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Antioxidant and Cytotoxic Activities of Plectranthus *amboinicus* (Lour.) Spreng. Extracts

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Research Article

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Abstract

Objectives: to evaluate the antioxidant and cytotoxic activities of *Plectranthus amboinicus*, (Lour.) Spreng.
Methods: The leaves of *Plectranthus amboinicus*, (Lour.) Spreng. were extracted using *n*-hexane, aetylacetate and ethanol by maceration method. The antioxidant activity was tested using DPPH and Beta Carotene-Linoleic Acid methods, and compared to those of BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene) and quercetin. Cytotoxic assay was performed on MCF7 breast cancer cell line, using MTT [3-(4,5-dimetiltiazol-2-il)-2,5 difeniltetrazolium bromide) assay.
Results: The aetylacetate and ethanolic extracts of *Plectranthus amboinicus*, (Lour.) Spreng. leaves were found to exhibit antioxidant activity with IC_{50} value 350.74 µg/mL and 281.26 µg/mL by DPPH method. The ethanolic and aetylacetate extracts showed a high antioxidant activity by ß-Carotene-Linoleic Acid Method. *N*-hexane and aetylacetate extracts showed potent cytotoxic effect on MCF7 with IC_{50} 63.644 µg/mL, 7.647 µg/mL respectively.
Conclusion: All extracts exhibited different potency in antioxidant activity which depending on extracting solvent used and secondary metabolites occur in each extract. The *n*-hexane and aetylacetate extracts exhibited strong cytotoxic effect that suggest to further study.

Key words: Antioxidant, *Plectranthus amboinicus*, (Lour.) Spreng., DPPH method, Beta Caroten-Linoleic Acid Method,Cytotoxic

INTRODUCTION

Effective anticancer drugs with selectivity againts only malignant cells are desired. Some plants have potential anticancer. As candidate for anticancer drug, it is necessary to evaluate its antioxidant and cytotoxicity activities. Free radical is a reactive species having one or more unpaired electrons, and attempt to get electron from another molecule or release the unpaired electron [1-2]. The human body has endogen antioxidant, but if there are too many free radicals exist around then the exogen antioxidant will be needed. Antioxidant will give one or more electrons to the free radical so that it can no longer cause damage [3]. The antioxidants in general are compounds that can delay or inhibit oxidation process [4].

Plectranthus amboinicus (Lour) Spreng., (*Coleus amboinicus*, Lour., *Coleus aromaticus,* Benth.), is one of plant used as lactagogue by native people in Indonesia. This plant has also been traditionally used for the treatment of inflammation [5], heart disease [6], as diuretic [7], immunomodulator [8] and hepatoprotector [9]. The pharmacognostical study of (*Plectranthus amboinicus* (Lour) Spreng., found that it contains flavonoid, terpenoid, saponin, steroid, tannin, protein, carbohydrate and volatile oil [10]. The study conducted on the antioxidant activity and total phenolic content of *Coleus forksholii* Briq., *Coleus aromaticus* Benth., dan *Coleus zeylanicus* Benth showed that *Coleus forksholii* has highest polyphenol content and antioxidant acitivity among others [11]. Phytomedicines derived from plants have shown great promise in the treatment of cancer disease. The aim of this study is to evaluate the antioxidant activity of *Plectranthus amboinicus* (Lour) Spreng. leaves extracts prepared with different solvents by using 2 methods i.e. DPPH (*1,1- diphenyl-2-picrylhydrazyl*) and ß-Carotene-Linoleic Acid Methods. The cytotoxic effect of extracts on MCF7 cell line was also evaluated.

MATERIAL & METHODS

Chemicals and reagents

Butylated hydroxianisole (BHA), butylated hydroxytoluene (BHT), ß-Carotene, linoleic acid, *1,1-*

diphenyl-2-picrylhydrazyl, quercetin, were purchased from Sigma (St. Louis MO, USA). *n*-hexane, aetylacetate and ethanol were purchased from Merck (Darmstadt, Germany).

