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PHYTOCHEMICAL SCREENING AND BIOACTIVITY OF *Angiopteris evecta* LEAVES FROM EAST KALIMANTAN

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Abstract: This study aimed to evaluate the bioactivity and screen the phytochemicals presenting in *Angiopteris evecta* leaves in East Kalimantan. The leaves of *A. evecta* were extracted by using methanol at room temperature. *Sallmonela thypi, Propionibacterium acnes,* and *Bacillus cereus* were chosen for testing the antibacterial activities of the plant extract. The results showed that *S. thypi*, *P. acne* and *B. cereus* could be inhibited with the inhibition zone at 11.22 ± 1.02 mm, 10.22 ± 1.26 and 10.30 ± 0.00 mm. Chloramphenicol was selected as positive control with inhibition zone 22.39 ± 0.79 mm, 30.00 ± 0.00 and 19.33 ± 1.15 mm, the least concentration of the plant extract at 5 mg/mL. The results of testing antioxidant activities showed that the IC_{50} value of DPPH radical scavenging assay was 0.08 ± 0.00 mg/mL while OH radical-scavenging activity assay was 3.48 ± 0.09 mg/mL. As compared to ascorbic acid, the plant extract showed slightly less antioxidant activity against DPPH. IC_{50} value of the plant extract for anti-tyrosinase activity was found to be 0.76 ± 0.02 mg/mL. The results of phytochemical screening confirmed the presences of active compounds, which were alkaloids, flavonoids, saponins, steroids, and carbohydrates, in the plant extract.

1. Introduction

Angiopteris evecta, the largest ferns on the planet, is widely distributed in Australia, New Guinea, Polynesia, India, Malaysia, Thailand and Indonesia [1,2]. In East Kalimantan, Indonesia, this species has been used as traditional medicine to treat various diseases [3].

Previous study reported that *A. evecta* had the trace amount of quercetin as flavonoid types and di-*C*-glycosylapigenins as violanthin (6-*C*-glucosyl-8-*C*-rhamnosylapigenin), isoviolanthin (6-*C*-rhamnosil-8-*C*-glucosylapigenin) and vicenin-2 [4,5].

Another report found the antibacterial activity of methanol extract against *Xanthomonas campestris* pv. *centellae* [6] and exhibited to inhibit tuberculosis activity [7]. Ethyl acetate and dichloromethane fractions of stem and root also demonstrated good inhibition against bacterial activity and fungal activity [8].

The study aims to evaluate phytochemical properties, antibacterial activity, antioxidant activity and tyrosinase inhibition. This research is the first data report of methanol crude extract of *A. evecta* leaves from East Kalimantan.

2. Materials and Methods

2.1 Crude Preparation

The leaves of *A. evecta* were collected at Samarinda, Indonesia and identified by Technology Research Institute of Natural Resources Conservation, Indonesian Forestry Ministry. A voucher specimen (AE-DD-1) was deposited at the Forest Product Chemistry Laboratory, Forestry Faculty, Mulawarman University, Indonesia.

Dried leaves (498.76 g) of *A. evecta* were extracted by methanol at room temperature. Rotary evaporator was used to afford a crude extract (84.76 g). Ten gram of the methanol crude extract was used for bioactivity assay.

2.2 Chemicals

$Bi(NO_3)_3$, CH_3COOH, KI, NaOH, HCl, $(CH_3COO)_2Pb$, H_2SO_4, 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol, ascorbic acid, kojic acid, phosphate buffer, ethanol 20%, tyrosinase, 3,4-dihydroxy-L-phenylalanine (L-DOPA), $FeSO_4$, H_2O_2, salicylic acid, chloramphenicol and acetone were all purchased from Sigma–Aldrich and TCI. All others unlabeled reagents were analytical grade and high purity.

2.3 Phytochemical Screening

Secondary metabolites of crude methanol extract were identified using standard procedures [9-11]. Protocols are described as mentioned below:

Alkaloids: Smal quantity of extracts was warmed with 2% H_2SO_4 for two minutes. Few drops of Dragendorff's reagent were added. Orange red precipitate indicates the presence of alkaloids.

Flavonoids: Small quantity of extract was dissolved, NaOH and HCl were added. Yellow solution to change colorless indicates the presence of flavonoids.

Saponins: Small quantity of extract was mixed with warm distilled water in a test tube and shaken vigorously

for 30 seconds. The stable foam (1 cm height) indicates of saponins.

Steroids: Small quantity of extract was added with a few drops of acetic anhydride and H_2SO_4. The color changed from violet to blue or green in some samples indicate of steroids.

Triterpenoids: Small quantity of extract was added with a few drops of acetic anhydride and H_2SO_4. The color changed to reddish or violet in some samples indicating positive result for triterpenoids.

Tannins: Small quantity of extract was mixed with $Pb(CH_3COO)_2$ 1%. Yellow precipitate indicates the presence of tannins.

Carbohydrate: Small quantity of the extract was dissolved and mixed with Molisch reagent. Sulfuric acid was added carefully by slowly dripping. The layer of sulfuric acid indicating result has the carbohydrates.

