Evaluation of Antioxidant and Cytotoxic Activities of *Vernonia Amygdalina* Del. Leaves

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Keywords : Antioxidant, Cytotoxic, *Vernonia amygdalina* Del., leaves, fraction.

Abstract : The excessive production of oxygen free radicals and the unbalanced mechanism of antioxidant protection results in the onset of many diseases such as breast cancer. Antioxidant activity was determined by 1-1-diphenyl-2-picrylhydrazil (DPPH) method and cytotoxic activity was determined with MTT method towards T47D cell line. Antioxidant activity from n-hexane, ethylacetate, ethanol fractions and quercetine as positive control with DPPH assay measured as IC_{50} were 297.33 ± 0.46; 177.99 ± 0.32; 37.92 ± 1.03 and 2.32 ± 0.01 µg/mL respectively. Cytotoxic activity from n-hexane, ethylacetate, ethanol fractions and doxorubicin as positive control with MTT assay measured as IC_{50} were 327.89 ± 1.13; 64.92 ± 0.72; 1591.75 ± 37.05 and 1.82 ± 0.05 µg/mL respectively. The results reveal that fractions of *Vernonia amygdalina* Del. leaves have antioxidant and cytotoxic activities. Our further study is to asses anticancer mechanism of *Vernonia amygdalina* Del. leaves.

1 INTRODUCTION

Oxidation is a natural process in living organisms. Free radicals producing by metabolism process or enviromental sources which interact with biological system. Reactive species are atoms or molecules which have instability and relatively reactive. The uncontrolled production of free radicals and the unbalanced mechanism process of antioxidant protection cause of many degenerative diseases, such as heart diseases, cancer, Alzheimer's, diabetes, and aging (Jamuna, et el., 2012; Nagmoti, et al., 2012; Rosidah, et al., 2008; Yang, et al., 2004).

There are some factors that influence the risk of breast cancer such as the length period of exposure to hormones, dietary factors, reproductive factors and first pregnancy at an advanced age, and lack of physical activity, hormone replacement therapy on chronic use, as well as congenital genetic factors associated with breast cancer like the presence of gene mutations and radiation during breast development, (Barnett, et. al., 2008).

Vernonia amygdalina Del. from family of Asteraceae come from West Africa. Several studies find some chemical constituents such as flavonoids, sesquiterpene lactones, fatty acids and steroidal saponins (Ohigashi, et al., 1991; Igile, et al., 1994; Sinise, et al., 2015; Igile, et al., 1995; Jisaka, et al., 1992; Quasie, et al., 2016; Erasto, et al., 2007) and indicated some of pharmacological activity such as anti-malaria, anti-inflammation, anti-tumor, anti-obesity, and other activities (Eyong, et al., 2011; Adedapo, et al., 2008; Egedigwe, et al., 2016; Atangwho, et al., 2012; Erasto, et al., 2008; Adaramoye, et al., 2008; Luo, et al., 2010; Sonibare, et al., 2009). The aim of this study was to evaluation of antioxidant and cytotoxic activities of n-hexane, ethylacetate and ethanol fraction of *Vernonia amygdalina* Del. leaves.

2 MATERIALS AND METHODS

2.1 Plant and Chemicals Material

Fresh leaves of *Vernonia amygdalina* Del. was collected from Faculty of Pharmacy, University of Sumatera Utara, Medan, Indonesia. *Vernonia amygdalina* Del. was identified in Herbarium Medanense, Department of Biology, Faculty Mathematic and Natural Sciences, University of

Hasibuan, P., Harahap, U., Sitorus, P. and Satria, D.
Evaluation of Antioxidant and Cytotoxic Activities of Vernonia amygdalina Del. Leaves.
DOI: 10.5220/0009844800002406
In *Proceedings of BROMO Conference (BROMO 2018) - Symposium on Natural Product and Biodiversity*, page 1
ISBN: 978-989-758-347-6

Sumatera Utara and the voucher specimen was deposited in herbarium (No. 1712/MEDA/2017). Chemicals used were distilled water, DMSO (Sigma), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma), [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT) (Sigma).