Plant and preparation of Extracts

The *Plectranthus amboinicus* was obtained from Pematang Siantar, Sumatera Utara, Indonesia. The leaves of *Plectranthus amboinicus* were dried at 45^{0}C and ground into powder. The dried leaves powder (500 g) extracted with *n*-hexane by maceration method. After three days of maceration at room temperature, the supernatant separated by decantation and the marc was remacerated twice. the marc of *n*-hexane extract was extracted with etylacetate by maceration. The same procedure was applied to ethanolic extract. Extract from each solvent were concentrated by a rotary evaporator (Heidolph VV-200) and the concentrated extract was dried by freeze-dryer (Edwards).

Determination of DPPH scavenging activity

The free radical scavenging activity of *Plectranthus amboinicus* extract, and BHT were measured in terms of hydogen donating or radical scavenging ability using the stable DPPH (Rosidah, 2008). Each of 7.5 ml; 8.75; 10 ml; 11.25 ml from the extract of *Plectranthus amboinicus* and each of 0.25 ml; 0.5 ml; 0.75 ml from BHT (in methanol) were placed in different test tubes. To this mixture, 5 ml 0.5 mM DPPH was added. After 30 minutes of incubation at room temperature (22-24^{0}C), absorbance was measured at 517 nm by using spectrophotometer (Shimadzu 1800) using methanol as the blank. A control contained 1 ml methanol and 5 ml 0,5 mM DPPH. Free radical scavenging activity of the extracts were calculated according to the following formula:

Free radical scavenging activity (%) = $(A_c - A_s)/Ac \times 100$

Where A_s is the absorbance of DPPH and sample, and A_c is the absorbance of control.

Determination of antioxidant by using the ß-carotene-linoleic acid method

One milliliter of ß-carotene (1 mg in 1 ml chloroform) was added to a conical flask with 20 mg linoleic acid and 200 mg polyoxyethylene sorbitan monopalmitate (Tween-40). Chloroform was removed under vacuum at 40^{0}C (using a rotary evaporator), and the resultant mixture was diluted with 10 ml of water, mixed well, and followed by addition of oxygenated water (40 ml) to form a solution. The aliquots (4 ml) were pipetted into different test tubes containing 0.2 ml of extracts, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and quercetin (0.2 mg/ml, in ethanol), respectively, and the absorbance was measured with a spectrophotometer (Shimadzu 1800) at 470 nm immediately and to 120 min (15 minutes intervals), againts a blank solution without the ß-carotene. All determinations were carried out in triplicate. The antioxidant activity (AA) was calculated according to the following formula

$$AA = 100\left[1 - \frac{(A_0 - A_t)}{(A_0^0 - A_t^0)}\right]$$

Where A_0 and A_0^0 are the absorbance value measured at zero time of the incubation for test sample and control, respectively, and A_t and A_t^0 are the absorbance value measured after incubation for test sample and control, respectively. The results were expressed in percentage.

Cell line and culture medium

Human breast adenocarcinoma cell line MCF7 was obtained from Faculty of Medicine, Universitas Gadjah Mada, Indonesia. MCF7 cells were cultured in RPMI medium, suplemented with 10% (v/v) fetal bovine serum (FBS), 2% penicillin-streptomycin and 0.5% fungizone in a 37^{0}C incubator with 5% CO_2.

Cytotoxicity assay

Cytotoxicity was determined by the MTT assay. Briefly, MCF7 cells were plated at 10^4 cells/well in a 96-well plate. After incubation for 24 h at 37^{0}C, cells were treated by 3 extracts *(n*-hexane extract, etylacetate extract, ethanol extract) with different concentration and incubated for 24 h. MTT solution was added to each well and further incubated for 4 h at 37^{0}C, optical density was read with an ELISA reader at 595 nm.

