

Antioxidant Activity from Perepat Plant (*Sonneratia alba*) Ethanol Leaf Extract with Cap-e Methods to Overcome Oxidative Stress in Thalassemia

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Abstract

Thalassemia is a genetic disease with symptoms of anemia as a result of partial or complete disorder of the hemoglobin synthesis in red blood cells. Consumption of blood supplementation and routine blood transfusion to overcome the anemia symptoms in thalassemia patients can lead to excessive absorption of iron (Fe) content that can trigger the occurrence of oxidative stress. Therefore, antioxidant supplements are also important for thalassemia patients. Natural antioxidants derived from plants and fruits can be used to meet the needs of antioxidants in thalassemia patients. The diversity of mangrove plants has the potential to be explored and tested for its bioactivity, one of which is the herbaceous plant (*Sonneratia alba*), which has the potential as a source of antioxidant compounds. Preliminary test of antioxidant activity of ethanol extract has been done by using DPPH method. Furthermore, in-vitro testing with CAP-e (Cellular Antioxidant Protection in erythrocytes) method in normal blood, carrier of thalassemia and thalassemia patient. The results of the test using the ethanol extract from the leaf showed optimum antioxidant activity at a concentration of 300 ppm in normal blood and carrier properties, and 100 ppm in the blood of patients with thalassemia.

Keywords: Mangrove plants, antioxidant, Cap-e, thalassemia

INTRODUCTION

Genetic inherited hemoglobin disorders were originally found in tropical and subtropics area, but are now commonly found worldwide for migration. The World Health Organization (WHO) has identified hemoglobin disorders as a disease with high impact on public health systems in Western and developing countries. About 7% of the global population is the carrier of the disorder. Among the disorders of hemoglobin, thalassemia clearly contributes (Wheatherall, 2005; Modell and Darlison, 2008; de Franceschi *et al.*, 2011). Due to spontaneous mutations, hemoglobin gene variants are present at low prevalence in all large populations. They fall into two major groups - a structural variant that alters the amino acid sequence resulting in unusual hemoglobin, and thalassemia that decreases or eliminates the production of globin chains (Livingstone, 1985; Modell *et al.*, 2007).

Thalassemia is a genetic disease passed down by Mendel's law in an autosomal recessive manner from parents to his children. This disease is caused by a deficiency of the α and β globin chains that make up hemoglobin (Thein, 2013). Thalassemia is divided into thalassemia minor, intermedia and major. Major thalassemia is the worst type of thalassemia because it can cause severe anemia with ineffective hemolysis and erythropoiesis. Patients suffering from thalassemia major should undergo a monthly blood transfusion to maintain Hb about 9-12 g / dL and increase growth, reduce hepato-splenomegaly and bone deformation (Borgna-Pignati and Galanello, 2004). However, persistent transfusions can cause side effects caused by the accumulation of iron in the organs and cell damage. The deposited iron is responsible for the formation of reactive oxygen species (ROS) such as superoxide anion (O_2^-),

hydroxyl radical (OH \cdot), singlet oxygen and hydrogen peroxide (H_2O_2), which induces oxidative stress in thalassemia major patients (Mahdi, 2014). To handle the accumulation of iron, iron sailing agent is required that serves as an iron binder. but the use of deferoxamine in the long term can cause side effects such as impaired vision, hearing, cardiovascular, digestive, hematology, liver, and nerves (Milena *et al.*, 2010). Because of side effects in the use of iron chelating compounds in the treatment of thalassemia patients, it is necessary alternative therapy that reduces side effects by utilizing natural ingredients that have

antioxidant activity. Antioxidants are protective agents that can quell ROS activity and play an important role in the protection of cells from oxidative damage. Some antioxidant agents such as enzymes (glutathione peroxidase, superoxide dismutase, and catalase), large molecules (ferritin, albumin), small molecules (uric acid, glutathione, bilirubin, ascorbic acid, α -tocopherol, and vitamin E) and some important minerals such as zinc, copper and selenium. Enzymatic antioxidant activity, ie, catalase, glutathione peroxidase and glutathione reductase are found to be drastically reduced in untreated β -thalassemic patients when compared with normal individuals. However, superoxide dismutase activity was found to increase in untreated thalassemia patients when compared with normal individuals (Baumgartener and Van Way, 1999; Dhawan *et al.*, 2005; Simsek *et al.*, 2005).

