

Total Phenolic, Antioxidant and Cytotoxic Activities of *Saurauia vulcani* (Korth.) Leaves towards RAW 264.7 Cells

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Keywords: Total phenolic, Antioxidant, Cytotoxicity, *Saurauia vulcani* Korth. Leaves

Abstrak : This study aims to evaluate total phenolic content (TPC), antioxidant and cytotoxic activities of *Saurauia vulcani* Korth leaves towards RAW 264.7 cells. *Saurauia vulcani* Korth. leaves powder was extracted by maceration method, TPC were determined by Folin-Ciocalteu and antioxidant capacity was assessed by DPPH assay and cytotoxicity resolved by MTT assay. *n*-hexane extract of *Saurauia vulcani* Korth leaves (NESL) was found to contain high levels of phenolic (88.16±0.71 mg GAE/g), ethylacetate extract of *Saurauia vulcani* Korth leaves (EEASL) (153.22 ±0.71 mg GAE/g); and ethanol extract of *Saurauia vulcani* Korth leaves (EESL) (242.21±1.11 mg GAE/g), antioxidant capacity NESL; EEASL; EESL from DPPH assay was measured as inhibitory concentration (938.33±41.08 ppm; 94.66±0.63 ppm; 48.88±0.18 ppm). Viability of RAW 264.7 cell toward extracts of *Saurauia vulcani* Korth leaves showed no toxicity with best concentrations at 12.5 and 25 µg/mL. The results reveal that NESL, EEASL, EESL has high levels of phenolic and strong antioxidant capacity. Cell viability testing showed that extract of *Saurauia vulcani* Korth leaves didn't induce cytotoxicity to RAW 264.7 cells.

1 INTRODUCTION

In this modern era human population is very susceptible to various diseases, especially inveterate diseases such as cardiovascular, cancer, infectious diseases, diabetes, heart disease, Alzheimer's, aging and others. It is caused by an uncontrollable composition of oxygen free radicals and unequal process of anti-oxidant care outcome causes of much illness so it is necessary immunomodulator that can improve the human body's immune system that can prevent various diseases (Satria, 2017) (WHO, 2015) (Kusmardi, 2007). One of the potential plants is Pirdot (*Saurauia vulcani* Korth.). *Saurauia vulcani* Korth. Is one of the plant which used as antidiabetic traditionally in Tapanuli Utara, North Sumatera, Indonesia. *Saurauia vulcani* Korth. is efficacious as wound healing, hypoglycemic, antihyperlipidemic (Sitorus, 2015) (Sitorus, 2018) (Hutahaean, 2018). The results of phytochemical screening *Saurauia vulcani* Korth. contains flavonoids, steroidal/triterpenoid, glycosides, saponin, tannins (Marpaung, 2016) (Saragih, 2016). Steroidal/triterpenoid and flavonoid which is also believed to

be efficacious as an immunomodulator (Durga, 2014).

Therefore, the function of this research was to determine of total phenol value, activity of antioxidant and cytotoxicity of *Saurauia vulcani* Korth leaves extract toward RAW 264.7 cells.

2 MATERIALS AND METHODS

2.1 Plants and Chemicals Reagent

Fresh leaf of *S. vulcani* Korth. were obtained from Dolog Huluan Village, Raya District, Simalungun Regency, Sumatera Utara province, Indonesia. Chemicals used were AlCl₃.6H₂O (Merck), distilled water, DPPH (Sigma), Folin-Ciocalteu (Sigma), Quercetin (Sigma), gallic acid (Sigma), sodium acetate (Merck), and sodium bicarbonate (Merck), phosphate buffer saline (PBS), dimethylsulfoxide (DMSO), Dexamethasone (Harsen). RAW 264.7 cells used are from the Department of Parasitology Medical Faculty, University of Gadjah Mada

Yogyakarta. Penicillin/streptomycin (100×) and Phosphate buffer saline (PBS).

2.2 Preparation of *n*-hexane Extract of *Saurauia vulcani* Korth Leaves (NESL), ethylacetate Extract of *Saurauia vulcani* Korth Leaves (EEASL) and Ethanol Extract of *Saurauia vulcani* Korth. Leaves (EESL)

The air-dried and powdered of *S. vulcani* Korth. leaves (1 kg) were repeatedly extracted by maceration with *n*-hexane (3×3 day, 8 L), the dust was dried in the air and extracted with ethylacetate (3×3 day, 8 L), the dust was dried in the air and extracted with ethanol (3×3 day, 8 L) at 25-30°C with periodical intrusion. The liquid was congregated, and then vaporized to get an viscid extract and then freeze dried to dry (Satria, 2015) (Satria, 2017).

