In vitro cytotoxicity of Alstonia scholaris (R.Br) bark on Vero and HeLa cell lines

Zuraida¹ and S Mariya²

¹ Forest Research and Development Centre, Environment & Forestry Research Development and Innovation Agency (FORDA), Ministry of Environment and Forestry, Jalan Guning Batu no 5 Bogor 16610, Indonesia
² Primate Research Center, Bogor Agricultural University, Jalan Lodaya II/5, Bogor 16151, Indonesia

E-mail: zuraidaus21@gmail.com

Abstract. Alstonia scholaris R.Br is one of forest plants that have medicinal properties used to treat various diseases. This study aimed to determine the activity of in vitro cytotoxicity of Alstonia scholaris bark against HeLa and Vero cells. Alstonia scholaris bark was macerated with 70% ethanol and fractionated using n-hexane and chloroform to obtain n-hexane, chloroform, and ethanol fraction. All three fractions were tested using MTT assay against HeLa (cervix cancer) and Vero (normal) cells. The results showed that chloroform fraction was the most toxic to HeLa cells with IC₅₀ (125.06 μg/mL) and followed by ethanol fraction (200.07 μg/mL), and n-hexane fractions (238.47 μg/mL) respectively. On the other hand, ethanol fraction was the least toxic to Vero cell growth with IC₅₀ (579.93 μg/mL), followed by n-hexane fraction (459.47 μg/mL), and chloroform fraction was the most toxic (396.24 μg/mL). It was suggested that ethanol fraction was the best fraction because it had the least toxicity to a normal cell, but still had toxicity to HeLa cell.

1. Introduction

Cervical cancer is cancer induced by human papillomavirus (HPV) infection. In Indonesia, cervical cancer is the second rank causes death after breast cancer in women between 15 to 44 years old. There were 20,928 new cervical cancer cases diagnosed annually in Indonesia in 2012 [1]. Death caused by cervical cancer was 28% lower than the mortality rate in the world and 2.5% lower than the mortality rate in Southeast Asia. Risk factors for cervical cancer are obesity, lack of physical activity, alcohol, and smoking [2, 3]. Treatment for cervical cancer includes various options such as surgery, radiation, and chemotherapy depending on the stage of cancer. However, these treatments require expensive costs. Besides, resistance to cisplatin and carboplatin as cervical cancer therapy agents has been reported to affect DNA damage [4, 5].

Because of side effects and less successful radiochemotherapy treatment, natural products have been chosen as an alternative medicine for an anticancer drug. Active compounds in plants have a pleiotropic effect with many pharmacological activities. One of them is a cytotoxic agent against cervical cancer. Compounds that have been reported as anti-cancer agents are alkaloids, flavonoids, and polyphenols [3]. Active compounds in plants can be tested for their cytotoxic effects on cervical cancer using HeLa cells. HeLa was the first cultured cancer cell taken from epithelium cancer cell of a patient named Henrietta Lacks in 1951 [6]. Cell lines developed from human cancer are the basic model used in laboratories to study cancer and examine the therapeutic effects of anticancer agents [7].
Alstonia scholaris is one of the medicinal forest plants rich in the active compound. Parts of pulai plants usually utilized are leaves, stems, and bark. Pulai bark extract contained flavonoids, alkaloids, saponins, tannins, and terpenoids [8]. Alstonia scholaris bioactivity has been reported as antimicrobials, antioxidants [9], antidiabetics, anti hyperlipidemic [10], anti-inflammatory [11] and anticancer [12]. However, the anticancer activity of ethanol, chloroform, and n-hexane fractions of pulai bark from Indonesia was unknown. This study aimed to examine the cytotoxic effects of HeLa and Vero cells from all three fractions.