Based on the literature review, found that vitamin C is reported to inhibit the peroxidation of phospholipid membrane due to oxidative stress in thalassemia patients (Rajagukguk *et al.*, 2014). This suggests that antioxidants are indispensable for treating thalassemia patients. Natural antioxidants derived from plants and fruits can be used to meet the needs of antioxidants in thalassemia patients. Many types of laboratory methods involve red blood cells to evaluate oxidative damage as a result of inflammation, and to evaluate antioxidant protection by natural products (Luqman and Rizvi, 2006). Diversity of plants in Indonesia, especially the diversity of mangrove plants has the potential to be explored its phytochemical content and tested its bioactivity (Herawaty *et al.*, 2011).

One of the plants collected from the mangrove area of Jambi Province, which is a prepat (*Sonneratia alba*) has also been tested for its antioxidant activity against DPPH, shows that ethanol extract provides the best inhibition compared to hexane and acetone extract (Latief *et al.*, 2015). Therefore, in this study was tested in vitro using CAP-e method to see the antioxidant activity of ethanol extract in normal red blood cells, red blood cells of thalassemia carrier and red blood cells of thalassemia patients.

MATERIALS AND METHODS

General: Tools used include: glassware commonly used in chemical laboratories, analytical scales, and rotary evaporator. Meanwhile the tools for CAP-e are vortex, centrifuge, Thermo syringe DVR-3422, Nalgene 0.22 μ M Syringe filter, pipette

(multichannel pipette) and microtips, 96F Nunclon Delta black Microwell and Tecan Spectra Fluorescence plate reader.

The solvent used for the extraction of the precipitate leaves is distilled ethanol. In vitro antioxidant testing materials (Cap-e method) are normal red blood cells, red blood cells carrying thalassemia (trait), and thalassemia α and β , saline phosphate buffer (PBS 1X), fetal bovine serum (FBS), ascorbic acid (pse Emsure Merck), ddH₂O, a set of DCFDA Abuse Cellular ROS Detection Assay kit consisting of 20 mM DCFDA solution (in DMSO), 10X buffer and 55 mM tert-butyl hydrogen peroxide (TBHP) solution.

Plant Materials. Samples of leaf from Perepat plant (*Sonneratia alba*) (Harborne, 1987) as much as 10 kg were collected from mangrove forest area of Kab. Tanjung Jabung Timur, Jambi Province. The leaf sample is cleaned, cut into small pieces, and dried to a constant weight and finely ground.

Extraction and partition. A total of 10 kg of powdered dried leaves of Perepat are macerated with n-hexane solvent, followed by fractionation using acetone solvent and ethanol, to obtain the ethanol fraction of Perepat leaf (*S. alba*) (Muhaimin *et al.*, 2016 and 2017).

Antioxidant activity test by Cap-e method. Test the antioxidant activity of the precursor extract using CAP-e method for normal red blood cells, blood carrier thalassemia and blood thalassemia

patients. Total of 3 mL of fresh blood was centrifuged three times at a rate of 2400 rpm in a row for 2.5; 2.5; 10 minutes to separate red blood cells with white blood cells and blood plasma. Furthermore, red blood cells were given treatment using the Abcam DCFDA Cellular ROS Detection Assay (Kang *et al.*, 2010). The order of addition and the number of reagents is adjusted according to the kit instructions. There are 7 variables in test, blanks, negative control, positive control, vitamin C, and 3 variations of concentration from ethanol extract (50 ppm, 100 ppm, and 300 ppm). Blood that has been given each treatment is transferred in dark microplate and stored in dark space at room temperature. After 1 hour then ready to measure the intensity of fluorescence with instrument of Tecan Spectra Fluorescence Plate Reader.

RESULTS AND DISCUSSION

In this study, the leaf part of mangrove plants namely perepat (*S. Alba*) as much as 10 kg has been fractionated. Selected ethanol fractionate for CAP-e test was based on previous research which showed that ethanol extract gave the best value of antioxidant activity compared to hexane and acetone extract (Latief *et al.*, 2015). CAP-e test in normal red blood cells, in this test used 4 samples of normal red blood cells that had previously been screened. Antioxidant activity of ethanol fraction of the leaf shows an optimum free radical inhibition in fractionation of ethanol with 300 ppm constipation.