2.3 Determination of Total Phenol Value (TPV)

The TPV of sample was resolved used Folin reagent. Shortly, 100 µL of NESL; EEASL; EESL (500 µg/mL) was mingled with 7.9 mL of filtered water and 0.5 mL of Folin-Ciocalteu's reagent (1:10 v/v) and mixed using vortex for 1 minute. Total phenolic value was calculated as previously described (Satria, 2017).

2.4 Activity of Free Radical Scavenging

The DPPH testing was carried out according to the previous study with some modifications. 0.2 mM solution of DPPH in methanol was available, and 100 µl of this solution was attached to various concentrations of NESL; EEASL; EESL. Inhibitor concentration was calculated as previously described (Satria, 2017) (Jamuna, 2012).

2.5 Cell Culture

RAW 264.7 cells, cell line of a mouse macrophage, was achieved from the Department of Parasitology Medical Faculty, University of Gadjah Mada Yogyakarta. RAW 264.7 cells were preserved in DMEM enhanced with 100 units/mL of penicillin, 100 µg/mL of streptomycin, and 10% FBS at 37°C in a humidified environment containing 5% CO₂. Phosphate buffer saline (PBS) containing EDTA

0.02% and trypsin 0.25% was used to detach RAW 264.7 cells (Susanto, 2018) (Yuandani, 2017).

2.6 Viable Cell Assay

An MTT cell proliferation test was used to appraised the cytotoxicity of *n*-hexane extract (NESL), ethylacetate extract (EEASL) and ethanol extract (EESL) of *Saurauia vulcani* Korth. leaves on RAW 264.7 cells. Briefly, RAW 264.7 cells (3×10³ cells/well) were planted into a 96-well plate (Iwaki) and incubated for 24 h. The cells were then medicated with NESL, EEASL and EESL of *Saurauia vulcani* Korth. leaves at series (12.5; 25; 50; 100 and 200 µg/mL) concentrations. After 24 h stimulation, MTT was attached to each well to a final concentration of 12.5 µg/mL. The plates were further incubated for 3-6 hrs, and then the produced formazan crystals were soluble in DMSO. The absorbance at 595 nm was quantified in a microplate reader Benchmark. (Yuandani, 2017) (Nugroho, 2013).

2.7 Analysis of Statistical

Data were presented as mean ± standard deviation, which were analyzed using the SPSS 22 edition.

3 RESULTS AND DISCUSSION

3.1 Total Phenolic Value (TPV)

TPV was determined by the Folin-Ciocalteu method. The *n*-hexane extracts (NESL), ethylacetate extracts (EEASL) and ethanol extracts (EESL) of *Saurauia vulcani* Korth. leaves was found to include high rate of phenolic value (88.16 ± 0.71 mg GAE/g; 153.22±0.71 mg GAE/g; 242.21±1.11 mg GAE/g). Phenolic composite are known as an antioxidant, and they are very necessary plant constituents because of their free radical scavenging capability due to their hydroxyl groups (Satria, 2017) (Jamuna, 2017) (Nugroho, 2013) (Sitorus, 2017). Total phenol content for extract and fractions in DPPH assay were shown on Figure 1.

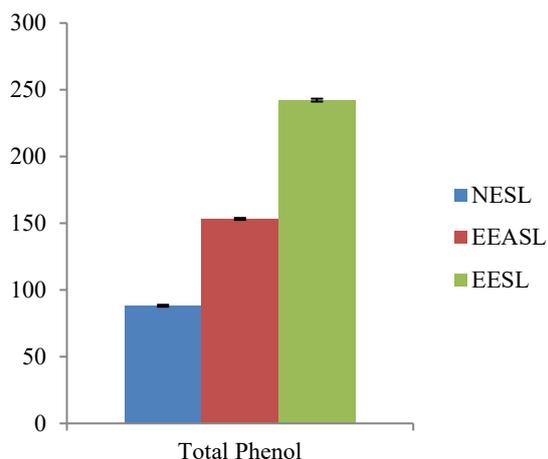


Figure 1. Total phenol value of *n*-hexane extract (NESL), ethylacetate extract (EEASL) and ethanol extract (EESL) of *Saurauia vulcani* Korth. leaves

