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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF CRUDE METHANOLIC EXTRACT OF *Polyscias guilfoylei* LEAVES

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Abstract: The present study aimed to investigate the preliminary phytochemical analysis, antioxidant and antimicrobial activities of crude methanolic extract from *Polyscias guilfoylei* leaves. Preliminary phytochemical screening was carried out using standard methods. Its antioxidant activity was evaluated by using five different methods including DPPH radical scavenging assay, hydrogen peroxide scavenging assay, reducing power assay, Fe^{2+}chelating assay, and hydroxyl scavenging assay. All methods were used ascorbic acid as a standard. Furthermore, the methanolic extract of *Polyscias guilfoylei* leaves was also tested for antibacterial activity by the agar well diffusion method. In preliminary phytochemical analysis the crude methanolic extract was consisted of tannins and saponins. Total phenolic content of the methanolic extract was 10.36 mg gallic acid equivalent/g of plant extract. For the antioxidant activities, the result showed that, the activities of plant extract against DPPH, H_2O_2, and $OH^{\cdot}$ radical were depended on the concentration with IC_{50} value of 1.88, 1.32, and 2.70 mg/ml while those for ascorbic acid were 0.23, 0.28 and 0.23 mg/ml, respectively. Fe^{2+}chelating activity of the plant extract was also found with IC_{50} value about 0.88 mg/ml and that for ascorbic acid was 0.23 mg/ml. The Fe^{3+} reducing power of the plant extract was 3.65 mg/ml and that for ascorbic acid was 0.25 mg/ml which were studied from absorbance at 0.500. The plant extract at the concentration of 5 mg/ml showed a promising activity against *S. thypii* with the size of inhibition zone of 10.0 mm ± 0.0, *P. acne* 9.3 mm ± 0.7 and *B. cereus* 9.0 mm ± 0.2. Chloramphenicol was used as a positive control with the size of inhibition zone of 21.0 mm ± 0.6; 29 mm ± 1.4 and 20.0 mm ± 1.2 at the same concentration, respectively. Our findings provide important evidence that the methanolic extract of *Polyscias guilfoylei* is one promising source of natural antioxidants as well as antimicrobial agents.

1. Introduction

A free radical is defined as any atom or molecule possessing unpaired electrons. The reactive oxygen species (ROS), such as the superoxide radical ($\cdot O^{2-}$), hydroxyl radicals (·OH), hydrogen peroxide (H_2O_2) might be generated during normal metabolic processes or due to the exogenous factors and agent. Various diseases such as cancer, cardiovascular disease, osteoporosis, and degenerative diseases may occur due to the formation of ROS, that can cause oxidative damage to human cells [1]. Some compounds known as antioxidants have ability to delay or inhibit the initiation or propagation of oxidative chain reaction and thus prevent or repair oxidative damage done to the body's cells by oxygen [2].

Antimicrobial properties of medicinal plants are being increasingly reported from different part of the world. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc, which have been found *in vitro* to have antimicrobial properties [3].

In Indonesia, there are plenty of medicinal plants which are called *jamu* and it becomes popular [4]. *Polyscias guilfoylei* (synonim *Geranium aralia*) is one of the medicinal plant and a member of the family Araliaceae. *P. guilfoylei* originates in Indonesia and Malaysia that this plants are widely spread in the areas of Sumatera, Java, Sulawesi and Ambon. In Indonesia this plant is cultivated and used as a fence plant [5] . *P. guilfoylei* is known as a traditional herbal plants and vegetables. The local name is puding leaf (Figure 1) [6]. Selections of the plant sample based on the limited use this plant in early research study and its traditional uses in treatment of some diseases.

In view of these findings, the aim of the present study was to evaluate screening phytochemical, antioxidant, and antimicrobial of methanolic extract of *Polyscias guilfoylei*.