2.2 Preparation of Fractions

The air-dried and powdered leaves of *Vernonia amygdalina* Del. (500 g) were repeatedly fractionated by maceration method with n-hexane (3x3 d, 7.5 L). The powder was dried in the air and fractionated with ethylacetate and continued with ethanol 96% (3x3 d, 7.5 L) at room temperature with occasional stirring. The filtrate was collected, and then evaporated to give a viscous fraction and then freeze dried to dry (Satria, et al., 2015; Anggraeni, et al., 2015; Hasibuan, et al., 2015; Harahap, et al., 2018).

2.3 Free Radical Scavenging Activity Test

The free radical scavenging activity was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH•). 0.2mM solution of DPPH• in methanol was prepared and 100µl of this solution was added to various concentrations of fractions. After 60 minutes, absorbance was measured at 516 nm. Quercetin was used as the standard. All the tests were performed in triplicate and percentage of inhibition was calculated by comparing the absorbance values of the control and test samples (Rosidah, et al., 2018; Satria, et al., 2017; Dalimunthe, et al., 2018).

2.4 Cytotoxicity Assay

The cells were treated with n-hexane, ethylacetate, ethanol fractions and doxorubicin. 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 37ºC. 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 fractions and doxorubicin. After incubation 24 h, the cells were incubated with 0.5 mg/mL MTT for 4 h in 37ºC. 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 ELISA reader at λ 595 nm. The data which were absorbed from each well were converted to percentage of viable cells (Hasibuan, et al., 2016; Harahap, et al., 2018).

2.5 Statistical Analysis

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

3 RESULTS AND DISCUSSION

3.1 Antiradical Activity

Antiradical power of the plant samples was measured in term of hydrogen donating ability using DPPH which is a stable, nitrogen-centered free radical and produces deep purple colour in methanol solution. Antioxidants either transfer an electron or a hydrogen atom to DPPH, thus neutralizing its free radical character (Pan, et al., 2008). DPPH test, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action. The DPPH assay has been largely used as a quick, reliable and reproducible parameter to search the in vitro general antioxidant acitivity of pure compounds as well as plant extracts (Koleva,et al., 2002). The reducing capacity of compounds could serve as indicator of potential antioxidant property (Meir,et al.,1995). It is very important to point out that a low IC_{50} value reflects a high antioxidant activity of the fraction, since the concentration necessary to inhibit the radical oxidation in 50% is low. Antioxidant activity from n-hexane, ethylacetate, ethanol fractions and quercetine as positive control with DPPH assay measured as IC_{50} were 297.33 ± 0.46; 177.99 ± 0.32; 37.92 ± 1.03 and 2.32 ± 0.01 µg/mL respectively.

3.2 Inhibitory Concentration 50% (IC_{50})

MTT method was used to determine cell viability after incubation for 24 h. In every treatment fractions and doxorubicin was shown to inhibit cells growth. The IC_{50} from n-hexane, ethylacetate, ethanol fractions and doxorubicin as positive control were 327.89 ± 1.13; 64.92 ± 0.72; 1591.75 ± 37.05

and 1.82 ± 0.05 µg/mL respectively.The cytotoxicity estimate of natural product is related to content of active compound in these plants including *Vernonia amygdalina Del.* Flavonoids and triterpenoids/steroids estimated as active compounds (Yadav, et al., 2012; Igile, et al., 1994; Quassie, et al., 2016). Doxorubicin is one of chemotherapeutic agent showed strong activity on T47D cell lines with IC_{50} value of 1.82 ± 0.05 µg/mL. T47D cells line underwent resistant to doxorubicin pass through to p53 mutation (Di Leo, et al., 2007; Vassade, et al., 2005).

ACKNOWLEDGEMENTS

We gratefully thank to Directorate of Higher Education, Ministry of Research Technology and High Education, Indonesia through "Hibah Penelitian Dasar Unggulan Perguruan Tinggi" Research Grant 2018 for financial support in the study.

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