INTRODUCTION

Many products of natural medicines from plants with secondary metabolites have potential as anticancer and are used clinically12. *Durio kutejensis* is known as *lai, durian kenyak, durian pudu, poken*, and *pampoken* in Kalimantan. Kalimantan people usually use fruits and its flower for consumption and traditional medicine3. *Durio kutejensis* contains terpenoid, tannin, and phenols46. Previously, *D. kutejensis* leaves was tested its activity as an antioxidant28, and stem bark of *D. kutejensis* has potential as antidiabetic activities8. Some genus of Durio, such as *Durio zibethinus* and *Durio affinis*, were tested on MCF-7, T47D, and HeLa cells for anticancer activity30,31. However, there is no research before on root bark for secondary metabolite and its activity. This study aims to investigate its phytochemical and anticancer activities of *D. kutejensis* root bark on MCF-7 cell lines based on references.
MATERIALS AND METHODS

Materials
Ethanol 96%, silica gel GF254 plate (Merck), silica gel 60 (Merck), ethyl acetate (Merck), methanol, n-hexane, Mayer’s, Wagner’s, and Dragendorff’s reagents, chloroform, H2SO4, HCl, FeCl3, acetic acid, MCF-7 cells, phosphate buffered saline (PBS) (Gibco), MTT solution, SDS 10%, DMSO 1%, Dulbecco’s Modified Eagle’s Medium (DMEM), and trypsin-EDTA 0.25% (Gibco).

Methods
Extraction and fractionation
The root bark of *D. kutejensis* was collected from Pulang Pisau, Central Kalimantan, as shown in Figure 1. The plant sample was determined at the Laboratory of Biology Department, Universitas Negeri Sebelas Maret (No. 209/UN27/9.6.4/Lab/2017). The root bark was cleaned, chopped, dried in direct sunlight, and powdered. Root bark (5 kg) is extracted with ethanol 96% for three days by the maceration method. The filtrate was filtered and evaporated with a rotary evaporator at 50°C.

A schematic of the extraction and fractionation processes is presented in Figure 2. As much as 30 g of ethanol extract was fractionated using hexane and ethyl acetate solvents with a liquid-liquid partition. The extract was dissolved first with 50 mL ethanol solvent and put into a separating funnel. Then, ethyl acetate and hexane were mixed with a volume of 50 mL and then shaken. The mixture is allowed to stand for some time and separate to hexane fraction on the top, ethyl acetate fraction in the middle, and the bottom as ethanol. The layers were separated using a separating funnel that was carefully accommodated, and the partitioning process was repeated 2-3 times. The result of partition separation is evaporated and weighed.

Phytochemical screening
The ethanol extract was tested by a qualitative test with the procedure as reported by previous studies as follows:

1. Alkaloid test
   Mayer’s test: The extract was treated with Mayer’s reagent and will form a yellow cream precipitate.
   Wagner’s test: The extract was treated with Wagner’s reagent and will form a brown or reddish-brown precipitate.
   Dragendorff’s test: The extract was sprayed or dropped with a small Dragendorff’s reagent and will show an orange spot.

2. Terpenoid test
   Salkowski’s test: As much as 5 mg of the extract was mixed with 2 mL of chloroform, and 3 mL of concentrated H2SO4 was carefully added to form a
3. Flavonoid test
Shinoda’s test: A piece of magnesium ribbon and 1 mL of concentrated HCl was added to the extract. Then, it will show the pink-red or red coloration of the solution.

H₂SO₄ test: Some of the extracts were treated with few drops of H₂SO₄ and will form orange color.

4. Phenol test
FeCl₃ test: 10 mg of extracts were treated with few drops of FeCl₃ solution and will show the formation of bluish-black color.

5. Saponin test
Frothing/Foam test: About 0.5 mg of the extract was shaken with 0.5 mL distilled water and will form frothing or some bubbles for a long time.

6. Steroid test
Liebermann-Burchard test: The extract was added with 1 mL of chloroform, 2-3 mL of acetic acid anhydride, 1-2 drops of concentrated H₂SO₄ and will show the dark green coloration.

7. Tannin test
Braemers test: A small extract was mixed with distilled water and heated on a water bath. Then filtered and added some FeCl₃. A dark green color was formed as the presence of tannins.

Cytotoxic assay
The MCF-7 cells were cultured in the Laboratory of Pharmaceutical Biology, Universitas Muhammadiyah Surakarta. For incubation, DMEM was used as a medium after adding 10% PBS, 2% penicillin/streptomycin, and 0.5% fungizone. Cell lines were adapted and subcultured in mediums at 37°C and 5% CO₂ incubator. The cell lines were then added with the extract, n-hexane fraction, ethyl acetate fraction (triplicate). Doxorubicin was used as a comparison for positive control. After 48 hours of incubation for MCF-7, MTT reagent was added and incubated in a CO₂ incubator for 2 to 4 hours at 37°C. Then, 100 µL SDS 10% was added in 0.01 N HCl and stored in a dark place (covered with aluminum foil) overnight. The absorbance results were checked with an ELISA reader at wavelengths 594 nm. After that, % cell viability calculated with the following equation:

\[
\% \text{Cell viability} = \frac{\text{Absorbance of sample} - \text{Absorbance of control media}}{\text{Absorbance of control cell} - \text{Absorbance of control media}} \times 100 \%
\]

The IC₅₀ value was calculated with Microsoft Excel 2010. The value of IC₅₀ is obtained by the probit log calculation with \( y = bx + a \), where \( y \) is % cell viability, and \( x \) is log concentration.

RESULTS AND DISCUSSION

Extraction and fractionation
The ethanol extract obtained was 123 g with the ethyl acetate and the n-hexane fraction obtained were 2.17 g and 0.89 g, respectively. According to the result, ethanol solvent is quite effective in extracting secondary metabolites and is commonly carried out, especially for extracting polar, semipolar, and non-polar metabolites. Previous research on D. kutejensis also carried out the extraction of plant parts using ethanol, n-hexane, and ethyl acetate solvents.

Phytochemical screening
The result showed that ethanol could extract various compounds from D. kutejensis, as presented in Table I. The ethanol solvent is known to extracts various polar, semipolar, to non-polar compounds in a plant. Previous research also showed that D. kutejensis contains terpenoid, tannin, and phenols.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Method/Test</th>
<th>Result</th>
</tr>
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<tbody>
<tr>
<td>Alkaloid</td>
<td>Mayer’s test</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Wagner’s test</td>
<td>-</td>
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<tr>
<td></td>
<td>Dragendorff’s test</td>
<td>-</td>
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<tr>
<td>Terpenoid</td>
<td>Salkowski’s test</td>
<td>+</td>
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</table>
**CONCLUSION**

In conclusion, *D. kutejensis* root bark contains flavonoid, tannin, terpenoid, phenol, and saponin. However, *D. kutejensis* root bark has no potential activity against MCF-7 cells. Further research is needed to explore the other secondary metabolite for the anticancer activity in some cancer cells and pharmacological activity.

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