Cytotoxicity of *Alstonia scholaris* (R.Br) bark on MCF-7 and Vero cell lines

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**Abstract.** *Alstonia scholaris* R.Br (pulai) is one of traditional forest plant to treat many kinds of disease such as arthritis, diabetes, hypertension, and hyperlipidaemia. On the other hand research about this species as anti cancer is very limited. This study aimed to determine in vitro cytotoxic activity of three fraction of *A. Scholaris* bark against breast cancer (MCF-7) and normal (vero) cells. Three fractions of *A. Scholaris* bark (n-hexane, chloroform, and ethanol fractions) derived from 70% ethanol crude extracted pulai bark. MTT (3- 4,5-dimethylthiazol-2- -2,5-diphenyl tetrazolium bromide) assay applied tot MCF-7 and vero cells. The result showed that n-hexane fraction had the strongest cytotoxic effect on MCF-7 cells, followed by chloroform and ethanol fraction with IC₅₀ values 109.01; 163.33; and 264.19 µg/mL respectively, but ethanol fraction is the least toxic on vero cells growth compared to n-hexane and chloroform fraction with IC₅₀ value 579.93; 459.47; and 396.24 µg/mL respectively. It’s suggested that ethanol fraction is the best recommended fraction as anticancer agent because it was the least toxicity to the growth of normal cells, but still toxic to cancer cell. It was concluded that ethanol fraction was the best fraction to breast cancer cells.

**1. Introduction**

Cancer is one of disease cause death in the world. Breast cancer is the first rank cancer for women in the world with 1.671.100 cases and 521.900 of them died [1]. Prevalence of breast cancer in Indonesia was 21.4% compare to other cancers cases, and breast cancer was the main cause of death in women [2]. Breast cancer is included invasive and non-invasive. Invasive breast cancer is cancer cells that invade until tissues and organ, while non-invasive breast cancer is cancer cells that only attack lobular epithelial tissue [15]. Breast cancer can be controlled and cannot be controlled. Controlled factors are lack of exercise, obesity, food additive, smoking, alcohol consumption, and hormone replacement therapy, while uncontrollable factors are age, gender and heredity [9]. Treatments such as radiotherapy, surgery, hormone therapy, and chemotherapy have been tried to treat breast cancer. Unfortunately, there is no effective treatments to treat advanced cancer. Nowadays, it has been identified that new findings, biomarkers, and agents were considered to be used effectively as cancer chemoprevention [3]. Using natural or synthetic compounds to prevent, slow down, suppress, or reverse carcinogenic processes is called cancer chemoprevention. New natural drugs discovery is important to reduce side effects, high selectivity, low toxicity, and kill cancer cells [8]. It has been proved that, more than 60% of medicines are from natural products e.g. medicinal forest plants.
Pulai is one of medicinal forest plant species has secondary metabolite that functions pharmacological activities. Leaves, roots and stems contain secondary metabolite. Pulai bark ethyl acetate crude extract contained flavonoids, alkaloids, saponins, tannins, and terpenoids [14]. Pulai leaves ethanol extract, and bark acted as anticancer activity on MCF-7 cells with IC₅₀ 15.50 and 298.85 μg/mL respectively [4], while alkaloid fraction had activity on MCF-7 cells with IC₅₀ 29.76 μg/mL [7]. However, studies on comparing cytotoxic activity of pulai bark fractions against MCF-7 and Vero cells had not been reported yet. This study aimed to examine cytotoxic activity of ethanol, chloroform, and n-hexane fractions of pulai bark against MCF-7 cells and Vero cells.

2. Method

2.1 Extraction and fractionation

Simplicia of A. scholaris R.Br bark obtained from CIFOR, Bogor. This simplisia dried, and milled to 80 mesh, then extracted and fractionated follow the method of [5]. Filtrate obtained by maceration in 70% ethanol for 3x24 hours, then evaporated at 50°C until the volume reached 10%. filtrate was fractionated with n-hexane and chloroform to get three fractions : ethanol, chloroform and n-hexane.

2.2 Cytotoxicity test of pulai bark fractions on Vero and MCF-7 cells

MTT method used in cytotoxicity tests on Vero and MCF-7 cells according Phonnok et al. (2010) methode. MCF-7 (ATCC HTB 22) and Vero (CCL ATCC 81) cells were cultured separately in Dulbecco's modified eagle's medium (DMEM), supplemented with 100 U/ml penicillin, 10% Bovine Serume Fetal (FBS), and 100 µg streptomycin/ml. In cell culture plates 96 wells grown 2000 Cells per well in100 μL medium growing, incubated at 37 °C 5% CO2 for 24 hours, then 100 µL each fractions added to each concentration, and incubated for 48 hours. After that 5 mg/mL 10 μL MTT reagent dropped to each well, then incubated for 4 hours. Finally, 100 µL 0.1 N HCl-isopropanol dropped to formazan crystals. Each treatment was carried out triplo, and absorbance measured using spectrophotometer 595 nm wavelength

3. Result and discussion

Therapeutic on cancer cells using natural products has been expanding. The aimed of this study is to investigate cytotoxic activity of pulai bark ethanol, chloroform, and n-hexane fractions on MCF-7 (breast cancer) and Vero (normal) cells from African green monkey's kidney using MTT method. Result research shown in inhibition and percentage viability of both cells. Percentage inhibition is inhibition activity of sample on growth cells, while viability cell is ability of the cell to maintain its life. Cytotoxic activity tested on Vero cells to know the effect of three fractions on cell growth, because commonly, anticancer agents have negative impact on normal cell growth. MTT results of three fractions pulai's bark against Vero cells presented in Table 1.

