INTRODUCTION Many products of natural medicines from plants with secondary metabolites have potential as anticancer and are used clinically. Durio kutejensis is known as lai, durian kenyak, durian pulu, paken, and pampaken in Kalimantan. Kalimantan people usually use fruits and its flower for consumption and traditional medicine. Durio kutejensis contains terpenoid, tannin, and phenols. Previously, D. kutejensis leaves were tested its activity as antioxidant, and stem bark of D. kutejensis has potential as antidiabetic activities. Some genus of Durio, such as Durio zibethinus and Durio affinis, were tested on MCF-7, T47D, and HeLa cells for anticancer activity.

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)
- 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 sunlight, and powdered. Root bark (5 kg) was extracted with ethanol 96% for three days by the maceration method. The filtrate was filtered and evaporated with a rotary evaporator at 50°C. / Figure 1. Root bark of D. kutejensis A schematic of the extraction and fractionation processes was 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 was allowed to stood 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 was evaporated and weighed. / Figure 2.

Extraction and fractionation process scheme of D. kutejensis Phytochemical screening

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

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 layer. It will show an appearance of reddish-brown color in the inner face. Flavonoid test Shinoda’s test: A piece of magnesium ribbon and 1 mL of concentrated HCl was added to the extract. It will show the pink-red or red coloration of the solution. H2SO4 test: The extracts were treated with few drops of H2SO4 and will form orange color. Phenol test FeCl3 test: About 10 mg of extracts were treated with few drops of FeCl3 solution and will show the formation of bluish-black color.

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. 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. Tannin test Braemer’s test: A small extract was mixed with distilled water and heated in a water bath. Then filtered and added some FeCl3. A dark green color will form 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 was calculated with the following equation: / 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 was 2.17 g and 0.89 g, respectively.
According to the result, ethanol solvent was quite effective in extracting secondary metabolites and was commonly carried out, especially for extract 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 was 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.

Cytotoxic assay: The IC50 value for ethanol extracts of root bark, n-hexane, and ethyl acetate fractions on MCF-7 cells were 761.29; 280.5; and 207.08 µg/mL, respectively. The extract had the highest IC50 value, while the lowest is ethyl acetate fraction, which shows that the fraction had better cytotoxic activity than the extract. The possible cause was because the fraction contains certain compounds in a higher concentration than the extract. The solvent in the extraction process could affect some compound content in the extract.

Ethyl acetate, as a semipolar solvent, could dissolve steroids and alkaloids and effectively extract steroids, terpenoids, and flavonoids. Simultaneously, n-hexane solvents were effective for non-polar metabolites such as steroids and terpenoids. However, the IC50 values shown by the three were still much lower than doxorubicin at only 0.25 µg/mL, as shown in Table II. The cytotoxic test showed that the anticancer activity of ethanol extract, ethyl acetate, and n-hexane fraction was included in the inactive category (IC50 > 100 µg/mL) based on The National Cancer Institute (NCI) category.

Previously, research on an ethanol extract of D. affinis on T47D cells and HeLa cells had IC50 values of 828.3 and 300.5 µg/mL, respectively. The research showed that the ethanol extract of D. kutejensis root bark had no anticancer activity against MCF-7 cells.

Table I. Phytochemical screening of D. kutejensis root bark extract

<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>
</tr>
<tr>
<td></td>
<td>Dragendorff’s test</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>Salkowski’s test</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>Shinoda’s test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>H2SO4 test</td>
<td>+</td>
</tr>
<tr>
<td>Phenol</td>
<td>FeCl3 test</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>Frothing/Foam test</td>
<td>+</td>
</tr>
<tr>
<td>Steroid</td>
<td>Liebermann-Burchard test</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>Braemer’s test</td>
<td>+</td>
</tr>
</tbody>
</table>

Table II. The IC50 values of D. kutejensis root bark on MCF-7 cells

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC50 ± SD (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol extract</td>
<td>761.29 ± 6.06</td>
</tr>
<tr>
<td>n-hexane fraction</td>
<td>280.5 ± 3.05</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>207.08 ± 3.82</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>0.25 ± 0.12</td>
</tr>
</tbody>
</table>

CONCLUSION: In conclusion, D. kutejensis root bark contains flavonoid, tannin, terpenoid, phenol, and saponin. However, D.
kutejensis root bark had no potential activity against MCF-7 cells. Further research was needed to explore the anticancer activity of secondary metabolites on some other cancer cells as well as for other pharmacological activities. ACKNOWLEDGMENT The authors thank Allah SWT, family, Pharmacy Faculty of Universitas Muhammadiyah Surakarta, and Faculty of Applied Sciences, Universiti Teknologi MARA, Sarawak, for the facilities and accommodations in this research. REFERENCES Solowey E, Lichtenstein M, Sallon S, Paavilainen H, Solowey E, Lorberboum-Galski H. Evaluating medicinal plants for anticancer activity. ScientificWorldJournal. 2014;2014:721402. doi:10.1155/2014/721402 Prakash O, Kumar A, Kumar P, Ajeet.


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