2. Materials and Methods

2.1 Plant Material

The leaves of *Geranium Aralia* (*Polyscias guilfoylei*) were collected from the Local Farm Tanah Merah (Samarinda, East Kalimantan) in January 2013. The plant was identified by Technology Research Institute of Natural Resources Conservation, Indonesian Forestry Ministry. A voucher specimen no. GA-DD-4 was deposited at the Forest Products Chemistry, Faculty of Forestry, Mulawarman University, Indonesia.

2.2 Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), thrichloro-acetic acid, galic acid and ascorbic acid (Vitamin-C) were also purchased from Sigma Aldrich GmbH (Germany). Eethylenediaminetetraacetic acid (EDTA), 35% hydrogen peroxide, potassium hexacyano-ferrate(III), salysilic acid, sodium bicarbonate, were purchased from Ajax Finechem (Australia). Folin-ciocalteu reagent, iron(II) sulfate heptahydrate,

iron(III) chloride hydrogen sodium 4,4'-[3-(2-pyridinyl)-1,2,4-triazine-5,6-diyl] dibenzene-sulfonate (ferrozine), potassium dihydrogen phosphate, quercetine, potassium sodium hydrogen phosphate, were purchased from Merck KGaA (Germany).

2.3 Preparation of Methanolic Extract.

The dried leaves (521.50 g) was powdered with a blender and extracted in methanol at room temperature for 3 days. The extract was filtrated and evaporated to 500 ml in rotary evaporator under reduced pressure, yielding the crude of *Polyscias guilfoylei* (PME) 80.81 g.

2.4 Phytochemical Analysis

The major secondary metabolite classes such as triterpenoids, steroids, flavonoids, alkaloids, saponins, carbohydrates, and tannins were screened according to common methods described by Harborne and Kokate [7, 8].

2.5 Determination of Total Phenolic Content in Extract

Total phenolic content was estimated according to literature procedure [9]. PME (0.2 ml, 3 mg ml^{-1}) was mixed with 1 ml of 2 N *Folin-Ciocalteau* reagent for 5 min and 0.8 ml of 7.5% Na_2CO_3 were then added. The absorbance was measured at 760 nm after 2 h of incubation at room temperature. Result was expressed as gallic acid equivalent in mg/ gram crude extract and all the samples were measured in triplicate.

2.6 DPPH Radical Scavenging Activity Assay

One mililiter of methanolic extract of samples at various concentrations (0.5, 1, 2, 3, 4 and 6 mg ml^{-1}) were added to one mililiter of methanolic solution of DPPH and allowed to stand for 20 min at room temperature. The absorbance of the sample was measured at 517 nm [10]. All experiments were repeated three times. Ascorbic acid was used as a control. The percentage scavenging effect was calculated using the formula:

Scavenging rate = [1- (A_1-A_2)/A_0] X 100%

where A_0 was the absorbance of the control (without extract) and A_1 was the absorbance in the presence of the extract, A_2 was the absorbance without DPPH.

2.7 H_2O_2 Scavenging Activity Assay

According to a literature procedure [11] with little modifications, H_2O_2 scavenging activity against the sample was measured. Phosphate buffer (1.2 ml, pH 7.4) and 0.5 ml of various concentrations of the extract were mixed, followed by 0.3 ml 40 mM of H_2O_2 solution. The mixed solution was homogenized and incubated at room temperature for 10 min. The absorbance of the sample was measured at 230 nm. Ascorbic acid was used as control. The H_2O_2 scavenging activity was calculated as follows:

Scavenging rate = [1- (A_1-A_2)/A_0] X 100%

where A_0 was the absorbance of the control (without extract) and A_1 was the absorbance in the presence of the extract, A_2 was the absorbance without H_2O_2.

2.8 OH Radical Activity Assay

The assay described by Du et al. [12] was used to test OH radical activity. One mililiter of PME with different concentrations (0.5, 1, 2, 3, 4 and 6 mg ml-1) was mixed with 1 ml of 4 mM $FeSO_4$, 1 ml of 4 mM salicylic acid and 1 ml of 4 mM H_2O_2. After the reaction mixture was homogenized, incubated at 37°C for 30 min, and centrifuged at 3000 rpm for 10 min. The absorbance of supernatant was then measured at 510 nm. Triplicate measurements were performed and the antioxidant activity was expressed as

Scavenging activity (%) = = [1- (A_1-A_2)/A_0] X 100%

where A_0 was the absorbance of the control (without extract) and A_1 was the absorbance in the presence of the extract, A_2 was the absorbance without hydrogen peroxide.