2. Method

2.1. Extraction and fractionation
Pulai bark obtained from the Center for International Forestry Research (CIFOR), Bogor, Indonesia, was washed, dried and milled to 80 mesh simplicia. Extraction and fractionation followed the method of Egua et al. [13]. The powder was extracted by maceration using 70% ethanol at a ratio of 1:10. Maceration was carried out for 3x24 hours until the filtrate was obtained. The filtrate was evaporated at 50°C until the volume reached 1/10 of the initial volume. The filtrate was fractionated with n-hexane to obtain hexane and ethanol fractions. Ethanol fraction was then fractionated back with chloroform to obtain chloroform and ethanol fraction. Both fractions were concentrated with evaporator at 50 °C.

2.2. Cytotoxicity test of pulai bark fractions on Vero and HeLa cells
The method used in cytotoxicity tests on Vero and HeLa cells was 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium bromide (MTT) [14]. Vero (CCL ATCC 81) and HeLa cells (CCL ATCC 2) were cultured separately in Dulbecco's modified eagle's medium (DMEM), supplemented with 10% Bovine Serum Fetal (FBS), 100 U/ml penicillin, and 100 μg streptomycin/ml. There were 5000 Cells grown per well in cell culture plates 96 wells in medium growing 100 μL, then incubated at 37 ºC 5% CO2, for 18-20 hours. Each fraction of 100 μL was added to each concentration then incubated for 48 hours. Reagent MTT 5 mg/mL 10 μL was dropped to each well, then incubated for 4 hours; then 0.1 N HCl-isopropanol 100 μL was dropped to dissolve formazan crystals. Absorbance measurements were carried out at a wavelength of 595 nm using a spectrophotometer; each treatment was carried out triplo.

3. Results and discussion
This analysis aimed to evaluate cytotoxicity activity of ethanol, chloroform, and n-hexane fraction of pulai bark against HeLa and Vero cells from African green monkey’s kidney. Data are presented by percentage of viability and inhibition. Cell viability is the cell's ability to maintain its life. Percentage of inhibition shows the amount of inhibition of the sample on the growth of cancer and normal cells. Cytotoxicity activity was tested on Vero cells to identify the effect of the pulai’s fractions on cell growth because anticancer drugs generally have a negative impact on normal cell growth.

Table 1 shows data on MTT results from three fractions of pulai’s bark against Vero cells. The higher IC50 value shows the ability of the fraction to inhibit cell growth at high concentrations; it was supposed that in low concentrations, it would not affect cell growth. Ethanol fraction has the highest IC50 value followed by the n-hexane and chloroform fraction with value of 579.93, 459.47, and 396.24 µg/mL respectively. It indicated that ethanol fraction was the least toxic to Vero cells compared to the other two fractions.

Based on table 2, chloroform fraction has the smallest IC50 value followed by ethanol and n-hexane fraction with values of 125.06, 200.07, and 238.47 µg/mL respectively. It showed that chloroform fraction was able to inhibit the highest cell growth against HeLa cells. Patel et al. [15] reported that Solanum nigrum methanol extract had IC50 value of 265 µg/mL, while Moringa oleifera ethanol extract in Hermawan et al. [16] had an IC50 value > 250 µg/mL, and Vithya et al. [17] evaluated that the cytotoxicity activity of Sophora interrupted methanol extract to produce IC50 values of 211.5 µg/mL. Cytotoxic activity of the three pulai bark fractions compared to several other studies showed stronger toxicity effect on HeLa cells.
According to the above results, it was found that the IC$_{50}$ value of Vero cells from three fractions was higher than those of IC$_{50}$ value from HeLa cells. It showed that ethanol, chloroform, and n-hexane fraction of pulai bark could inhibit the growth of HeLa cancer cells but did not inhibit normal cell growth at the same concentration. Ethanol fraction is the best fraction as a candidate for inhibiting Hela cells cancer.

4. Conclusion
According to the information above, it is suggested that ethanol fraction is the best fraction because it has the least toxicity to a normal cell, but still has toxicity to HeLa cell with IC$_{50}$ 200.07 µg/mL.
5. References

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