Based on Table 1 shown that IC₅₀ value for each fractions. The higher IC₅₀ value the lower toxicity to inhibit cell growth. It is supposed that in low concentrations, it won’t affect cell growth. Ethanol fraction had the highest IC₅₀ value compare to n-hexane and chloroform fractions with IC₅₀ value 579.93, 459.47, and 396.24 μg/mL respectively. This indicated that ethanol fraction was the least toxic to Vero cells compared to the other two fractions.

Based on Table 2, each fraction had significant inhibition and good viability and on MCF-7 cells at certain concentrations. Ethanol fraction was able to survive up to 73.42% at 150 μg/mL and significantly inhibited 85.66% (300 μg/mL) and 91.11 (600 μg/mL) with IC₅₀ 264.19 μg/mL. Chloroform fraction had viability up to 63.87% at 100 μg / mL and significantly 77.42% (200 μg/ mL) and 92.97 (400 /mL) with IC₅₀ 163.33 μg/mL. N-hexane fraction could survive 73.74% at concentration 62.5 μg/mL and significantly inhibited 85.80% (125 μg/mL) and 90.64 (250 μg/mL) with IC50 109.01 μg /mL. The n-hexane fraction has the strongest cytotoxic effect against MCF-7 cells because it has the smallest IC₅₀ value, followed by chloroform and ethanol fractions.
Table 1. Result of MTT test of three pulai’s bark fractions on Vero cell

| Fraction     | Extract concentration [µg/mL] | % Viability | % Inhibition | IC50 (µg/mL) |
|--------------|--------------------------------|-------------|--------------|--------------|
| Ethanol      | 25                             | 90.32       | 9.68         | 579.93       |
| Fraction     | 50                             | 89.67       | 10.33        |              |
|              | 100                            | 85.36       | 14.64        |              |
|              | 200                            | 79.64       | 20.36        |              |
|              | 800                            | 33.37       | 66.63        |              |
|              | 25                             | 94.40       | 5.60         |              |
| Chloroform   | 50                             | 86.06       | 13.94        | 396.24       |
| Fraction     | 100                            | 78.94       | 21.06        |              |
|              | 200                            | 69.72       | 30.28        |              |
|              | 800                            | 7.35        | 92.65        |              |
|              | 25                             | 95.97       | 4.03         |              |
| n-hexane     | 50                             | 84.60       | 15.40        | 459.47       |
| Fraction     | 100                            | 92.88       | 7.12         |              |
|              | 200                            | 83.72       | 16.28        |              |
|              | 800                            | 12.19       | 87.81        |              |

Table 2. Result of MTT test of three pulai’s bark fraction on MCF-7 Cells

| Fraction     | Extract concentration [µg/mL] | % Viability | % Inhibition | IC50 (µg/mL) |
|--------------|--------------------------------|-------------|--------------|--------------|
| Ethanol      | 4.68                           | 98.37       | 1.63         | 264.19       |
| Fraction     | 18.75                          | 95.16       | 4.84         |              |
|              | 150                            | 73.42       | 26.58        |              |
|              | 300                            | 14.34       | 85.66        |              |
|              | 600                            | 8.9         | 91.11        |              |
|              | 12.5                           | 88.97       | 11.03        |              |
| Chloroform   | 25                             | 85.15       | 14.85        | 163.33       |
| Fraction     | 50                             | 74.86       | 25.14        |              |
|              | 100                            | 63.87       | 36.13        |              |
|              | 200                            | 22.58       | 77.42        |              |
|              | 400                            | 7.03        | 92.97        |              |
| n-hexane     | 3.91                           | 95.48       | 4.52         | 109.01       |
| Fraction     | 15.62                          | 90.64       | 9.36         |              |
|              | 62.5                           | 73.74       | 26.26        |              |
|              | 125                            | 14.20       | 85.80        |              |
|              | 250                            | 9.36        | 90.64        |              |

Pulai fractions were also tested for its viability and inhibition against vero cells. Viability of the three fractions were very good at a concentration 25; 50; 100; and 200 µg / mL. Ethanol fraction, chloroform fraction, and n-hexane fraction were able to survive well until concentration 200 µg / mL.
with viability 79.64%, 69.72%, and 83.72%, respectively. IC$_{50}$ values ethanol, chloroform, and n-hexane fraction were 579.93, respectively; 396.24; and 459.47 µg / mL. Ethanol fraction is known to be the least toxic to vero cell growth because it has the highest IC$_{50}$ value, followed by the n-hexane and the chloroform fraction. IC$_{50}$ values for Vero cells in this study showed higher values than IC$_{50}$ for MCF-7 cells in all three fractions.