3.2 Free Radical Scavenging Activity

Antiradical ability of the plant samples was measured in term of hydrogen donating capability using DPPH which is a constant, nitrogen centered free radical and produces deep purple color in methanol solution (Pan, 2008). The capacity of reducing composite could serve as an indicator of potential antioxidant property (Meir, 1995) (Dalimunthe, 2016) (Shah, 2015); Satria, 2017). DPPH assay, which is based on the ability of DPPH, a steady free radical, to decolourize in the existence of antioxidants, is a direct and dependable method for determining radical scavenging behavior (Hasan, 2006) and has been mainly used as a fast, credible and reproducibile at in vitro antioxidant activity assay (Koleva, 2002). Inhibitory concentration (IC₅₀) for NESL; EEASL; EESL and Quercetin in DPPH assay was (938.33±41.08 ppm; 94.66±0.63 ppm; 48.88±0.18 ppm and 4.94±0.05 µg/mL, respectively. NESL is a weak antioxidant, EEASL strong antioxidant, EESL as a powerful antioxidant. IC₅₀ for extract and fractions in DPPH assay were shown on Figure2.

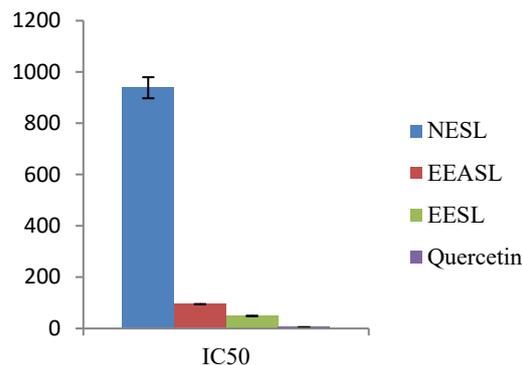


Figure 2. Activity of antioxidant, IC₅₀ of *n*-hexane extract (NESL), ethylacetate extract (EEASL) and ethanol extract (EESL) of *Saurauia vulcani* Korth. leaves and Quercetin as positive control.

3.3 Cells Viability

The results of cell viability test on extract of *n*-hexane, extract of ethylacetate and extract ethanol of *Saurauia vulcani* Korth. leaves and dexamethasone as positive control. The best results were shown on extract *n*-hexane of *Saurauia vulcani* Korth. leaves with concentration at 12.5 and 25 µg/mL which resulted in the highest viability percentage. The higher result of viability percentage then the greater the viability of the cell. Percent of viable cells of RAW 264.7 cells for extract and fractions in DPPH assay were shown on Figure 3.

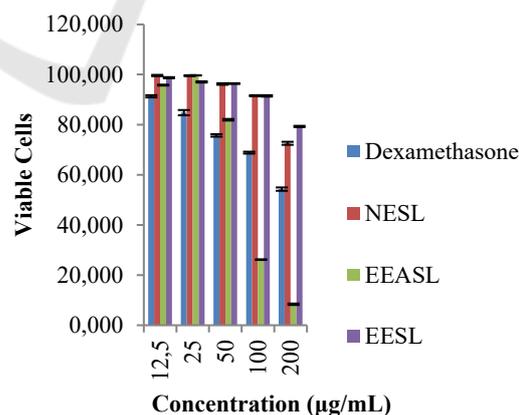


Figure 3. Percentage of viable cells of RAW 264.7 cells treated by *n*-hexane extract (NESL), ethylacetate extract (EEASL) and ethanol extract (EESL) of *Saurauia vulcani* Korth. leaves and dexamethasone as a positive control

The effects of extract of *Saurauia vulcani* Korth. leaves on survival of RAW 264.7 cells.

Cell viability testing was performed to determine the cytotoxicity of that *Saurauia vulcani* Korth leaves extract toward RAW 264.7 cells. RAW 264.7 cells were medicated with culture media containing that extract of *Saurauia vulcani* Korth leaves with various concentrations. After 24 hours of incubation, culture medium was aspirated and cell viability was measured using an MTT solution. As shown in Figure 3, cell viability testing showed that extract of *Saurauia vulcani* Korth leaves didn't induce toxicity toward RAW 264.7 cells even at the maximum concentration (Susanto, 2018) (Yuandani, 2017).

4 CONCLUSION

The results reveal that NESL, EEASL, EESL has high levels of phenolic and weak, strong and powerful antioxidant capacity. Cell viability testing showed that extract of *Saurauia vulcani* Korth leaves didn't induce toxicity toward RAW 264.7 cells.

ACKNOWLEDGEMENTS

This research was funding by PDUPT 2018 ministry of research technology and higher education.

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