Cytotoxic Compound From Leaves Of Irvingia Malayana

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Abstract—Chemical constituents investigation from leaves of Irvingia malayana resulted flavonoid showed cytotoxic activity against HeLa cell line. Separation use column chromatography resulted others compound no active. The structure were determined by physical, chemical and spectroscopy techniques.

Keywords—Irvingia malayana, cytotoxic compound, leaves, HeLa cell line.

I. INTRODUCTION

Medicinal plant used as anticancer and antimalaria in West Kalimantan, Indonesia is Irvingia malayana, one of the 5 species of Irvingia found in family Simaroubaceae. In West Kalimantan, Indonesia, the leaves are used for traditional medicine of cancer, malaria, and inflation.

Irvingia malayana is distributed in Indonesia, Malaysia, Thailand, Laos and Cambodia [1]. Bandelier et al., [1] found sterols and tocopherols of the fruit of Irvingia malayana. Phenolic compounds reported have isolated from stem bark of Irvingia malayana [2]. Friedelin, a terpenoid that reported from stem bark of Irvingia malayana showed antifeedant [3, 4]. Ng et al., [5] found betulinic acid from bark of Irvingia malayana that showed cytotoxic activity.

The cytotoxic activity of leaves extract from Irvingia malayana was previously reported [6]. It was no information about secondary metabolite which posses cytotoxic activity.

Based on previos research about cytotoxic activity of fraction from leaves of Irvingia malayana showed that n-hexane fraction have higher cytotoxic activity against HeLa cell line (IC50 125 µg/mL). Others fraction are methylene chloride, ethylacetate and methanol fraction showed IC50 325 µg/mL, 201 µg/mL and 600 µg/mL, respectively. Methanol extract there is no activity [7].

II. PROCEDURE

A. Material

Leaves of Irvingia malayana were used as sample in this research. It was taken from Palung Mountain National Park, Ketapang, West Kalimantan, Indonesia. Accuracy of sample species was determined in Herbarium Bogoriense, LIPI, Indonesia. Sample was dried in room temperature for a week. Then, the sample was blended and filtered (use filter 60 mesh). The powder of the leaves is extracted by maceration. The chemical materials that used are organic solvent, silica gel G 60 (230-400 mesh), silica gel G 60 (70-230 mesh), TLC plates silica gel 60 F254 (Merck) and carcinoma cell of human servics (Hella cell line).

B. Extraction

The powder of leaves of Irvingia malayana (1 kg) was macerated with methanol 80% in room temperature (3 x 24 hours). Extracts are filtered and the filtrate is collected. Residue is remaceration by added methanol again. All the filtrate are collected and evaporated. Methanol extract of the sample was extrated (partition) by n-hexane, methylene chloride and ethylacetate. All fraction were evaporated, then the activity test will done by MTT assay.

C. Cell Culture

To growth HeLa cell (ATTCC CCI 2.1) collection of Cancer Chemoprevention Research Centre, Faculty of Pharmacy, Gadjah Mada University, it can do in the first day. Media that used is DMEM with 10% PBS and Penstrep. Cell concentration is 1.10^5/mL, used 12 source, each volume is 4 mL. Then, cell will incubated in incubator (temperature 37°C). In second day, cell is washed and added with compound from leaves extracts. Cell washing by take 2 mL from each source then added by 2 mL from new media (two source as controls) and added by 2 mL compounds 125, 250, 500 and 750 ppm in concentration that has dissolved use culture media completely. Incubation is continued in 37°C until fifth day. In fifth day trypsinization is done. After that, we will count total of the cell that still alive. It used count room Neubauer under the microscope.

D. Viability of Cell

Viability of cells was measured by the MTT assay, which gives the concentration at which 50% of the cell remains viable after any compounds treatment (IC50). In this assay, MTT is converted to a blue formazan dye by mitochondrial enzyme in actively respiring but not neccessarily proliferating.
cells. The intensity of the colour formed can be converted to untreated controls to obtain the IC$_{50}$ value by reading the absorbance at 590 nm. Cancer cell were seeded with various concentrations of the test compounds in a 96-well plate in a total volume of 200 µL of medium. Sterile MTT (25 mL of 5 mg/mL in PBS) was added to each well and the plates were covered in foil and incubated at 37°C. After 3 h, 100 µL of extraction buffer consisting of 20% SDS dissolved in a dimethylformamide/water (1/1, v/v) solution at pH 4.0 was added. Blue colour formation was measured at 590 nm with a Dynatech MRX microplate reader. The percentage of cells surviving was determined by comparing the absorbance of the solutions recovered from the treated cells with that of the control cell.

III. RESULT AND DISCUSSION

Powdered leaves (1 kg) of * Irvingia malayana* was successively extracted with methanol then to partition with n-hexane, methylene chloride and ethylacetate, yielding 70.5 g methanol extract, 6.3 g n-hexane fraction, 10.2 g methylene chloride fraction, 20.2 ethylacetate fraction and 11.9 g methanol fraction. n-hexane fraction showed highest cytotoxic activity.

A portion (6.3 g) of the n-hexane fraction was fractioned by VLC eluted with methylene chloride - n-hexane mixture of increasing polarity (3:7, 4:6, 5:5, 6:4 and 7:3) to give fractions A-E (1.3 g; 1.5 g; 0.9 g; 1.2 g and 1.1 g respectively). Fraction B was further fractioned by column chromatography eluted with n-hexane-methylene chloride (5:5) to give six fractions B1-B6 (20 mg; 10 mg; 19 mg; 9 mg; 17 mg and 40 mg, respectively). Fraction B3 subjected to column chromatography eluted with n-hexane-methane chloride (2:8) and after crystallization, compound 1 (12 mg) was obtained.

Amount (10 g) of the ethylacetate fraction was fractioned by VLC eluted with ethylacetate-methylene chloride - n-hexane mixture of increasing polarity (4:6, 5:5, and 6:4) to give fractions A-E (1.3 g; 2.0 g; 1.9 g; 1.4 g and 1.6 g respectively). Fraction C was further fractioned by column chromatography eluted with ethylacetate-methylene chloride (5:5) to give five fractions C1-C5 (20 mg; 17 mg; 29 mg; 20 mg and 40 mg, respectively). Fraction C3 separated by preparative chromatography eluted with ethylacetate-methylene chloride (6:4) and after crystallization, compound 2 (15 mg) and compound 3 (9 mg) were obtained. Identification compound (1) is β-sitosterol, compound (2) is gallocatechin –(4α-8) epigallocatechin–4′,4‴-dimethylether and compound (3) is benzophenone (have reported before).

Three compounds from leaves of * Irvingia malayana* were analyzed by MTT assay method against HeLa cell line. Compound 2 showed higher cytotoxic activity (IC$_{50}$ 27.5 µg/mL) and compounds 1 and 3 there is no activity.

IV. CONCLUSION

Flavonoid from leaves of * Irvingia malayana* showed potential activity against HeLa cell line.