Antiproliferative and Apoptotic Induction of n-Hexane Fraction of *Picria fel-terrae* lour. Herbs on T47D Cell Line

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Abstract: A recent research reported that breast cancer is leading to the estimated new cancer cases, and the second most incidence death cause of women affictioning from cancer. This research aim is to evaluate cytotoxic, antiproliferative and apoptotic induction activities of n-hexane fraction (nHF) of *Picria fel-terrae* Lour. herbs. Cytotoxic activity of nHF was determined with MTT method, cell cycle and apoptotic analysis were determined with flow cytometry method towards T47D cell line. Cytotoxic activity from nHF with MTT assay measured as IC_{50} was 75.87 ± 0.75 µg/mL, nHF at 15 µg/mL caused accumulation in G_2-M (37.47%) and S phase accumulation (19.41%) and increased early (24.25%) and late apoptosis (4.26%). The results reveal that nHF of *Picria fel-terrae* Lour. herbs have antiproliferative and apoptotic induction activities. Our further study is to isolation anticancer compounds from *Picria fel-terrae* Lour. herbs.

1 INTRODUCTION

Breast cancer take place when breast cells start to grow with uncontrollably. Cells could invade nearby tissues and spread pass through the body. Each kind of tissue in the breast can form a cancer, but the cancer generally arises in the milk ducts or glands. Factors which influence the risk of breast cancer are reproductive factors (e.g. no children and first pregnancy at an advanced age), the length of exposure to hormones, dietary factors and lack of physical activity, radiation during breast development, hormone replacement therapy, as well as congenital genetic factors (Barnett, et. al., 2008). WHO reported that breast cancer is one of the main cause of death and the most common incidence of cancer type amongst women worldwide in 2012 (WHO, 2015).

Poguntano (*Picria fel-terrae* Lour.) have been used for treat of colic, diuretic, fever, malaria, and skin disease (Perry, 1980). The modern pharmacological assessment indicated that the *Picria fel-terrae* Lour. exerts antidiabetic, antioxidant, anti-inflammatory, anthelmintic, diuretic, antipyretic, hepatoprotective, cardioprotective, and analgesic activities (Sitorus, et al., 2014; Dalimunthe, et al., 2015; Sihotang, et al., 2016;Huang, et al., 1994; Thuan, et al., 2007; Zhong, et al., 1979; Zou, et al., 2005; Harfina, et al., 2012; Patilaya and Husori, 2015). Moreover, *Picria fel-terrae* inhibits hepatitis B (HB) e-antigen excreted by HepG2 2215 cell lines, suggesting to have anti-HB virus activity (Zheng, et al., 2010). It can be developed as co-chemotherapeutic regimen and inhibit metastasis for breast cancer by inducing apoptosis, cell cycle arrest, suppressing cyclin D1 and Bcl-2 expression, suppressing expression of COX-2 and VEGFR2 based on the recent studies (Satria, et al., 2015; Lestari, et al., 2013, Harahap, et al., 2018). The aim of this study was to assess the antiproliferative and apoptosis induction activities of n-hexane fraction of *Picria fel-terrae* Lour. Herbs.

2 MATERIALS AND METHODS

2.1 Plant and Chemicals Material

Fresh herbs of *Picria fel-terrae* Lour. was collected from Tiga Lingga village, Dairi regency, Sumatera Utara province, Indonesia. *Picria fel-terrae* Lour. was identified in Research Centre for Biology, Indonesian Institute of Science, Bogor, and the voucher specimen was deposited in

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herbarium. Chemicals used were annexin-V (BioLegend), distilled water, DMSO (Sigma), [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT) (Sigma), propidium iodide kit (BioLegend).

2.2 Preparation of n-Hexane Fraction (nHF)

The air-dried and powdered herbs of *Picria fel-terrae* Lour. (1 kg) were repeatedly fractionated by cold maceration with n-hexane (3x3 d, 7.5 L) at room temperature with occasional stirring. The filtrate was collected, and then evaporated under reduced pressure to give a viscous fraction and then freeze dried to dry (Satria, et al., 2015; Anggraeni, et al., 2015; Hasibuan, et al., 2015).

2.3 Cytotoxicity Assay

The cells were treated with nHF. In this test, T47D cell line was grown in RPMI 1640 medium, medium containing 10% Fetal Bovine Serum (Gibco), 1% penicillin-streptomycine (Gibco), and fungizone 0.5% (Gibco) in a flask in a humidified atmosphere (5% CO_2) at 37^{o}C. The inoculums seeded at $1x10^4$ cells/mL at an optimal volume of 0.1 mL per well. After 24 h incubation, the medium was discharged and treated by nHF. After incubation 24 h, the cells were incubated with 0.5 mg/mL MTT for 4 h in 37^{o}C. Viable cells reacted with MTT to produce purple formazan crystals. After 4 h, SDS 10% as stopper (Sigma) in 0.01N HCl (Merck) was added to dissolve the formazan crystals. The cells were incubated for 24 h in room temperature and protected from light. After incubation, the cells were shaken, and absorbance was measured using microplate reader at λ 595 nm. The data which were absorbed from each well were converted to percentage of viable cells (Hasibuan, et al., 2015; Satria, et al., 2014).

2.4 Preparation of Cells for Flowcytometry Analysis

T47D cells ($5x10^5$ cells/well) were seeded into 6-well plate and incubated for 24 h. After that, the cells were treated with nHF and then incubated for 24 h. Both floating and adherent cells were collected in conical tube using tripsin 0.025%. The cells were washed thrice with cold PBS and centrifuged 2500 rpm for 5 min. The supernatant was separated, while the sediment was collected (Satria, et al., 2015; Anggraeni, et al., 2015).