2.4 Antibacterial Activity

Modify agar well diffusion method was followed according to the method [12] for antibacterial assay. *Sallmonela thypi, Propionibacterium acnes,* and *Bacillus cereus* were applied on agar plate. Wells (7 mm) were made on the inoculated media. A hundred µL of sample solution (5 mg/mL) was applied in the wells then incubated for 18 hours at 37 ± 0.5 ^oC. Acetone and chloramphenicol were used as negative and positive control, respectively. Inhibition zones were measured in millimetre. Sample treatment was replicated three times.

2.5 DPPH Radical Scavenging Activity Assay

The assay was measured according to the procedure of [7,9] with minor modification. One mL of sample solution with variable concentrations (0.025-0.5 mg/mL) was mixed with 1 mL of DPPH (0.04 mg/mL) and incubated in the dark room at room temperature for 20 min. The reaction was monitored at 517 nm. The percent inhibition was calculated as follow:

$$\% \text{ inhibition} = \left[1 - \frac{A_1 - A_2}{A_0}\right] x\ 100$$

where A_1 is absorbance of sample, A_2 is absorbance color of sample without DPPH and A_0 is absorbance of control without sample.

2.6 OH Radical Scavenging Activity Assay

OH radical scavenging activity was followed according to the method [14] with little modification. About 0.5 mL sample was mixed with 0.5 mL $FeSO_4$ (4 mM), and added 0.5 mL salicylic acid (4 mM) and 0.5 mL H_2O_2 (4 mM), shook vigorously then incubating at 37 ^oC for an hour. The absorbance was measured at 510 nm. Ascorbic acid was used as positive control and the effect of OH radical scavenging was calculated:

$$\text{OH radical scavenging effect (\%)} = \left[1 - \frac{A_1 - A_2}{A_0}\right] x\ 100$$

where A_1 is absorbance of sample, A_2 is absorbance color of sample without H_2O_2 and A_0 is absorbance of control without sample.

2.7 Tyrosinase Inhibition Assay

The assay was evaluated by previous method [15,16]. The assay medium consisted of 0.25 mL of PBS (0.02 M and pH 6.8), 0.25 mL of tyrosinase (135 U/ml), and 0.25 mL of sample solution (diluted in 20% ethanol) was mixed and preincubated at room temperature for 10 min. Then 0.25 mL of L-Dopa (0.34 mM) solution was added. The tyrosinase activity was measured at the 492 nm after 2 min incubation. Kojic acid was used as a positive control and the percent inhibition of tyrosinase activity was calculated as follow the formula:

% inhibition = [(A – B) – (C – D) / (A – B)] x 100

where A is control without sample, B is control without sample and tyrosinase, C is sample and D is sample without tyrosine [15,16].

3. Results and Discussion

3.1 Phytochemical Screening

The result of phytochemical assay indicate both physiological activity and medical activity [12]. Based on the result (Table 1) of the phytochemical screening from methanol crude extract; contained secondary metabolites constituent such as alkaloids, flavonoids, saponins, steroids and carbohydrates. It is not surprisingly due to the same report with previous research [6]. In contrast of screening alkaloid for Thai plants were reported that *A. evecta* had negative test for alkaloid [17] nevertheless, It might be happend due to the various physiological and biosynthetic reactions [18].

Based on the literature, secondary metabolite constituent have been developed as drug to treat of many diseases [19]. For instance, alkaloid was indicated as the central nervous system activity, antihelmintic activity, aphrodisiac, antibiotic activity, treatment of venereal diseases and antimalarial activity [20].

3.2 Antibacterial Activity

Three pathogenic bacteria were tested to the crude methanol extract of *A. evecta* leaves and the results were presented in Table 2. Inhibition activity against *P. acne, B. cereus and S. typi* showed that extract had inhibition zone 10.22 ± 1.26 mm, 10.3 ± 0.00 mm and 11.22 ± 1.02 mm, respectively.

The inhibitions of sample test were significantly different compared with the reference; however each bacterium provided similar response significantly against sample. Chloramphenicol as a reference was chosen as antibacterial strain because is active against broad range of organisms i.e. gram posistive and gram negative bacteria (including anaerobes bacteria) and its effects on

gram negative is better than gram positive bacteria, especially Salmonella typhi.

3.3 DPPH Radical Scavenging Activity

Antioxidants play an important role in counteracting free radicals cause various diseases. In vitro analysis enables facile screening for antioxidants [21]. The role of antioxidant is to remove free radicals in which hydrogen donate electron to free radical till being unreactive species [22]. The antioxidant scavenging activity of methanol extract of *A. evecta* reported that increasing concentration showed increasing inhibition of free radical (Figure 1). Ascorbic acid as positive control showed the excellent scavenging activity with IC_{50} = 0.01 ± 0.00 mg/mL and the methanol extract of *A. evecta* had a strong scavenging activity as well, with IC_{50} value of 0.08 ± 0.00 mg/mL. This result related to phytochemical result which contained secondary metabolites that responsible to antioxidant activity.