Graph of free radical inhibition activity on normal red blood cells

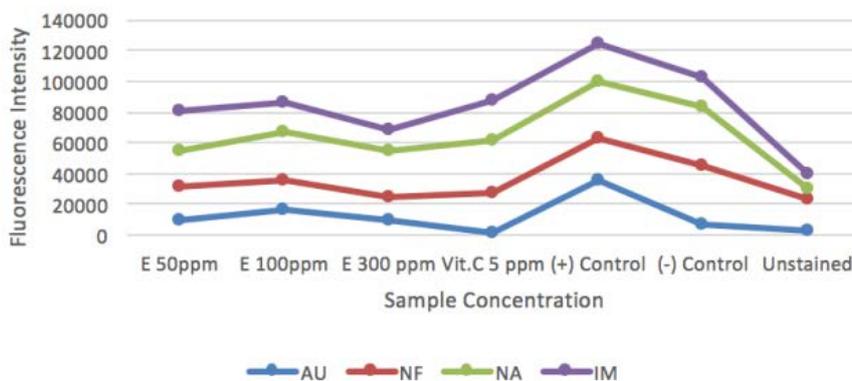


Figure 1. Graph of antioxidant activity in normal red blood cells.

Graph of Free Radical Inhibition Activity in Red Blood Cells of Carriers Thalassemia

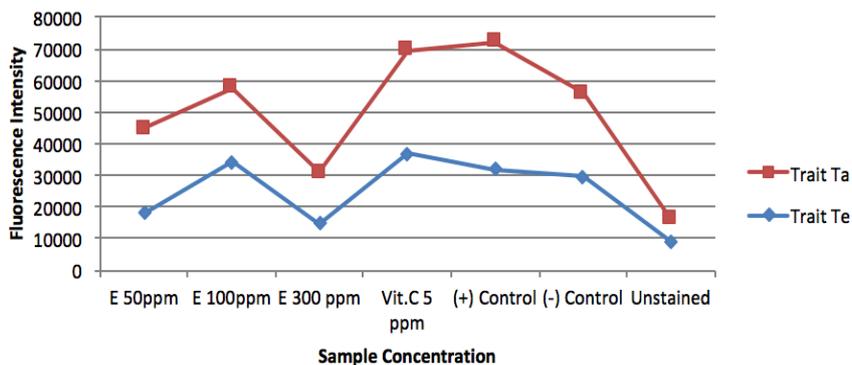


Figure 2. Graph of Antioxidants Activity in red blood cells from carriers thalassemia.

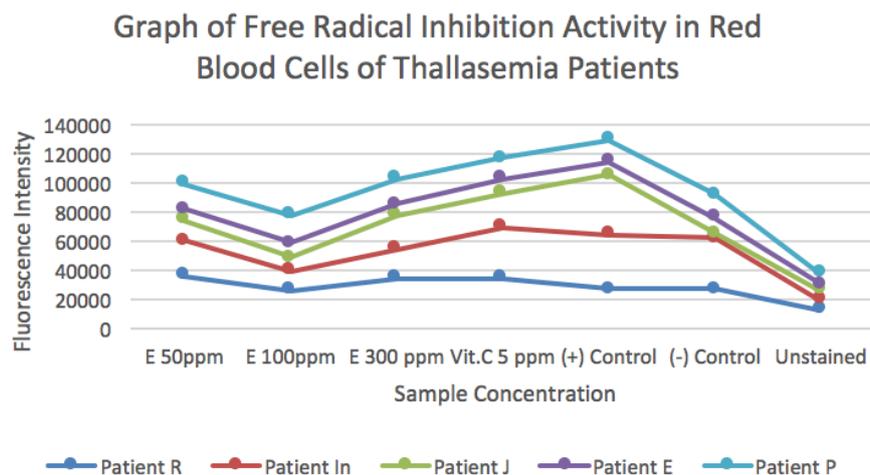


Figure 3. Graph of antioxidant activity in red blood cells of thalassemia patients.

In ethanol fractionate concentration of 300 ppm, able to reduce the activity of radical bebas, indicated by the low fluorescence value in the measurement. Low fluorescence values indicate at least free radicals that can bind to fluorescence compounds (DCFDA), because free radical activity has been suppressed by antioxidant compounds contained in the ethanol fraction of the leaf.

