Antioxidant Activity from *Lygodium microphyllum* Aerial Parts

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Abstract: *Lygodium microphyllum* is an invasive plant known as another name "Old World Climbing Fern" or considered a weed plant. Research on *L. microphyllum* related pharmacological activity is limited. This study aims to evaluate antioxidant activity from Methanol extract and Fractionation using n-hexane, Ethyl acetate and water solvent with a different polarity from *L. microphyllum* plant. Methods: Aerial part of L. *microphyllum* was macerated with methanol solvent, the extract obtained in fractionation using solvents with different polarity levels and was tested in invitro free radical using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The IC50 result was 49.328 ± 0.242 ppm for methanol extract, n-hexane extract was 32.041 ± 0.770 ppm, and ethyl acetate extract 8,732 ± 0.204 ppm and water extract 93.330 ± 1.931 ppm. The conclusion from this study reveals the results of extraction using ethyl acetate solvent showed the highest value antioxidant activity against DPPH.

1 INTRODUCTION

Lygodium microphyllum is an invasive plant known as "Old World Climbing Fern" or is considered a weed plant. *L. microphyllum* plants develop and growing fast in large colonies that dominate an area and cause disruption of the ecosystem the original flora and degrades critical ecosystem services and habitats of rare and endangered species (Ferriter 2001; Langeland & Hutchinson, 2001; Pemberton, 1998).

L. microphyllum spread to the nearby plant and live in an open place to get sunshine (Bower, 2010). Some studies showed *L. microphyllum* have medicinal properties including antibacterial (Wang et al 2014), Hepatoprotector (Gnanaraj et al, 2017), Cytotoxic activity against P-388 Murine Leukemia cells (Kuncoro, et al 2017), and antiplasmodial against *Plasmodium falciparum* (Ratri et al, 2018). The chemical composition of *L. microphyllum* has been reported including steroid and glycoside flavonoids (Kuncoro, et al 2017).

Reports of the antioxidant activity from other species Lygodium in one genus have been reported. Methanol extract from *L. flexuosum* has antioxidant activity (Jeetendra & Manish, 2011).

The aim of this studies to evaluated Antioxidant activities aerial part from *L. microphyllum* against DPPH.

2 MATERIAL AND METHOD

2.1 Material

L. microphyllum aerial part was obtained from Lempake Village, Samarinda, Kalimantan Timur. Specimens were identified and deposit by staff at the Dendrology laboratory, Foresty Faculty, Mulawarman University.

2.2 Extraction Process

Aerial parts of *L. microphyllum* are cleaned and dried and powdered to obtain 3.52 Kg. The dry powder was macerated with methanol solvent at room temperature. The methanol extract was obtained and concentrated 526 g with a Rotary Evaporator (Buchi®). The methanol extract obtained was dissolved in water (1:1) and partitioned successively with n-hexane solvent, ethyl acetate solvent, and Aqua solvent. The resulting extract with solvent was evaporated and obtained n-hexane extract (59 g), ethyl acetate extract (72 g), and aqua extract (296,5 g).

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2.3 Antioxidant Assay

2.3.1 Preparation

The DPPH crystals were weighed 4 mg to be dissolved in 100 mL of methanol in the measuring flask for obtaining a DPPH solution with a concentration of 0.004% or 40 ppm (part per million) used in the test. The solution is stored in a tightly sealed place and protected from light.

2.3.2 Concentration Series Determination

The concentration series used was 0.2,4,6,8,10 ppm.. These concentration variations were used in antioxidant activity testing by DPPH method. Preparation of stock solution with a concentration of 1000 ppm (10 mg dry extract diluted with methanol to 10 mL). Furthermore, dilution to obtain the concentration and Duplo repetition or two repetitions and a negative control that DPPH solution and methanol (without the addition of extract).

2.3.3. Determination of the Maximum Absorption Wavelength DPPH

2 mL of 0.004% DPPH solution was added with 2 mL of methanol. After being left for 30 minutes in the dark, Absorption of the solution was measured by UV-Vis spectrophotometer at 515 -520 nm wavelength to obtain the maximum wavelength.

2.3.4 Antioxidant Assay

The antioxidant test was carried out through a series of sample solutions from the methanol extract and the three fractions with 2 repetitions using a methanol solvent. Each solution plus 2 mL of DPPH solution, in order to obtain a solution of a predetermined concentration, was allowed to stand for 30 minutes (calculated after addition of DPPH solution), measured its absorbance at the maximum wavelength. The absorbance data obtained is used to determine % inhibition (damping). Through sample concentration curve versus % inhibition, IC_{50} extract value can be obtained with statistical analysis using linear regression. There is also a measure of absorbance of blanks (methanol). The antioxidant test indicator is the color change of DPPH. The data in this study is the percentage of DPPH radical reduction obtained using the formula:

$$\% \text{ AA} = \frac{Abs.Control - Abs.S}{Abs.Control} \times 100\%$$

Data analysis using linear regression equation using the formula:

$$y = b\,(x) + a$$

Explanation:
y: % AA
x: log concentration
a: intercept
b: slope

3 RESULT AND DISCUSSION

In this study conducted testing of antioxidant activity of aerial parts of *L. microphyllum*. Dry powder aerial parts of *L. microphyllum* macerated using methanol to obtain methanol extract. Methanol extract in fractionation based on polarity level using n-hexane, ethyl acetate, methanol and water. Each extract obtained was tested for its antioxidant activity using DPPH method. This method was chosen because is simple, easy, fast and sensitive and requires only a little extract. This method is often used to detect the anti-radical ability of a compound because the result proves to be accurate, reliable, relatively fast and practical. The principle of this method is the measurement of synthetic free radical capture in polar organic solvents such as ethanol or methanol at room temperature by a compound having antioxidant activity. This process of radical capture through the mechanism of taking hydrogen atoms from antioxidant compounds by free radicals so that free radicals capture an electron from antioxidants. With the arrest of these radicals, the diazo double bond in DPPH decreases, resulting in decreased absorbance. The free radical used is DPPH (2,2-diphenyl-1-picrylhydrazyl).

