Antioxidant activity of an Epiphyte Fern in Palm Oil Tree

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Abstract. Vitaria elongata is an epiphytic plant in the Pteridaceae family, which usually grows on palms and wetlands. Other species of this genus have been reported to have cytotoxic and antioxidant activity, but their biological activity was not reported in this species. Therefore, the research aimed at antioxidant activity from V. elongata extracts. The extract of methanol was obtained by maceration (3 x 24 h). The sample was subsequently brought with partition containing various organic solvents (n-hexane, dichloromethane and ethyl acetate). The n-hexane, dichloromethane, ethylacetate, and water extracts tested for antioxidant activity were performed using 1.1-diphenyl-2-picrylhidrazil (DPPH) methods. The IC50 value of dichloromethane, ethyl acetate and water extract was 472.35 μg / mL, 94.72 μg / mL, and 13.63 μg / mL, while there was no antioxidant activity of the n-hexane extract and component VE-DCM-01, IC50 > 1,00 μg / mL.

1. Introduction

Epiphytes are plants grown on the other plant that prevent them from rooting in soil. These plants live within the plantae kingdom. Epiphytes can be divided into vascular and non-vascular epiphytic plants inhabited by plant divisions including marchantiophyte (liverworts), anthocerotophyte (hornworts) and bryophyte (mosses). Vascular epiphytic plants comprise divisions of pteridophytes, cycadophytes, gnetophytes and magnoliophytes formed by epiphytes up to 1,000 plant genera.[1] Various disease therapies, including infectious or non-infectious diseases, have been used for epiphytes. Infectious illnesses which can be recognized in traditional cultures, including skin disorders (wounds, burns, ulcers, abscesses, smallpox) and non-skin diseases (fever, diarrhoea, ulcers, colds, worms, and malaria).[2]

Fern is a pteridophyte botanic group. This group alternates very distinctly from separate unnoticed and sort-lived gamethophytes (sexual) to prominent and dominant (asexual) stages. In comparison to moose, ferns have a vascular bundle of xylem and flame, and are thus classified into vascular plants without any seed or flora. Fern reproduction uses spore. Almost 10 percent of leptosporangiate ferns are epiphytic ferns.[3] Such plants require a special microhabitat on the bark or trunk of the host.[4] Oil palm is one of the hosts of epiphytic plants. Different forms of epiphyt plant growing in oil palms, from mosses like plants Calymperaceae, Dicranaceae to ferns, covering species like Aipeniaceae, Blechnaceae, Davallia, and Vittaria.[3]

We have an ongoing interest in the biological activities of epiphyte ferns, focusing particularly on palm oil. In this report, details on the extraction and antioxidant activity of Vitaria elongata Sw are presented. This species was selected due to its abundance of palm oil and lack of information regarding its antioxidant activity.
2. Experimental

2.1. Extraction

_V. elongata_ Sw was taken from the trunks of oil palm trees growing in the area, Kampar Regency, Province of Riau. Samples were dried at room temperature for 3 weeks. The leaves and stems are cut into small pieces and then crushed using a blender until the powder is ready to be macerated. The powder produced was as much as 3 kg.

A sample (3 kg) was extracted with MeOH (3X 12 L, 24 h) and the combined MeOH extracts were sonicated, concentrated and the water content adjusted to approximately 1:9 (H₂O/MeOH, 500 mL). The extract was partitioned with hexane (2X 500 mL) to give hexane extract (24 g). The aqueous MeOH layer was adjusted with water (2:3 H₂O/MeOH) and extracted with dichloromethane (CH₂Cl₂) (2X 500 mL) to yield CH₂Cl₂ extract (11 g). The methanol was evaporated, and resultant aqueous layer was partitioned against ethyl acetate (EtOAc) (2X 500 mL) to provide EtOAc extract (24.7 g) and water extract (24.5 g).

