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Research Article

Phytochemical and Cytotoxic Test of *Durio kutejensis* Root Bark on MCF-7 Cells

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Abstract

Durio kutejensis has known potential as herbal medicine in Kalimantan. Many things can be explored from *D. kutejensis* related pharmacological activity in every part of the plant included root bark. This study investigated the phytochemical content with a qualitative test and tested cytotoxic activities of *D. kutejensis* root bark on MCF-7 cell lines. Cytotoxic activity tested on MCF-7 cells with MTT assay method. The result showed that *D. kutejensis* root bark contains flavonoid, tannin, terpenoid, phenol, and saponin. IC_{50} value for ethanol extracts of root bark, *n*-hexane fractions, ethyl acetate fractions, and doxorubicin on MCF-7 cells were 761.29; 280.5; 207.08; and 0.25 µg/mL, respectively. In conclusion, *D. kutejensis* root bark has some secondary metabolites but no cytotoxic activity on MCF-7 cells. Further research is needed to explore the anticancer activity of secondary metabolites on some other cancer cells and other pharmacological activities.

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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 medicine[3]. *Durio kutejensis* contains terpenoid, tannin, and phenols[4-6]. Previously, *D. kutejensis* leaves were tested its activity as antioxidant[7,8], and stem bark of *D. kutejensis* has potential as antidiabetic activities[9]. 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 GF_{254} plate (Merck), silica gel 60 (Merck), ethyl acetate (Merck), methanol, *n*-hexane, Mayer's, Wagner's, and Dragendorff's reagents, chloroform, H_2SO_4, HCl, $FeCl_3$, 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 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.

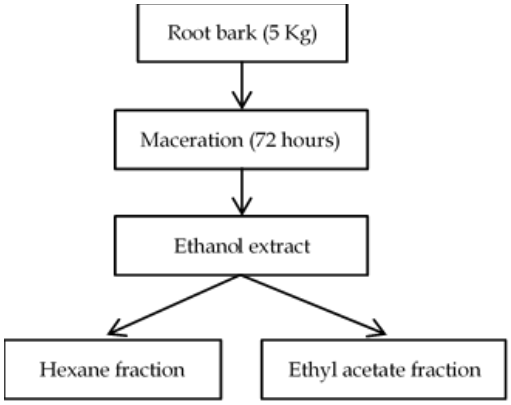

Figure 2. Extraction and fractionation process scheme of *D. kutejensis*

Phytochemical screening

The ethanol extract was tested by a qualitative test with the procedure as reported by previous studies[12,13] 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 H_2SO_4 was carefully added to form a layer. It will show an appearance of reddish-brown color in the inner face.

3. 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.

   H_2SO_4 test: The extracts were treated with few drops of H_2SO_4 and will form orange color.

4. Phenol test

   $FeCl_3$ test: About 10 mg of extracts were treated with few drops of $FeCl_3$ 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 H_2SO_4, and will show the dark green coloration.

7. Tannin test

   Braemer's test: A small extract was mixed with distilled water and heated in a water bath. Then filtered and added some $FeCl_3$. 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% CO_2 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 CO_2 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:

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

The IC_{50} value was calculated with Microsoft Excel 2010. The value of IC_{50} 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[14]. Previous research on *D. kutejensis* also carried out the extraction of plant parts using ethanol, *n*-hexane, and ethyl acetate solvents[8].

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[14]. Previous research also showed that *D. kutejensis* contains terpenoid, tannin, and phenols[4-6].

Cytotoxic assay

The IC_{50} 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 IC_{50} 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[15]. 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[14]. However, the IC_{50} 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 (IC_{50} >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 IC_{50} values of 828.3 and 300.5 µg/mL, respectively[11]. 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

Phytochemicals	Method/Test	Result
Alkaloid	Mayer's test	-
	Wagner's test	-
	Dragendorff's test	-
Terpenoid	Salkowski's test	+
Flavonoid	Shinoda's test	+
	H_2SO_4 test	+
Phenol	$FeCl_3$ test	+
Saponin	Frothing/Foam test	-
Steroid	Liebermann-Burchard test	+
Tannin	Braemer's test	+

Table II. The IC_{50} 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.

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