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INTRODUCTION Many products of natural medicines from plants with secondary metabolites have potential as anticancer and are used clinically^{1,2}. *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 medicine³. *Durio kutejensis* contains terpenoid, tannin, and phenols⁴⁻⁶. Previously, *D. kutejensis* leaves were tested its activity as antioxidant^{7,8}, and stem bark of *D. kutejensis* has potential as antidiabetic activities⁹. Some genus of *Durio*, such as *Durio zibethinus* and *Durio affinis*, were tested on MCF-7, T47D, and HeLa cells for anticancer activity^{10,11}.

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 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 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 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^{12,13} 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 metabolites¹⁴. Previous research on *D. kutejensis* also carried out the extraction of plant parts using ethanol, n-hexane, and ethyl acetate solvents⁸. 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 extract various polar, semipolar, to non-polar compounds in a plant¹⁴. Previous research also showed that *D. kutejensis* contains terpenoid, tannin, and phenols⁴⁻⁶.

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 extract¹⁵. 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 terpenoids¹⁴. 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^{16,17}.

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, respectively¹¹. 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

Method/Test	Result
Alkaloid	Mayer's test -
Wagner's test	-
Dragendorff's test	-
Terpenoid	Salkowski's test +
Flavonoid	Shinoda's test +
H ₂ SO ₄ test	+
Phenol	FeCl ₃ test +
Saponin	Frothing/Foam test -
Steroid	Liebermann-Burchard test +
Tannin	Braemer's test +

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

Sample	IC ₅₀ ± SD (µg/mL)
Ethanol extract	761.29 ± 6.06
n-hexane fraction	280.5 ± 3.05
Ethyl acetate fraction	207.08 ± 3.82
Doxorubicin	0.25 ± 0.12

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.

Anticancer Potential of Plants and Natural Products: A Review. *Am J Pharmacol Sci*. 2013;1(6):104-15. doi:10.12691/ajps-1-6-1 Atmoko T. Potensi dan Konservasi Durian Hutan Kalimantan (Durio kutejensis). In: Soemargono A, editor. *Prosiding Seminar Nasional Buah Tropika Nusantara II: Dukungan Teknologi dan Hasil Penelitian dalam Membangun Pembangunan Bio-Industri Buah Tropika Berkelanjutan* [Internet]; 2014 Sep 23-25; Bukittinggi. Solok: Balai Penelitian Tanaman Buah Tropika; 2015. p. 437-46. Gorinstein S, Poovarodom S, Leontowicz H, Leontowicz M, Namiesnik J, Vearasilp, et al. Antioxidant properties and bioactive constituents of some rare exotic Thai fruits and comparison with conventional fruits: In vitro and in vivo studies.

Food Res Int. 2011;44(7):2222-32. doi:10.1016/j.foodres.2010.10.009 Sardans J, Llusia J, Owen SM, Niinemets Ü, Peñuelas J. Screening Study of Leaf Terpene Concentration of 75 Borneo Rainforest Plant Species: Relationships with Leaf Elemental Concentrations and Morphology. *Rec Nat Prod*. 2015;9(1):19-40. Khoo HE, Azlan A, Kong KW, Ismail A. Phytochemicals and Medicinal Properties of Indigenous Tropical Fruits with Potential for Commercial Development. *Evid Based Complement Alternat Med*. 2016;2016:7591951. doi:10.1155/2016/7591951 Ikram EHK, Eng KH, Jalil AMM, Ismail A, Idris S, Azlan A, et al. Antioxidant capacity and total phenolic content of Malaysian underutilized fruits. *J Food Compos Anal*. 2009;22(5):388-93. doi:10.1016/j.jfca.2009.04.001 Arung T, Suwinarti W, Hendra M, Supomo S, Kusuma IW, Puteri DCN, et al. Determination of Antioxidant and Anti-Melanogenesis Activities of Indonesian Lai, Durio kutejensis [Bombacaceae (Hassk) Becc] Fruit Extract. *Trop J Pharm Res*. 2015;14(1):41-6. doi:10.4314/tjpr.v14i1.7

Yusro F, Ohtani K, Kubota S. Inhibition of α -Glucosidase by Methanol Extracts from Wood Bark of Anacardiaceae, Fabaceae, Malvaceae and Phyllanthaceae Plants Family in West Kalimantan, Indonesia. *Kuroshioken Kagaku*. 2016;9(2):108-22. Jayakumar R, Kanthimathi MS. Inhibitory effects of fruit extracts on nitric oxide-induced proliferation in MCF-7 cells. *Food Chem*. 2011;126(3):956-60. doi:10.1016/j.foodchem.2010.11.093 Rudiyanasyah, Masriani, Mudianta IW, Garson MJ. Isolation and Absolute Configuration of Boehmenan from Durio affinis Becc. *Rec Nat Prod*. 2014;8(2):195-8. Santhi K,

Sengottuvel R. Qualitative and Quantitative Phytochemical analysis of *Moringa concanensis* Nimmo. *Int J Curr Microbiol App Sci*. 2016;5(1):633-40.

doi:10.20546/ijcmas.2016.501.064 Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha LY. Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts. *Afr J Tradit Complement Altern Med*. 2011;8(1):1-10. Widyawati PS, Budianta TDW, Kusuma FA, Wijaya EL. Difference of Solvent Polarity to Phytochemical Content and Antioxidant Activity of *Pluchea indica* Less Leaves Extracts. *Int J Pharmacogn Phytochem Res*. 2014;6(4):850-5. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. *Plants*. 2017;6(4):42. doi:10.3390/plants6040042 Vijayarathna S, Sasidharan S.

Cytotoxicity of methanol extracts of *Elaeis guineensis* on MCF-7 and Vero cell lines. *Asian Pac J Trop Biomed*. 2012;2(10):826-9. doi:10.1016/s2221-1691(12)60237-8 Çoruh N, Özdoğan N. Wild-growing *Rosa heckeliana* Tratt.: phenolic constituents with cytotoxic and antioxidative properties. *Turk J Biol*. 2017;41:195-212. doi:10.3906/biy-1605-89

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