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Tropical Bio-resources for Sustainable Development

"The Role of Innovation to Enhance German Alumni in Scientific and Professional Capacities"

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Natural products exploration in frame of tropical plant bio-resource conservation and utilization

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Abstract The extracts and raw materials from mangroves have been used by coastal communities for natural medicines, one of these is Api-Api (*Avicennia lanata* Ridley) leaves. In this research, phytochemical screening, total phenolic content analysis, toxicity test, and antioxidant activity test of methanol crude extract, *n*-hexane fraction, ethyl acetate fraction and methanol-H₂O fraction of *A. lanata* leaves have been done. Total phenolic content was analyzed by Folin-Ciocalteu method. Based on this analysis was known that the total phenolic content on crude methanol extract, ethyl acetate fraction and methanol-H₂O fraction of *A. lanata* leaves are 3.27; 5.35 and 7.59 mg GAE/g sample, respectively. On toxicity test by using Brine Shrimp Lethality Test (BSLT) method, was obtained the LC₅₀ values of crude methanol extract, *n*-hexane fraction, ethyl acetate fraction and methanol-H₂O fraction of *A. lanata* leaves are 104.79; 128.69; 268.33 and 324.70 ppm, respectively. The antioxidant activity test was carried out by radical scavenging using 2,2-diphenyl-1-picrylhydrazyl (DPPH) which act as free radical DPPH was reduced by antioxidants of extract samples. The IC₅₀ values obtained for the crude methanol extract, *n*-hexane fraction, ethyl acetate fraction and methanol-H₂O fraction of *A. lanata* leaves were 37.89; 172.12; 51.71 and 39.00 µg/mL, respectively. Based on the IC₅₀ values could be known that the crude methanol extract, ethyl acetate fraction and methanol-H₂O fraction of *A. lanata* leaves were belonging to the extracts that had a very strong antioxidant activity, while *n*-hexane fraction relatively had a weak antioxidant activity.

Keywords Antioxidant, *Avicennia*, phenolic, toxicity

1. Introduction

Indonesia has the second largest tropical forest in the world rich in biodiversity. The bio diversity of natural resources in Indonesia, one of which, is the source of secondary metabolites compounds. A movement back to nature has led to the increasing in the usage of natural products as

natural medicine. For example, medicinal herbs currently widely used either as a remedy alternative and for the maintenance of health. Some medicinal plants are distributed in Mangrove forest. Mangrove Forest in Indonesia is the highest in the world ($\pm 42.550 \text{ km}^2$ with ± 45 species) [1]. Mangrove forest plays role as ecological function, food resources, and natural medicine (*Sonneratia*, *Avicennia*) based on local wisdom.

2. Material and Methods

In this research were conducted phytochemical screening [2], total phenolic content analysis [3], toxicity test, and antioxidant activity test of methanol crude extract, *n*-hexane fraction, ethyl acetate fraction and methanol-H₂O fraction of *A. lanata* leaves. Total phenolic content was analyzed by Folin-Ciocalteu method. This analysis was carried out by using spectrophotometer UV-Vis at 765 nm wavelength and gallic acid as standard. The toxicity test by using Brine Shrimp Lethality Test (BSLT) method [4]. The antioxidant activity test was carried out by radical scavenging using 2,2-diphenyl-1-picrylhydrazyl (DPPH) which act as free radical DPPH was reduced by antioxidants of extract samples. The absorbancies were measured by Vis spectrophotometer at the maximum wavelength ($\lambda = 517 \text{ nm}$) [5].

3. Results and Discussion

Based on phytochemical screening was known the secondary metabolites content in extract *A. lanata* leaves. In crude methanol extract was found alkaloid, steroid, saponin, flavonoid and phenolic compounds. In *n*-hexane fraction was found alkaloid and steroid compounds. In ethyl acetate fraction was found alkaloid, steroid, saponin, flavonoid and phenolic compounds. In methanol-H₂O fraction was found saponin, flavonoid and phenolic compounds.

Total phenolic content was analyzed by Folin-Ciocalteu method. This analysis was carried out by using spectrophotometer UV-Vis at 765 nm wavelength and gallic acid as standard. Based on this analysis was known that the total phenolic content on crude methanol extract, ethyl acetate fraction and methanol-H₂O fraction of *A. lanata* leaves are 3.27; 5.35 and 7.59 mg GAE/g sample, respectively.

