

Cytotoxic Activity of Crude Extracts and Fractions from *Irvingia malayana*

Ari Widiyantoro¹, Thamrin Usman², Edy Meiyanto³, Sabirin Matsjeh⁴

¹Ph.D Student, Department of Chemistry, Faculty of Mathematic and Natural Sciences, Gadjah Mada University, Yogyakarta, Indonesia

²Department of Chemistry, Faculty of Mathematic and Natural Sciences, Tanjungpura University, Pontianak, Indonesia

³Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia

⁴Department of Chemistry, Faculty of Mathematic and Natural Sciences, Gadjah Mada University, Yogyakarta, Indonesia

Abstract : *Irvingia malayana* has been utilized for traditional medicine in West Kalimantan, Indonesia. The aim of this research is to detect cytotoxic activity of extract of *Irvingia malayana*. The extraction with methanol and partition by varied eluent polarity. This extraction produced methanol extract, n-hexane fraction, methylene chloride fraction, ethylacetate fraction and methanol fraction. Cytotoxic test using MTT assay method was done to each extract and fraction. Cell viability profiles produced from MTT assay showed that methanolic extract, the other fractions (n-hexane, methylene chloride, ethylacetate and methanol) from bark of *Irvingia malayana* decrease HeLa cells viability compare to control cells in the concentration dependent manners with IC_{50} of 59, 92, 30, 22, and 33 $\mu\text{g/mL}$ respectively. The strongest cytotoxic activity was showed by ethylacetate fraction of bark of *Irvingia malayana*, but methylene chloride fraction of root of *Irvingia malayana* also potential to find bioactive compound against HeLa cell line.

Keywords - *Irvingia malayana*, cytotoxic activity, crude extracts, fractions, HeLa cell line

I. INTRODUCTION

Irvingia malayana is a plant in the Simaroubaceae family. It is distributed in Southeast Asia e.i Indonesia, Thailand, Vietnam, Cambodia and Laos. This plant species is widely distributed in Sumatera and Kalimantan, Indonesia. West Kalimantan people used stem bark extract this plant as traditional medicine. In Sambas and Ketapang District, extract of *Irvingia malayana* used it to cure cancer and malaria, especially extract of bark. How to use it is to soak 3-5 pieces bark of *Irvingia malayana* in one cup of hot water and then allowed to stand for 5 minutes to be drunk as cancer and malaria drugs.

This plant has been reported containing compounds as antimalaria [1], [2], antimutagenic [3], antiinflammation [4] and antifeedant [5]. Widely studied plant parts are leaves, wood and seeds. This research investigates the cytotoxic activity of all parts of the plant against HeLa cells.

Based on literature studies, the biological activity of plant extracts of *Irvingia malayana* against HeLa cells showed that ethanol crude extract of the leaves of *Irvingia malayana* have cytotoxic activity (IC_{50}) 11.7 $\mu\text{g/mL}$ and methanol fraction (IC_{50}) 14.8 $\mu\text{g/mL}$ against HeLa cells. While the fraction of cyclohexane and methylene chloride showed no cytotoxic activity against HeLa cells. This literature has not revealed what compounds is contained in the leaves of *Irvingia malayana* which is cytotoxic against HeLa cells [1].

Several chemical compounds have been isolated from various plant parts of *Irvingia malayana*. Chemical compounds have been isolated from the wood of *Irvingia malayana* are 2-hydroxy-3, 2', 6'-trimethoxy-4'-(8', 9'-epoxy-7'-hydroxypropyl)-5-ethenyl-biphenyl; 9-hydroxy-7-methoxy-8-(4'-hydroxy-3'-methoxyphenyl)-4-hydroxy-3-methoxyphenylpropane; 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone; caffeic aldehyde; ferulaldehyde; n-hexacosyl ferulate; 7 β -hydroxy- β -sitosterol; β -sitosterol- β -D-glucoside; glutinol and oleanic acid [6]. While the methanol extract of the stem bark of *Irvingia malayana* have been isolated betulinic acid which showed cytotoxic activity and antiangiogenic [7]. Others secondary metabolites that friedelin; friedelinol; 3,3',4'-tri-O-methylellagic acid; methyl 3,4,5-trihydroxybenzoate; 3,3',4'-tri-O-methylellagic-xyloside- β -glucoside; 3,3'-di-O-methylellagic acid-4- β -acid-6"-acetoxo-4-glucoside; 3,3',4'-tri-O-methylellagic acid-4-glucopyranoside; β 5,7,4'-trihydroxyflavone-8-C-glucopyranoside and β 5,7,3',4'-tetrahydroxyflavone-8-C-glucopyranoside-7-O-linkage also have been isolated from *Irvingia malayana*. The isolated β 5,3',4'-trihydroxyflavone-6-C- compounds were tested for cytotoxicity and anti-HIV reverse transcriptase. Compound 3,3',4'-tri-O-methylellagic acid showed activities against several human cell lines i.e. KB, KB-V(+VLB), KB-V(-VLBL), Lu-1 and ZR-75-1 [8].

