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Cytoprotective Activity of Ethylacetate Fraction of *Picria fel-terrae* Lour. Herbs

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Abstract

AIM: Free radicals produce from metabolism or enviromental which interact continously with biological system. *Picria fel-terrae* Lour. herbs have been used as antioxidant and treat various diseases. The aim of this study was to evaluate cytoprotective activity of ethylacetate fraction (EAF) of *Picria fel-terrae* Lour. herbs.

METHODS*:* Cytoprotective activity were determined by MTT asay and flow cytometry assay on Vero cells which induced with H_2O_2 0.8 mM.

RESULTS*:* EAF at 100 µg/mL were showed highest viability (88.83 ± 2.90%) and ROS expression (66.75%) on Vero cells.

CONCLUSION*:* EAF of *Picria fel-terrae* Lour. herbs have cytoprotective activity.

Introduction

The steady increase of free radicals in cells creates the conditions for so-called oxidative stress. Free radicals produce from metabolism or enviromental which interact continously with biological system. Reactive species are molecules or atoms that have an electronic instability and highly reactive. The uncontrolled amount of oxygen free radicals and the unbalanced mechanism of antioxidant scavenging results in the onset of many diseases, such as cancer, diabetes, Alzheimer's, heart diseases and aging [1], [2], [3], [4].

Picria fel-terrae Lour. have been show to exerts diuretic, hepatoprotective, cardioprotective, antidiabetic, inhibits hepatitis B (HB) e-antigen, antioxidant, anti-inflammatory, co-chemotherapeutic for breast cancer, anthelmintic, and analgesic [5], [6], [7], [8], [9], [10], [11], [12], [13], [14], [15].

Material and Methods

Fresh herbs of *Picria fel-terrae* Lour. was collected from Tiga Lingga village, Dairi regency, Sumatera Utara province, Indonesia. Chemicals used were DCF-DA (Immuno Chemistry), distilled water, hydrogen peroxide (Merck), MTT (Sigma).

Preparation of Ethylacetate Fraction (EAF). The ethylacetate fraction was prepared based on previous study [16], [17], [18].

MTT Assay. Cytoprotective activity of EAF against H_2O_2 (0.8 mM) induced oxidative stress was determined by MTT assay based on [19], [20]. The data which were absorbed from each well were converted to percentage of viable cells.

ROS Expression Analysis. Vero cells ($1x10^6$ cells/well) were seeded into 6-well plate and incubated for 24 h. After that, the cells were treated

with extract and then incubated for 24 h. Each of well was added 10 µL DCFDA and incubated for 30 m, and then 1000 µL medium with or without H_2O_2 (0.8 mM) was added and incubated for 3h. Cells were harvested and analyzed based on previous study [17], [18], [19], [20], [21].

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

Results

The effect of EAF to viability cells was shown on Figure 1.

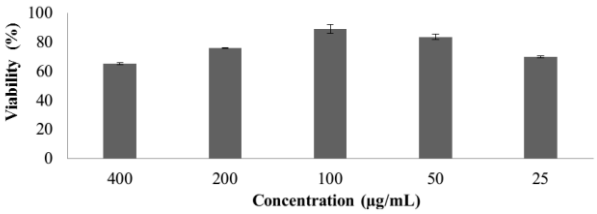

Figure 1: Effect of EAF towards viability of Vero cells which induced by H_2O_2 0.8 Mm

Analysis of percentage of ROS was shown on Table 1.

Table 1: Percentage of ROS expression

Perlakuan	ROS Expression (%)
Control cells	3.02
Control cells + H_2O_2 0.8 mM	87.80
EAF + H_2O_2 0.8 Mm	66.75

Analysis of ROS expression was performed using flow cytometry method with DCF-DA reagent (Figure 2).

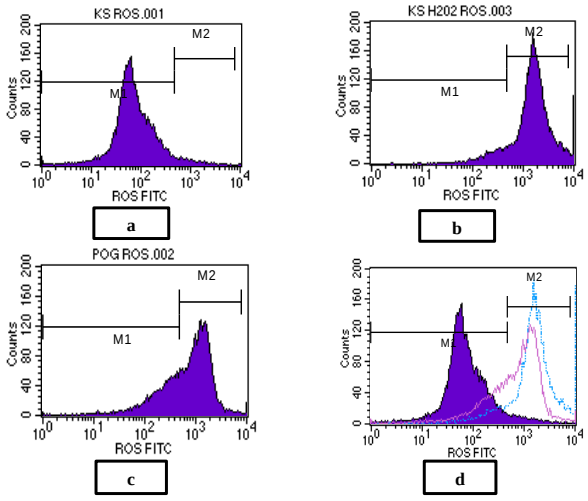

Figure 2: Analysis of ROS Expression; A) Control cells unstaining; B) Control cells; C) EAF 25 µg/mL; D) Overlay all of treatments

Discussion

MTT method is a quantitative analysis of viable cell which conducted to the amount of formazan crystal where life cells reduce MTT salts [19], [20], [21], [22].

Picria fel-terrae Lour. contains flavonoids and tannins which contribute as antioxidant and could prevent chelating of metal ions to form free radicals and protect towards oxidative stress Antioxidant flavonoids and tannins have responsible for the production of ROS. It is believed that antioxidants exert their protective effect by decreasing oxidative damage to DNA and by decreasing abnormal increases in cell division [23], [24]. Flavonoids inhibit the enzymes responsible for superoxide anion production, such as xanthine oxidase and protein kinase. A number of flavonoids efficiently chelate trace metals, which play an important role in oxygen metabolism. Free iron and copper are potential enhancers of ROS formation, as exemplified by the reduction of H2O2 with the generation of the highly aggressive hydroxyl radical [25].

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