Testing of Brine Shrimp Lethality Test (BSLT) test revealed the methanolic crude extract is the most toxic compared to the fractions from leaves of *A. lanata* LC₅₀, because it has the smallest LC₅₀ value of 104.79 ppm (table

1).

Table 1. LC₅₀ values from extracts and fractions of *A. lanata* leaves

Extracts and Fractions	LC ₅₀ (ppm)
Crude methanolic extracts	104,79
n-hexane fraction	128,69
Ethyl acetate fraction	268,33
Methanol-H ₂ O fraction	324,70

Dealing with the classification level of the toxicity (table 2), based on this test was known that the most toxic extract is crude methanol extract because it has the lowest LC₅₀ value.

Table 2. The classification level of the toxicity of an extract based on LC₅₀ value

Range of LC ₅₀ value	Remark
LC ₅₀ ≤ 30 ppm	Very toxic
30 ppm < LC ₅₀ ≤ 1000 ppm	Toxic
LC ₅₀ > 1000 ppm	No toxic

Crude methanol extracts at concentration range of 6 – 100 µg/mL revealed an antioxidant activity with the DPPH radical scavenging in range of 22.84 – 90.73% with the scavenging average value of 50,64%. The fraction of n-hexane have antioxidant activity with the DPPH radical scavenging in range of 4.74 - 28% with average value of radical scavenging of 14.25%. The fraction of ethyl acetate gave an antioxidant activity with the DPPH radical scavenging in range of 6.47 – 86.21% with the average value of 39,83%. Fraction methanol-H₂O has an antioxidant activity with the DPPH radical scavenging percentage in range of of 20.91 – 90.30% with the average value of radical scavenging of 49.83%. Whereas, in vitamin C, DPPH radical scavenging revealed in value of 35,42 - 97,99% with the average value of scavenging of 61,97% .

The assay of antioxidant activity carried out with the DPPH radical scavenging methods. The DPPH free radical scavenging activities of crude methanolic extract and each fraction from *A. lanata* leaves extracts as well as vitamin C revealed the inhibitory concentration 50 (IC₅₀) values, as shown in the following table 3.

Table 3. The value of IC₅₀ of *A. lanata* Leaves extracts measured by spectrophotometer at a maximum wavelength of 517 nm

Extracts and fraction of <i>A. lanata</i> Leaves	IC ₅₀ (µg/mL)
Crude methanolic extracts	37,89
n-hexane fraction	172,12
Ethyl acetate fraction	51,71
Methanol-H ₂ O fraction	39
Vit C	19,83

The concentration level of IC₅₀ indicated an antioxidant substance is needed to inhibit 50% free radical DPPH. Antioxidant substances that have high antioxidant activity will have the low IC₅₀ values. Specifically, the strength of antioxidant activity of a compound according to Mardawati [6] can be classified based on the values of IC₅₀ as presented on the table 4.

Table 4. Classification of antioxidant activity strength of a compound

Nr.	Range value of IC ₅₀	Remark
1.	IC ₅₀ ≤ 50 µg/mL	Very strong
2.	50 µg/mL < IC ₅₀ < 100 µg/mL	Strong
3.	100 µg/mL ≤ IC ₅₀ ≤ 150 µg/mL	Medium
4.	151 µg/mL ≤ IC ₅₀ ≤ 200 µg/mL	Weak

Based on the classification of the strength of the antioxidant activity can be known that crude methanolic extract, ethyl acetate and methanol-H₂O fractions are classified as having a very strong antioxidant activity, whereas the n-hexane fraction belongs to the weak antioxidant activity. From the results it can be estimated that the secondary metabolite compounds such as flavonoids and phenolic extracts contained in crude methanolic extract and ethyl acetate- and methanol-H₂O fraction have antioxidant activity. In addition, alkaloids compound contained in crude methanol extracts, the fraction of ethyl acetate and n-hexane fraction also have antioxidant activity.

4. Conclusion

The crude methanolic extract is the most toxic compared to the other fractions of *A. lanata* leaves. The crude methanolic extracts, ethyl acetate-, and methanol-H₂O fractions are classified as having a very strong antioxidant activity, whereas the n-hexane fraction belongs to the weak antioxidant activity. The methanol-H₂O fraction is recommended to be developed its utilization as an antioxidant.

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