II. MATERIALS AND METHODS

2.1 Material

Stem bark, bark, leaves and root 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 grinded and filtered (use filter 60 mesh). The powder of the sample is extracted by maceration. The chemistry materials that used are organic solvent, silica gel G 60 (230-400 mesh), silica gel G60 (70-230 mesh), TLC plates silica gel 60 F254 (Merck), and carcinoma cell of human services (HeLa cell line)

2.2 Methods

2.2.1 Extraction

The powder of stem bark of *Irvingia malayana* (2 kg) was macerated with methanol 80% in room temperature (4x24 hours). Extracts are filtered and the filtrate is collected. Residu is remaceration by added new methanol. All the filtrate are collected and evaporated. Methanol extract of the sample was extracted (partition) by *n*-hexane, methylene chloride and ethylacetate. All fraction were evaporated, then the activity test will done by MTT assay. The same procedure was also carried out on samples of bark (2 kg), leaves (1 kg) and root (2 kg) of *Irvingia malayana*

2.2.2 Cell culture

To growth HeLa cell (ATCC 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 1.10^4 /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 extract and fraction. Cell washing by take 2 mL from each source then added by 2 mL from new media (two source as control), and added by 2 mL extract and fraction 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, trypsination is done. After that, we will count total of the cell that still alive. It used count room Neubauer under the microscope.

2.2.3 Viability of Cell

Viability of cells was measured by the MTT assay, which gives the concentration at which 50% of the cells remains viable after any extract and fraction treatment (IC_{50}). In this assay, MTT is converted to a blue formazan dye by mitochondrial enzymes in actively respiring but not necessarily proliferating cells. The intensity of the color formed can be correlated to untreated controls to obtain the IC_{50} value by reading the absorbance at 590 nm. Cancer cells were seeded with various concentrations of the test compounds in a 96-well plate in a total volume of 200 μ L of medium. Sterile MTT (25 mL of 5 mg mL⁻¹ in PBS) was added to each well, and the plates were covered in foil and incubated at 37 °C. After 3 h, 100 μ L 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 cells.

III. RESULTS AND DISCUSSION

Powdered of stem bark (2 kg) of *Irvingia malayana* was extracted with methanol then to partition with *n*-hexane, methylene chloride and ethylacetate, yielding 120.3 g MeOH extract, 12.2 g *n*-hexane fraction, 23.5 g methylene chloride fraction, 50.2 ethylacetate fraction, and 25.6 g methanol fraction. While the bark (2 kg) of *Irvingia malayana* was extracted with methanol then to partition with *n*-hexane, methylene chloride and ethylacetate, yielding 130.5 g MeOH extract, 13.6 g *n*-hexane fraction, 24.1 g methylene chloride fraction, 52.1 ethylacetate fraction, and 27.2 g methanol fraction. Leaves (1 kg) of *Irvingia malayana* was extracted with methanol then to partition with *n*-hexane, methylene chloride and ethylacetate, yielding 70.5 g MeOH extract, 6.3 g *n*-hexane fraction, 10.2 g methylene chloride fraction, 20.2 ethylacetate fraction, and 11.9 g methanol fraction. Root (2 kg) of *Irvingia malayana* was extracted with methanol then to partition with *n*-hexane, methylene chloride and ethylacetate, yielding 118.9 g MeOH extract, 13.1 g *n*-hexane fraction, 22.5 g methylene chloride fraction, 49.3 ethylacetate fraction, and 23.9 g methanol fraction.

These activities were analyzed by monitoring cell viability of treated and untreated cells by their reduction of the tetrazolium substrate, MTT. Cytotoxicity test showed IC_{50} of ethylacetate fraction of bark of *Irvingia malayana* was good activity than the others fraction and extract. Specifically of bark, cytotoxic activity have been reported before in proceeding [9].

