Antioxidant Activity and Thin Layer Chromatography Profile of Achantus Ilicifolius L. Skin Barks Methanolic Extract, Water Fraction, Ethyl Acetat Fraction

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ABSTRACT

Acanthus ilicifolius L (local name jeruju) is widely found in mangrove forest like in Segara Anakan mangrove forest which is located in Cilacap, Central Java Indonesia. The aim of the research were to know the antioxidant activity of methanol extract (ME), water fraction (WF) and ethyl acetat fraction (EAF) of *A. ilicifolius* skin barks, to find the Thin Layer Chromatography profile of ME, WF and EAF. Antioxidant activity were determined by DPPH method for ME in serial concentration of 5, 4, 3, 2 and 1 ppm. Antioxidant activity of WF and EAF were measured in serial dilution of 1, 2, 4, 8, and 16 times. The result showed that IC50 of ME was 5.40 ppm which 6 times lesser than IC50 of positive control, buthylatedhydroxytoluene (BHT) was 1.02 ppm and IC50 of d-α-tokoferol was 0.93 ppm. The IC50 of WF was 25.444 % while EAF was 41.660 %. The antioxidant capacity of water fraction was higher than ethyl acetate fraction. In conclusion, ME, WF and EAF of *A. ilicifolius* exhibited an activity as scavenger of free radicals from DPPH. Thin layer chromatography method was used to identify the compound of ME, WF and EAF. Methanol extract of jeruju skin barks contained flavonoid and saponin. The WF only contained a flavonoid while EAF contained flavonoid, tannin and alkaloid.

Keywords: *Acanthus ilicifolius* L. skin bark, antioxidant, Thin Layer Chromatography

1 INTRODUCTION

Acanthus ilicifolius is a plant that widely found as weeds in Segara Anakan mangrove forest Cilacap, Central Java, Indonesia.

There were many report of biological activity of *A.ilicifolius*. Leaves of *Acanthus ilicifolius* are used as ethnomedicine for treatment of rheumatism, snakebite, paralysis and asthma in various places in the world.

2 MATERIAL AND METHODS

2.1 Plant materials and preparation of methanolic extract and fractions.

The plants were collected from Tritih area, Cilacap Central Java Indonesia on December 2009 and identified in Faculty of Biology, Jenderal Soedirman University, Purwokerto, Indonesia. The voucher specimen was kept in Laboratory of Pharmaceutical Biology, Muhammadiyah University of Purwokerto, Indonesia.

Skin barks of *A.ilicifolius* were shade dried for 7 days and pulverized using mechanical grinder. Thirty grams of powder were extracted by soxhlet 19 times circulations with total volume of 150ml methanol. After exhaustive extraction, methanolic extract was collected and concentrated under reduced pressure at 40°C. It was yielded 36.09% of concentrated viscous extract. Six grams of methanolic extract (ME) were washed with 100ml hot water several times and its water filtrate was extracted with ethyl acetate (90ml) using liquid-liquid extraction which were resulted two fractions and designated as water fraction (WF) and ethyl acetate fraction (EAF). ME, WF and EAF, then were used for antioxidant activity evaluation.

2.2 Identification by using Thin Layer Chromatography Method.

2.3 Antioxidant activity evaluation.

ME with concentration 5; 4; 3; 2; and 1ppm, were evaluated its antioxidant activity using DPPH (diphenylpicrylhidrazyl) method, as well as WF and EAF in serial dilution of 1; 2; 4; 8; and 16 times. Ability to donate electron from ME, WF and EAF were measured by its changing from purple color to yellow color of DPPH solution in methanol using UV-Vis Spectrophotometer.

Fifty microliter of ME, WF and EAF were diluted on methanol and was added 0.004% DPPH solution in methanol until 5 ml. After incubated at room temperature

for 30 minutes, absorbance was measured at 516.5nm using a blank solution. Free radical inhibition of DPPH in percent (%) was calculated using this formula:

I(%)=(Absblank–Abssample/Absblank)x100

3 RESULTS AND DISCUSSION

Based on the TLC chromatogram, ME contained flavonoid and saponin,WF only contained flavonoid, while EAF contained flavonoid, tannin and alkaloid.

Antioxidant activity of ME, BHT and Vitamin E were resumed in Table 1., whereas antioxidant activity of WF and EAF were shown in Table 2. It showed that there were positive correlation between concentration of samples and percentage of inhibition. IC50 value of ME was 5.40 ppm which was 6 times higher than Vitamin E (IC50 0.93ppm) and 5 times higher than BHT (IC50 1.02ppm). Meaning that activity to scavenge free radicals from DPPH of ME was six and five times weaker than Vitamin E and BHT, respectively. However, IC50 of ME exhibited greater value if it was compared with the IC50 of methanolic extract of *A. ilicifolius* leaves that was reported by Kumar and colleagues in 2008 (IC50 8.4g/ml $8.4x10^6$µg/ml). WF and EAF of ME of *A.ilicifolius* in this study showed quite similar activity which WF had 1.6 times stronger antioxidant activity than EAF.

Table 1. IC_{50} Value of ME, Vit E and BHT

Replicate	IC_{50} (ppm)		
	ME	**BHT**	**Vit E**
1	5.78	1.01	0.90
2	5.32	1.23	0.93
3	5.11	0.82	0.93
average	5.40	1.02	0.92

Table 2. IC_{50} Value of WF and EAF

Replicate	IC_{50} (ppm)	
	EAF	**WF**
1	0.40	0.24
2	0.43	0.27
3	0.41	0.25
average	0.42	0.25

ME contains various compounds from skin barks of *A. ilicifolius* parts that might have synergistic effect to scavenge free radicals from DPPH.

Stronger activity of WF than EAF could be explained by the presence of flavon glycosides that mainly found in skin barks of *A. ilicifolius*. These compounds had been more eluted in water rather than in ethyl acetate when it was extracted by liquid-liquid extraction.

CONCLUSION

Methanolic extract of skin barks of *Acanthus ilicifolius* and its water and ethyl acetate fraction exhibited activity to scavenge free radicals from DPPH. It needs further confirmation of compounds that is containing in methanolic extract as well as in water and ethyl acetate fraction.

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