Alkaloid fraction had inhibitory activity on MCF-7 cells with IC$_{50}$ value of 29.76 µg / mL [7]. This shown that the alkaloid fraction has a significant cytotoxic effect than those of three pulai fractions in this study. The alkaloid fraction in the study obtained from soxlet extraction using ethanol, then washed with methanol and ethanol residues were taken. Different extraction methods affect the active components of a plant [16]. In addition, ecological factors also affect the content of active ingredients [10].

Active component in plants influences their pharmacological activities. Ethyl acetate extract of pulai bark known contained flavonoids, alkaloids, saponins, tannins, and terpenoids [14]. According to [13] that chemical components that have antioxidant activity such as flavonoids contributed to anticancer. Alkaloid derivatives such as vinblastine and vincristine are the first anticancer agents [12]. Saponins and tannins were also known have anticancer activities [17]. According to [6] that terpenoids can be used as cancer therapy.

4. Conclusion
Three fractions of pulai bark had potential as anticancer and n-hexane fraction had the strongest cytotoxic effect on MCF-7 cells of all, but ethanol fraction is the least toxic of all. This result shown that ethanol fractions was chosen best fraction of all because it can be as anti breast cancer agent without inhibiting the growth of normal Vero cells.

References
[1] [GLOBOKAN] Global Burden of Cancer in Women (Current status, trends, and interventions) https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/global-cancer-facts-and-figures/global-burden-of-cancer-in-women.pdf. [13 Oktober 2017]
[2] [WHO] World Health Organization 2014 Cancer country profile (World Health Organization) http://www.who.int/cancer/country-profiles/idn_en.pdf?ua [13 Oktober 2017]
[3] Cazzaniga M and Bonanni B Breast cancer chemoprevention: old and new approaches J Biomed Biotechnol 2012;2012:985620
[4] Dhanya S, Kumar N V A, Nayak A S, Raj U S, Prabu D and Ravikikiran 2013 Cytotoxicity tsudies of microwave assisted natural product extract in HeLa and MCF-7 cell lines Int J Med Arom Plants 3 (1) pp 27-31
[5] Egua M O, Etuk E U, Bello S O and Hassan S W 2014 Antidiabetic potential of liquid-liquid partition fractions of ethanolic seed extract of Corchorus olitorious Journal of Pharmacognosny and Phytotherapy 6 (1) pp 4-9
[6] Huang M, Lu J J, Huang M Q, Bao J L, Chen X P and Wang Y T 2012 Terpenoids: natural products for cancer therapy Expert Opinion on Investigation Drugs 21 (12) pp 1801-18
[7] Jagetia G C and Baliga M S 2006 Evaluation of anticancer activity of the alkaloid fraction of Alstonia scholaris (Sapthaparna) in vitro and in vivo Phytoterapy Research 20 pp 103-9
[8] Khazaee Koohpar Z, Entezari M, Movafagh A and Hashemi M Anticancer activity of curcumin on human breast adenocarcinoma: role of Mcl-1 gene Iran J Cancer Prev 2015;8:e2331
[9] Lakshmi R, Athira R, Mary J T and Vijayalakshmi 2012 Breast cancer risk factors: preventable and non-preventable International Reserarch Journal of Pharmacy 3 (10) pp 48-52
[10] Liu W, Liu J, Yin D and Zhao X 2015 Influence of ecological factors on the production of active substances in the anti-cancer plant Sinopodophyllum hexandrum (Royle) TS Ying PLoSONE 10 (4) e0122981
[11] Phonnok S, Tanechpongtamb W U and Wongsatayanon B T 2010 Anticancer and apoptosis inducing activities of microbial metabolites Electronic Journal of Biotechnology 13 (5)
[12] Prakash O, Kumar A, Kumar P and Ajeet 2013 Anticancer potential of plants and natural products: a review *American Journal of Pharmacological Science* 1 (6) pp 104-15

[13] Raina H, Soni G, Jauhari N, Sharma N and Bharadjava N 2014 Phytochemical importance of medicinal plants as potential sources of anticancer agents *Turkish Journal of Botany* 38 pp 1027-35

[14] Saxena N, Shrivastava P and Saxena R 2013 Antibacterial efficacy of *Alstonia Scholaris* (L.) R. Br. stem bark extracts *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 4 (1) pp 964–70

[15] Sharma G N, Dave R, Sanadya J, Sharma P and Sharma K K 2010 Various types and management of breast cancer: an overview *Journal Advanced Pharmaceutical Technology and Research* 1 (2) pp 109-26

[16] Tiwari P, Kumar B, Kaur M, Kaur G and Kaur H 2011 Phytochemical screening and extraction *Internationale Pharmaceutica Sciencia* 1 (1) pp 98-106

[17] Yildirim I and Kutlu T 2015 Anticancer agents: saponin and tannin *International Journal of Biological Science* 9 (6) pp 332-40