2.5 Cell Cycle Analysis

Cells were fixed in cold 70% ethanol in PBS at -20^{o}C for 2 h. The cells were washed thrice with cold PBS and resuspended then centrifuged at 3000 rpm for 3 min and PI kit (containing PI 40 µg/mL and RNAse 100 µg/mL) added to sediment and resuspended and incubated at 37^{o}C for 30 min. The samples were analyzed using FACScan flow cytometer. Based on DNA content, the percentage of cells in each of stage in cell cycle (G1, S and G2/M) were calculated using ModFit Lt. 3.0.s.

2.6 Apoptosis Analysis

Annexin V kit was added to sediment and suspended and incubated at 37^{o}C for 30 min. The samples were analyzed using FACScan flow cytometer (Harahap, et al., 2018).

2.7 Statistical Analysis

Data were expressed as mean ± SD with descriptive analysis. All statistics were analyzed using the SPSS 21 software.

3 RESULTS AND DISCUSSION

3.1 Inhibitory Concentration 50% (IC_{50})

MTT method was used to determine cell viability after incubation for 24 h. In every treatment nHF was shown to inhibit cells growth. The IC_{50} value of nHF was 75.87 ± 0.75 µg/mL.The natural product is suspected to have cytotoxic properties based on their active compound in *Picria fel-terrae* Lour. Triterpenoids/steroids are suspected to be the main active compound (Yadav, et al., 2012). Triterpenoids are also considered as one of promising anticancer drugs (Petronelli, et al., 2009)

3.2 Effect on Cell Cycle and Apoptosis

To assess the activity of nHF to increase cell death by increasing cell cycle, we concentrated on it for further studies using flow cytometry method. The effect of nHF is given in Figure 1. Whereas treatment of nHF in 15 µg/mL caused cell accumulation at G_2-M phase (37.47%) and for control cell (30.11%). At S phase the accumulation

after nHF treatment (19.41%) and for control cell (16.80%). This fact was to indicate that nHF can inhibit cell grow that G_2-M and S phase. In the cell cycle analysis, nHF was exhibited higher G_2-M and S phase accumulation compared to control cells (Harahap, et al., 2018; Satria, 2015).

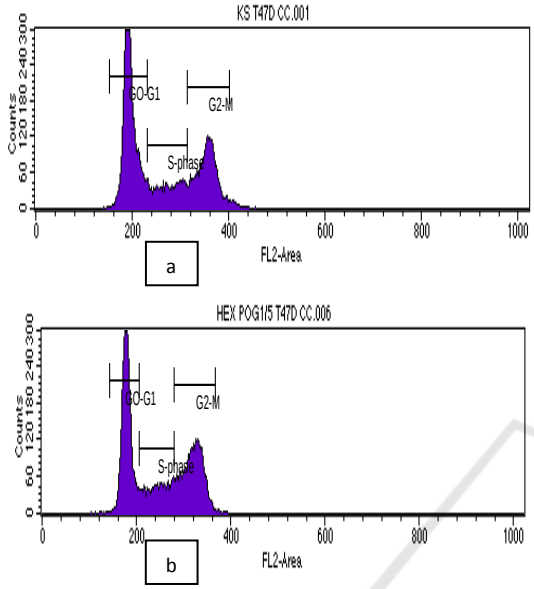
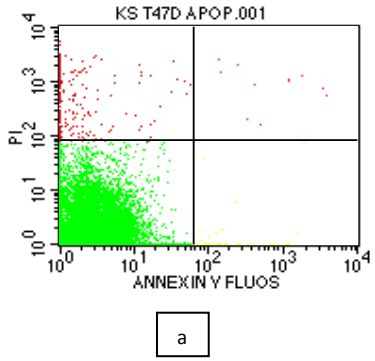


Figure 1. Cell cycle analysis using flowcytometry. T47D cells were treated by nHF for 24h and stained using propidium iodide. (a) control cells; (b) nHF 15 µg/mL. nHF exhibited G_2-M and S phase.

Evaluation of apoptosis induction was performed using flowcytometry method with Annexin-V. as shown in Figure 2.



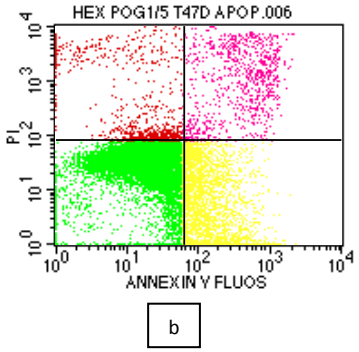


Figure 2. Apoptosis analysis using flowcytometry. T47D cells were treated by nHF for 24h and stained using Annexin-V. (a) control cells; (b) nHF 15 µg/mL.

As shown in Figure 2, the cells in the upper and lower right quadrants represent late apoptotic/ necrotic and early apoptotic cells, respectively. The percentage of control and nHF in early apoptotic 0.18% and 24.25%, in late apoptotic/early necrotic 0.06% and 4.26%. In apoptotic study, nHF increased the cells go through apoptosis in early apoptosis and late apoptosis if compared to control T47D cell lines. Apoptosis is a mechanism of programmed cell death with alterations on morphology, membrane blebbing and chromatin (Ruddin,et al., 1997).

4 CONCLUSIONS

The results suggest that n-hexane fraction of *Picria fel-terrae* Lour. herbs may exhibit an anticancer activity towards T47D cell lines through cell cycle inhibition and induction apoptosis.

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