RESULTS & DISCUSSION

Free radical scavenging activity

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [12]. The reduction in the amount of DPPH radical was determined by the decrease of its absorbance at 517 nm. The colour of the DPPH reagent changed from purple to yellow [13]. Figure 1 shows the DPPH radical scavenging effects of the extracts of *Plectranthus amboinicus* and standard compound increased in the order BHT>Ethanolic Extract>Aetylacetate Extract, but *n*-Hexane extract exhibited no antioxidant activity. At 400 and 450 µg/mL, aetylacetate and ethanol extracts showed similar scavenging activity to BHT at 10 µg/mL. The percentage inhibition of free radical by *Plectranthus amboinicus* etylacetate and ethanolic extracts due to hydrogen donation from the antioxidant. The colour of the DPPH reagent was significantly reduced form purple to yellow. It showed no dose-response relationship in DPPH radical scavenging activity wich suggest that there seem to be a synergism occurs among the several antioxidants compounds present in the mixture that make up the total antioxidant activity. The scavenging property of *Plectranthus amboinicus* may be due to hydroxyl groups existing in the chemical structure of the phenolic compound that can provide the necessary component as a radical scavenger and antioxidant. The antioxidant activity not only dependent on the concentration of antioxidant, but also on the structure and interaction among the antioxidants [13]. The antioxidant activity of putative antioxidants have been attributed to various mechanisms, namely, prevention of chain initiation, binding of transition

metal ion catalyst, decomposition of peroxides, prevention of hydrogen abstraction, and radical scavenging [14].

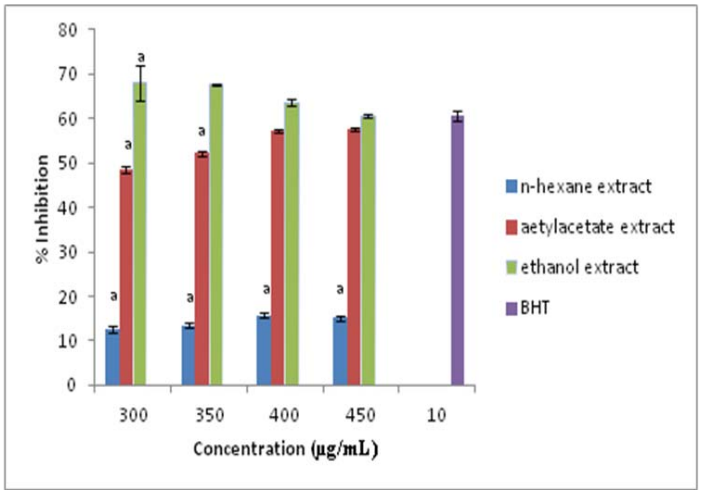

Figure1. Antioxidant activity of *Plectranthus amboinicus* extracts at different concentration analyzed by DPPH method. Each value represents a mean ± SD (n=3). a= p<0,05 with BHT

ß-carotene-linoleic acid antioxidant capacity

The antioxidant activity of *Plectranthus amboinicus* extracts, BHA, BHT, and quercetin, as measured by the bleaching of ß-carotene, is presented in Figure 2. It can be seen that *Plectranthus amboinicus* extracts exhibited varying degrees of antioxidant activity. The mechanism of bleaching of ß-carotene is a free radical-mediated phenomenon resulting from the hydroperoxides formed from linoleic acid. ß-carotene, in this model system, undergoes rapid discoloration in the absence of antioxidant. The linoleic acid free radical, formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups, attacks the highly unsaturated ß-carotene molecule. As ß-carotene molecules lose their double bonds by oxidation, the compound loses its chromophore (orange color). The antioxidant of the extracts of *Plectranthus amboinicus* and standard increased in the order BHA > BHT > quercetin> ethanolic extract > aetylacetate extract > *n*-hexane extract. The aetylacetate extract of *Plectranthus amboinicus*, at 400 µg/mL showed the highest antioxidant activity. The *n*-hexane extract also had antioxidant activity. The ethanol and aetylacetate extracts of *Plectranthus amboinicus* had the same antioxidant activity, it was supposed that there was quercetin content in those extracts. The antioxidant activity of *Plectranthus amboinicus* was affected by the extraction solvent and the analysis method. With the ß-carotene bleaching method, peroxyl radical scavenging and metal inactivation were major antioxidation factors. The polarity of the compound and the physical state of the lipid system also affected the behaviour of antioxidants [13].