Table 1. IC₅₀ Cytotoxicity against HeLa Cell (MTT assay)

Bark of <i>Irvingia malayana</i>	IC₅₀ (µg/mL)
Methanol Extract	59
<i>n</i> -Hexane Fraction	92
Methylene Chloride Fraction	30
Ethylacetate Fraction	22
Methanol Fraction	33

Table 2. IC₅₀ Cytotoxicity against HeLa Cell (MTT assay)

Stem Bark of <i>Irvingia malayana</i>	IC₅₀ (µg/mL)
Methanol Extract	-
<i>n</i> -Hexane Fraction	211
Methylene Chloride Fraction	162
Ethylacetate Fraction	-
Methanol Fraction	3161

Table 3. IC₅₀ Cytotoxicity against HeLa Cell (MTT assay)

Root of <i>Irvingia malayana</i>	IC₅₀ (µg/mL)
Methanol Extract	-
<i>n</i> -Hexane Fraction	172
Methylene Chloride Fraction	70
Ethylacetate Fraction	593
Methanol Fraction	-

Table 4. IC₅₀ Cytotoxicity against HeLa Cell (MTT assay)

Leaves of <i>Irvingia malayana</i>	IC₅₀ (µg/mL)
Methanol Extract	-
<i>n</i> -Hexane Fraction	125
Methylene Chloride Fraction	325
Ethylacetate Fraction	201
Methanol Fraction	600

Profiles obtained from cell viability MTT test showed that the methanolic extract and fractions (*n*-hexane, methylene chloride, ethyl acetate, and methanol) of bark of *Irvingia malayana* in general may decrease the viability of cells HeLa cells compared with controls. Given cytotoxic effect is the effect depends on the level concentration of sample where the greater concentration of sample so the greater the levels cytotoxic effect. Inhibitory activity shown by the largest cell viability ethyl acetate fraction of bark of *Irvingia malayana*. Active compounds contained in the bark of *Irvingia malayana* is estimated to have the potential chemopreventive pretty good views of its value IC₅₀ (<100 µg / mL)

IV. CONCLUSION

Ethylacetate fraction from stem bark of *Irvingia malayana* showed highest activity and potential to find bioactive compound against HeLa cell line

Acknowledgements

We are grateful to Cancer Chemoprevention Research Centre, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta Indonesia for cytotoxic test and the Directorate Higher Education, Ministry of Education and Culture, Republic of Indonesia for foundation this research by BPPS Programme

REFERENCES

- [1] Pouplin, J. N. and Tran.H., 2007. Antimalarial and cytotoxic activities of ethnopharmacologically selected medical plants from South Vietnam, *J. of Ethnopharm.* 109(2),417-427
- [2] Praptiwi and Chairul. 2008. The effect of pauh kijang (*Irvingia malayana* Oliv. ex. A. Benn) in decreasing parasitemia percentage in mice infected with *Plasmodium berghei*. *Biodiversitas*.9. 96-98
- [3] Nakahara, K., Roy. M. K., Alzoreky. N. S., and Thalang. N. V. 2002. Inventory of indigenous plants and minor crops in Thailand based on bioactivities, 9th *JIRCAS International Symposium*, 135-139
- [4] Widiyantoro, A., Destiarti, L., Kusharyanti, I., Supardi, Halim, D.G., Niwick and Willianti, V. 2012. Antiinflammation activity of bioactive compound from bark of *Irvingia malayana* against *Rattus norvegicus* induced by carrageenan, *J. Kaunia*, VII (2) :118-126
- [5] Widiyantoro, A., Kustiati, and Usman, T. 2006. Isolation and characterization of antifeedant compound from ethylacetate fraction of stem bark of *Irvingia malayana*, *J. Agripura*, 4 (2) : 523-529
- [6] Mitsunaga, K., Ouyang, Y., Koike, K., Sakamoto, Y., Ohmoto, T. and Nikaido, T.. 1996. Phenolic constituents of *Irvingia malayana*, *Natural Medicines*, 50 (5), 325-327
- [7] Ng, K.W., Salhimi, S.M., Majid, A.M.S.A and Chan, K.L.2010. Antiangiogenic and cytotoxic studies of some medicinal plants, *Planta Med.*, 76 : 935-940
- [8] Jaipetch, T. 1999. Chemical constituent of *Irvingia malayana* and *Clausena excavata*.Ph.D thesis. Faculty of Graduate Studies, Mahidol University
- [9] Kusharyanti, Widiyantoro, A., Maryati, and Meiyanto, E. 2008. Cytotoxic activity of bark extract of *Irvingia malayana*, Proceeding of XVI ISFI Congress, Yogyakarta