DPPH reacts with antioxidant compounds by taking hydrogen atoms from antioxidant compounds to obtain electron pairs. The compounds that can potentially as antioxidants are from the class of flavonoids, alkaloids, phenols, and tannins. Before the testing of antioxidant activity, the first determination of the maximum wavelength of DPPH used with the wavelength range 515-520 nm. The maximum wavelength is obtained from the maximum absorbance of 515 nm. This wavelength will be used in subsequent antioxidant testing. The compound that reacts as a radical catcher will reduce DPPH which can be observed by the color change of DPPH to yellow from purple when the odd electron from the DPPH radical has paired with hydrogen. From the free radical captured compound which will

form the reduced DPPH-H (Molyneux, 2004) seen in Figure 1.

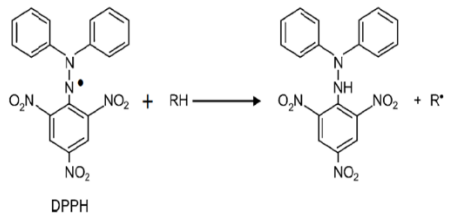

Figure 1: DPPH Radical Reaction with Antioxidant Compound (Molyneux, 2004)

All electrons in DPPH free radicals become in pairs, then the color of the solution changes to bright yellow from dark purple and absorbance at 515 nm wavelength will be lost. These changes can be measured stoichiometrically according to the number of electrons or hydrogen atoms captured by DPPH molecules due to the presence of antioxidants (Gurav, 2007). The calculation used in the determination of radical catch activity is the value of IC_{50} (Inhibition Concentration 50%). This value describes the concentration of test compounds that can capture radicals by 50%. IC_{50} values in this study were obtained by using a linear regression equation which expresses the relationship between sample concentrations (extract test) with the symbol (x) with radical capture activity (y) from series replication measurement. The smaller the value of IC_{50} obtained, the test sample has more effectiveness as a better radical catcher. Molyneux (2004) states that a substance has antioxidant properties if the IC_{50} value is less than 200 ppm. If the value of IC_{50} obtained ranges from 200-1000 ppm, then the material is less active but still potentially as an antioxidant substance.

From the test result on methanol extract, n-hexane, ethyl acetate, and water obtained absorbance value and% inhibition.

Table 1: Absorbance and % inhibition of antioxidant activity (DPPH) of methanol extract.

No.	ppm	Absorbance		% inhibition	
1.	0	0,7745	0,7745	0,0000	0,0000
2.	2	0,7559	0,7684	2,4015	0,7876
3.	4	0,7404	0,7605	4,4028	1,8076
4.	6	0,7160	0,7255	7,5533	6,3267
5.	8	0,7116	0,7116	8,1214	8,1214
6.	10	0,6973	0,6958	9,9677	10,1614

Table 2: Absorbance and % inhibition of antioxidant activity (DPPH) of n-hexane extract

No.	ppm	Absorbance		% inhibition	
1.	0	0,8478	0,8478	0,0000	0,0000
2.	2	0,8068	0,8241	4,8360	2,7955
3.	4	0,7678	0,7776	6,0156	8,2803
4.	6	0,7573	0,7549	10,6747	10,9578
5.	8	0,7478	0,7478	11,7952	11,7952
6.	10	0,7114	0,7114	16,0887	16,0887

Table 3: Absorbance and % inhibition of antioxidant activity (DPPH) of ethyl acetate extract

No	ppm	Absorbance		% inhibition	
1.	0	0,8820	0,8820	0,0000	0,0000
2.	2	0,7994	0,8033	9,3651	8,9229
3.	4	0,6788	0,7070	23,0386	19,7732
4.	6	0,5718	0,5749	35,1701	34,8186
5.	8	0,4704	0,4828	46,6667	45,2608
6.	10	0,3689	0,3849	58,1746	56,3605

Table 4: Absorbance and % inhibition of antioxidant activity (DPPH) of water extract

No.	ppm	Absorbance		% inhibition	
1.	0	0,9879	0,9879	0,0000	0,0000
2.	2	0,9849	0,9849	0,3037	0,3037
3.	4	0,9624	0,9624	2,5812	2,5812
4.	6	0,9607	0,9489	2,7533	3,9478
5.	8	0,9439	0,9454	4,4539	4,3021
6.	10	0,9396	0,9388	4,8892	4,9701

Table 5: Antioxidant activity (IC_{50})

No.	Extract	IC_{50} (ppm)
1	Methanol	49.328 ± 0.242
2	n-hexane	32.041 ± 0.770
3	Ethyl Acetate	8,732 ± 0.204
4	Water	93.330 ± 1.931

From data assay against DPPH obtained that the extract of ethyl acetate has the highest IC_{50} value 8,732 ± 0204 ppm. Based on this, the aerial part extract of *L. microphyllum* has an activity of an antioxidant with an IC_{50} value of less than 200 ppm.

4 CONCLUSIONS

Ethyl acetate fraction of aerial parts *L. microphyllum* has the highest antioxidant activity against DPPH with an IC_{50} value of 8,732 ± 0,204 ppm.

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