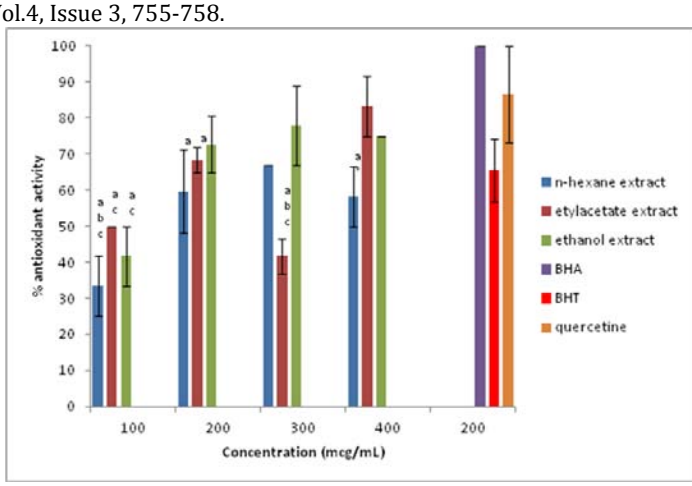

Figure2. The effect of concentration of *Plectranthus amboinicus* extracts on antioxidant activity analyzed by ß-carotene-linoleic acid method. Each value represents a mean ± SD (n=3). a= p<0,05 with BHA, b = p<0,05 with BHT, c = p<0,05 with quersetin

Cytotoxic assay

Cells were exposed to various concentration of Plectranthus amboinicus extracts (31.25 – 500 µg/mL) for 24 h. Cells treated with DMSO were used as control. In this MTT assay, decreased absorbance at 595 nm correlates with decreased viability of cells in culture. The result showed that aetylacetate extract had highest cytotoxic activity than the others. The cytotoxic activity potency were evaluated by IC_{50} value. The calculation of IC_{50} used probit analysis found that the IC_{50} value of *n*-hexane, etylacetate, and ethanol extract consecutively 63.644 µg/mL, 7.647 µg/mL, and 1382.806 µg/mL. The decreased of IC_{50} value correlated with increased of cytotoxicity. The potential cytotoxic activity of the extract is less than 100 µg/mL [15]. While the *n*-hexane and aetylacetate extracts had cytotoxic effect, the ethanol extract showed different result. Although the ethanol extract had the highest antioxidant activity, but it did not had the cytotoxic effect. It is possible that its cytotoxic compound is weakly soluble in ethanol.

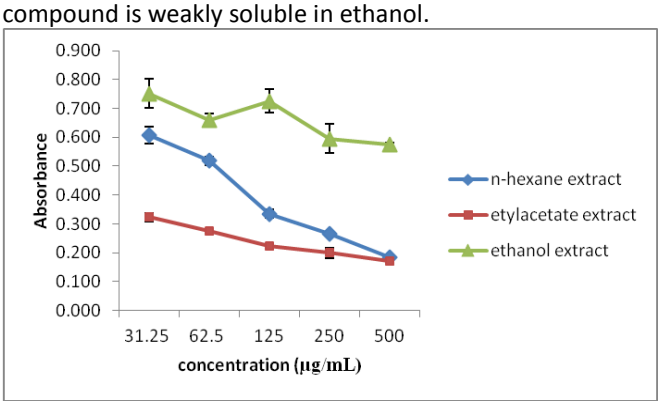

Figure3. Cytotoxic effect of *Plectranthus amboinicus* extracts on Human MCF7 Cells Line

CONCLUSION

In conclusion, the extracts of *Plectranthus amboinicus* in this research exhibited different extents of antioxidant activity. The result of the current study showed that the aetylacetate extract, which contain the highest amount of phenolic compounds, exhibited the greatest antioxidant activity by ß-carotene method. Whereas, the ethanol extract exhibited the greatest antioxidant activity by DPPH method. Different methods used to measure antioxidant activity with various mechanisms may lead to different observation. The extracting solvent significantly affected the yield, antioxidant activity of *Plectranthus amboinicus* extracts. Etylacetate extract of *Plectranthus amboinicus* is promising source as natural antioxidant and anticancer. *N*-hexane extract has cytotoxic effect but it does not have antioxidant activity by DPPH method. Ethanol extract of *Plectranthus amboinicus* has high antioxidant activity by DPPH and ß-carotene-linoleic acid methods, but it does not have cytotoxic effect. The *n*-hexane and aetylacetate extracts exhibited strong cytotoxic effect that suggest to further study.