2.2. Antioxidant Activity

The extracts were prepared with a certain concentration in methanol. Approximately 100 μL of the sample was transferred into row A of the microplate (plate consist of A-H rows, each row consists of 12 wells). Two-fold dilutions of the compound were added to the next row until the concentration was 31.25 μg/mL. Five μL Diphenylpicrylhydrazyl (DPPH) was added to each well of the sample. The microtiter plate was then vortexed and incubated for 30 min in the dark room.[5, 6] Then, Absorbance were measured by microplate reader (Berthold, Germany) at 520 nm. The same method was conducted for ascorbic acid as positive control.

The % Inhibition value is calculated by the following formula:

\[
\text{% Inhibition} = \frac{(A_0 - A_s)}{A_0} \times 100
\]

Where A₀ represents the absorbance of the DPPH radical solution without sample while Aₙ represents the absorbance of the sample with DPPH radical solution. A graph of inhibition percentages (I%) versus concentrations of the sample was plotted to provide value of IC₅₀.

Antioxidant activity index (AAI), calculated as follows as:

\[
\text{AAI} = \frac{\text{final concentration of DPPH (μg.ml⁻¹)}}{\text{IC}_{50} (μg.ml⁻¹)}
\]

3. Results and Discussion

In this research, the methanol extract was subjected into solvent-solvent partition with modified Kupchan partition.[7] The Kuphan partition is an efficient but easy way to start a purification protocol by separating a total MeOH extract from four large extracts, simplified mixes of different polarities—using only non-miscellaneous solvent partitions. Nothing about the sample is inferred and no information is lost. The distribution between extracts of compounds of different polarity can be significant.

To each extract was then analyze for their antioxidant activity by using free radical (DPPH) scavenging activity method. This method is a popular in antioxidant assay and the method is simple in determination of antioxidant in plant extracts and foods.[8] The method can be characterized by the formation of stable radicals with electrons that can be delocalised and give a deep purple colour, and the density of this colour can be reduced if reacted with a compound that can donate hydrogen atoms.[9] In antioxidant activity analysis, each extract was tested in inhibition of free radicals with a concentration of two-fold dilution and then IC₅₀ was obtained. The smaller the IC₅₀ value of a compound or extract, the stronger the antioxidant ability. Analysis of antioxidant activity from several extracts from this species can be seen in table 1.
### Table 1. Antioxidant Activity from several extracts of *Vittaria elongata* Sw

| Extract          | IC<sub>50</sub> (ppm) | AAI  |
|------------------|------------------------|------|
| Water            | 13.63                  | 5.87 |
| Ethyl acetate    | 94.73                  | 0.85 |
| Dichloromethane  | 472.35                 | 0.17 |
| n-Hexane         | >1000                  | 0.07 |
| Ascorbic acid    | 4.15                   | 19.26|

The extracts displayed various antioxidant activity in this analysis. There was weak antioxidant in n-hexane and dichloromethane while ethyl acetate and water extracts had antioxidant activity. The AAI was determined based on the mass of DPPH and the mass of the measured extracts in the reaction, resulting in a constant for each extract, independent of the DPPH concentration and extract used. In this study, the extracts were considered exhibit poor antioxidant activity with AAI < 0.5, modest antioxidant activity with AAI > 0.5 to 1.0, strong antioxidant activity with AAI between 1.0 and 2.0 and very high with AAI > 2.0. [10] Therefore, the water and ethyl acetate extracts are considered to have moderate and very strong activity, respectively. This might be due to the presence of Vittarilide-A, vittarilide-B, and ethyl 4-O-caffeoylquinate. Based on Wu and co-authors reported that the isolated compounds from *V. anguste-elongata* possessed DPPH scavenging activity with IC50 value of 91, 290, and 234 µM, respectively. [11]

### 4. Conclusions

The extracts responded differently toward free radical (DPPH) scavenging activity. The study showed that ethyl acetate and water extracts showed potential antioxidant activity.

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