and 4.11 µg/ml; and MCF-7/HER2 cell line with IC₅₀ 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 IC₅₀ value of less than 20 µg/ml.
REFERENCES