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References

1. Praptiwi, Dewi, P., Harapini, M. (2006). Peroxide Level and Free Radical Scavenger Activity of DPPH of *Knema Laurina* Methanol Extract. *Majalah Farmasi Indonesia*. 17(1): 32-36
2. Silalahi, J. (2006). *Functional Diets*. Yogyakarta: Kanisius Press.
3. Kaur, G., Jabbar, Z., Athar, M., Alam. M.S. (2006). *Punica granatum* (pomegranate) Flower Extract Possesses Potent Antioxidant Activity and Abrogates FE-NTA Induced Hepatotoxicity in Mice. *Food and Chemical Toxicology*. 444: 984-993
4. Diplock, A., T. (1994). Antioxidants and Free Radical Scavengers. In: Rice-Evans and Burdon editor. *Free Radical Damage and Its Control*. Volume 28. Amsterdam: Elsevier. 113-127.
5. Chang, J., Cheng, C., Hung, L., Chung, Y., dan Wu, R. (2007). Potential Use of *Plectranthus amboinicus* in The Treatment of Rheumatoid Arthritis. *eCAM*. 7(1): 115-120.
6. Hole, R.C., Juvekar, A.R., Roja, G., Eapen Susan, Souza, S.F. (2009). Positive Inotropic Effect of The Leaf Extracts of Parent and Tissue Culture Plants of *Coleus amboinicus* on an Isolated Perfused Frog Heart Preparation. *Food Chemistry*. 114(1): 139-141
7. Patel, R., Mahobia, N.K., Gendle, R., Kaushik, B., Singh, S.K. (2010). Diuretic Activity of Leaves of *Plectranthus amboinicus* (Lour) Spreng in Male Albino Rats. *Pharmacognosy Research*. 2(2): 86-88.
8. Santosa, C.M., Hertiani, T. (2005). Chemical Constituents and Effect of Coleus amboinicus, L. Aqueous Extract on Phagocytocyis Activity of Rat's Neutrofil (*Rattus norvegicus*). *Indonesian Journal of Pharmacy*. 16(3):141-148
9. atel, R. (2011). Hepatoprotective Effects of *Plectranthus amboinicus* (Lour.) Spreng Againts Carbon Tetrachloride-Induced Hepatotoxicity. *Journal of Natural Pharmaceuticals*. 2(1): 28-35
10. Kaliappan, N.D., dan Viswanathan, P.K. (2008). Pharmacognostical Studies on The Leaves of *Plectranthus amboinicus* (Lour) Spreng. *International Journal of Green Pharmacy*. 182-184
11. Rasineni, G. K., Siddavattam, D., Reddy, A.R. (2008). Free Radical Quenching Activity and Polyphenols in Three Species of Coleus. *Journal of Medicinal Plants Research*. 2(10): 285-291
12. Molyneux, P. (2004). The Use of The Stable Free Radical Diphenylpicryl-hydrazil (DPPH) for Estimating Antioxidant Activity. *Songklanakharin J.Sci.Technol*. 26(2): 211-219
13. Sun, T., Ho, C. (2005). Antioxidant Activities of Bucwheat Extracts. *Food Chemistry*. 90(2005): 743-749
14. Rosidah, Yam, M.F., Sadikun, A., Asmawi, M.Z. (2008). Antioxidant Potential of Gynura Procumbens. *Pharmaceutical Biology*. 46(9): 616-625
15. Kamuhabwa, A., Nshimo, C, and de Witte, P. (2000). Cytotoxic of Some Medicinal Plant Extracts Used in Tanzanian Traditional Medicine. *J. Ethnopharmacol*. 70(4): 143-149

AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.