3.4 OH Radical Scavenging Activity

OH radical is the highest reactivity because it has 1-electron with potential reduction 2310 mV [23]. OH radical can occur due of decomposition reaction of hydrogen peroxide with metal ions such as iron [14]. The scavenging abilities of methanol crude extract on hydroxyl radical inhibition are shown in Figure 2. In this study, inhibition ability of ascorbic acid was higher than sample with the IC_{50} at 0.46 ± 0.06 mg/mL and 3.48 ± 0.09 mg/mL, respectively.

Table 1: Phytochemical Properties of *Angiopteris evecta* in Methanol Crude Extract

Types of Compounds	Methanol Crude Extract of *Angiopteris evecta*
Alkaloids	+
Flavonoids	+
Saponins	+
Steroids	+
Terpenoids	-
Tannins	-
Carbohydrates	+

+ = present; - = absent

Tabel 2: Antibacterial activities of crude methanol extract from *A.evecta* leaves against *B.cereus, P.acne* and *S.typi*.

Bacteria	Inhibition Zone (mm)	
	Methanol Crude Extract (mm)	Chloramphenicol (mm)
P. acne	10.22 ± 1.26	30.00 ± 0.00
B. cereus	10.30 ± 0.00	19.33 ± 1.15
S.typhi	11.22 ± 1.02	22.39 ± 0.79

Data given are mean of three replicates ± standard deviation, $p < 0.05$

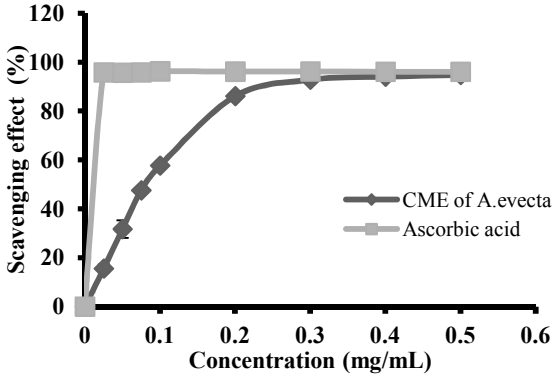

Figure1. The DPPH radical scavenging activity of crude methanol extract from *A. evecta* leaves.

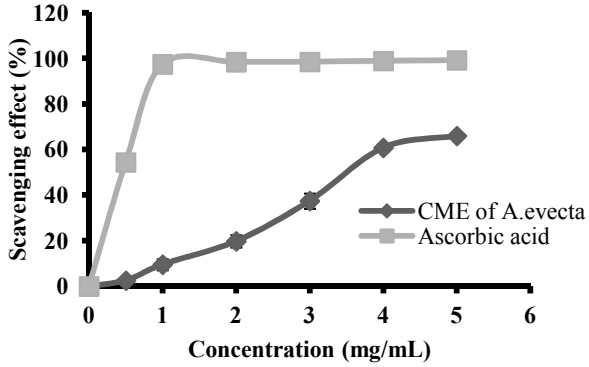

Figure2. OH radical scavenging activity of Methanol crude extract of *Angiopteris evecta.*

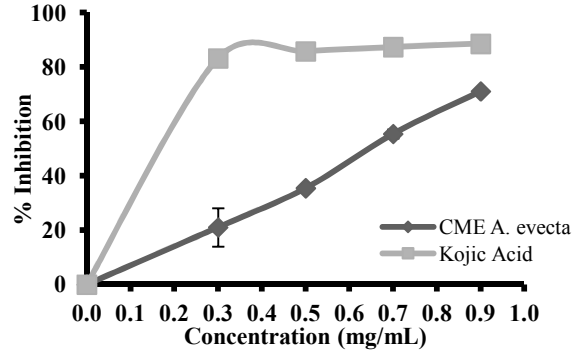

Figure3. Tyrosinase inhibitory activity of Methanol crude extract of *Angiopteris evecta.*

3.5 Tyrosinase Assay

Tyrosinase is a multifunctional copper-containing enzyme (mono- and di-phenolase activities) that is implicated in the synthesis of melanin [13]. Although melanin have a proto protective function in human skin but humans are still aware about their skin color because the objectionable skin discoloration or hyper pigmentation can cause aesthetic problem [23]. Tyrosinase inhibition activity is shown in Figure 3. The

result of tyrosinase inhibitory activity indicated that increasing concentration appropriated with increasing inhibition. The inhibition activity of sample showed up IC_{50} at 0.76 ± 0.02 mg/mL compared with kojic acid as the positive control with the IC_{50} at 0.17 ± 0.01 mg/mL. This could be due to greater solubility and the increased concentrations of active components in methanol extract.

4. Conclusions

Surprisingly, the best of our knowledge, the antioxidant and tyrosinase inhibitory are the first report for methanol extract of *A. evecta* leaves, particularly from East Kalimantan. Based on the result above, we concluded that the crude methanol extract of *A. evecta* contained secondary metabolites that sensitive to inhibit the growth of positive and negative bacteria as B.cereus, P.acne and S.typhi. Likewise, have potention as antioxidant that was exhibited through DPPH radical scavenging assay and OH radical scavenging assay. Moreover, have prospection as whitening agent.

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