The CAP-e test on red blood cells of carrier thalassemia. Similarly, the antioxidant activity test in normal red blood cells, antioxidant activity test on red blood cell carrier of thalassemia character also showed the optimum result at fractionation of ethanol with concentration of 300 ppm.

In the carrier of thalassemia trait, the number of red blood cells is less than normal red blood cells. However, its free radical inhibition activity gives a relatively similar pattern.

CAP-e test on red blood cells of thalassemia patients. Antioxidant activity of ethanol fraction in red blood cell of thalassemia patient showed the optimum free radical inhibition at concentration 100 ppm.

In the graph, the ethanol fraction from perepat leaf with a concentration of 300 ppm indicates an increase in fluorescence value. This indicates that at high concentrations, ethanol fractionates act as prooksidan. Thus, free radical inhibition activity that binds to fluorescent compounds is also inhibited. Further research is needed on the optimum concentration of ethanol fraction addition in its activity as an antioxidant in red blood cells of thalassemia patients.

CONCLUSIONS

The results of antioxidant activity test using Cap-e method showed that ethanol extract from mangrove plant from Jambi Province was good, has potency as antioxidant to inhibit oxidative stress found in normal red blood cell, carrier of thalassemia and thalassemia patients. Need further study to know the active compounds that act as antioxidants in the Cap-e method.

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REFERENCES

- [1] Baumgartener, T.G., Van Way, C.W., Vitamins in Nutrition Secrets, Philadelphia, Hanley and Belfus, 1999.
- [2] Borgna-Pignatti, C., Galanello, R., *Wintrobe's Clinical Hematology 1999*, 11, 1319-1365.
- [3] De Franceschi, L., Cappellini, M.D., Olivieri, O., *Seminars in Thrombosis and Hemostasis 2011*, 37, 226-236.
- [4] Dhawan, V., Kumar, Kh. R., Marwaha, R.K., Naravan, S., Kamgar, M., *Ind. Pedi. 2005*, 42, 1141-1145.
- [5] Harborne, J.B., *Phytochemical Methods Guides Modern Way of Analyzing Plants*. Institut Teknologi Bandung, 1987.
- [6] Herawati, N., Jalaluddin, N., La Daha, Zenta, F., *Majalah Farmasi dan Farmakologi 2011*, 15(1), 23-25.
- [7] Kang J., Li, Z., Wu, T., Jensen, G.S., Schauss, A.G., *Food Chemistry 2010*, 122, 3.
- [8] Latief, M., Nazaruddin and Nelson, *Prosiding SEMIRATA 2015 bidang MIPA BKS-PTN Barat Universitas Tanjungpura 2015*, 112 – 117.
- [9] Livingstone, F.B., *Frequencies of hemoglobin variants*. New York and Oxford: Oxford University Press, 1985.
- [10] Luqman, S., Rizvi, S.I., *Phytother Res 2006*, 20(4), 303-6.
- [11] Mahdi, E.A., *Acta Chim. Pharm. Indica 2014*, 4(3), 137-145.
- [12] Milena, R., Branka, Z., Biljana, S., Belton, N., Canat, B., *Thalassaemia Syndrome in Serbia, Hemoglobin 2010*, 34(5), 477-485.
- [13] Modell, B., Darlison, M., Birgens, H., Cario, H., Faustino, P., Giordano, P.C., *Scand J Clin Lab Invest 2007*, 67,39-69.
- [14] Modell, B., *Bulletin of the World Health Organization 2008*, 86(6), 480-487.
- [15] Simsek, F., Ozturk, G., Kemahl, S., *Oxidant and Antioxidant Status in Beta Thalassemia Major Patients 2008*, 58, 34-38.
- [16] Thein, S.L., *Molecular basis of β -thalassemia*. Cold Spring Harb Perspect Med, 2013.
- [17] Weatherall, D. J., *Annals of Human Biology 2005*, 32(2), 117-122.
- [18] Rajaguguk, R., Kosim, S., Tamam, M., *Medica Hospitalia 2014*, 2(2): 98-100.
- [19] Muhaimin, Syamsurizal, Chaerunisaa, A.Y., Sinaga, M.S., *International Journal of ChemTech Research 2016*, 9(5), 418-424.
- [20] Muhaimin, Syamsurizal, Latief, M., Anggraini, S., Mujahidin, D., *Research Journal of Pharmaceutical, Biological and Chemical Sciences 2017*, 8(1S), 141-148.