2.9 Ferrous Ion Chelating Assay

Fe^{2+}-chelating activity was measured according to literature procedure [13] with a few modifications. The reaction mixture contained 0.75 ml of PME with different concentrations (0.5, 1, 2, 3, 4 and 6 mg ml^{-1}), 0.75 ml $FeSO_4$ (0.1 mM) and shaked. After incubation for 1 min, 0.75 ml of ferrozine (0.25 mM in deionised water) added, shaked vigorously and left at room temperature for 10 min. The absorbance of the Fe^{2+}-ferrozine complex was measured at 562 nm against a blank. EDTA-2Na was used as the control. Triplicate measurements were performed and the chelating activity of the extract for Fe^{2+} was calculated as

Chelating rate = [1- (A_1-A_2)/A_0] X 100%

where A_0 was the absorbance of the control (without extract) and A_1 was the absorbance in the presence of the extract, A_2 was the absorbance without ferrozine.

2.10 Fe^{3+} Reducing Power Assay

Reducing power was determined by the method of Kilic et al. [14] Briefly, 0.3 ml with various concentrations of sample (0.5, 1, 2, 3, 4 and 6 mg ml^{-1}) were mixed with 0.5 ml of 0.2 M, pH 6.6 sodium phosphate buffer and 0.5 ml of 1 % potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50°C for 20 min. The reaction mixture was acidified with 0.5 ml of trichloroacetic (10%) after 20 min of the incubation. Lastly, the absorbance was measured at 700 nm after 0.5 ml of $FeCl_3$ (0.1 %) was added to this mixture. The ability of reduction great can be seen through the increasing of absorbance of the reaction mixture.

2.11 Antimicrobial Assay

Antimicrobial assays were conducted using method by Kusuma et al. with a few modifications [15]. *Sallmonela thypii, Propionibacterium acnes,* and *Bacillus cereus* were swab streaked on mixing of nutrient agar and potato dextrose agar. Then wells of 7 mm diameter were cut into agar plates and 5 mg/ml of sample solution, positive control (chloramphenicol) and blank (acetone) were added to each well. The plates were incubated for 18 hour at 37 ± 0.5 °C. The test materials having antimicrobial activity inhibited the growth of microorganism and a clear, distict zone

of inhibition was visualized surrounding the medium. The inhibition zones were measured in millimeters. Tests were carried out in triplicate and the results were shown as means.

3. Results and Discussion

Phytochemical screening of the crude extract of *Polyscias guilfoylei* revealed the presence of saponins and tannins (Table 1). Similar with previous results (Elya B et al.) reported that the *P. guilfoyle* leaves contained triterpenoids and saponins. Its also could reduce the concentration and the quality of spermatozoa of the male rabbits [6]. Saponins have also been reported in the *Polyscias fruticosa* [16-18], *Polyscias fulva* [19, 20] and *Polyscias dischroostachya* [21]. The genus *Polyscias* are reviewed by Gopalsamy et al [21] as a rich sources of triterpene glycosides. Tannins are another active compound found to be present in this extract. Tannins and their derivatives are phenolic compounds considered to be primary antioxidant or free radical scavengers [22].

The total phenolic content of PME leaves was determined with a linear gallic acid standart curve (y = 0.0096x; r^2= 0.9952) and the result was 10.36 mg GAE/ g extract. A linear calibration curve of gallic acid, in the range 10-60 µg/ ml, was constructed.

The antioxidants, in DPPH assay, were able to reduce the stable DPPH radical (purple) to the yellow colored diphenyl-picrylhydrazine. DPPH radical scavenging activities (RSA) of PME and ascorbic acid are shown in Figure 2. Ascorbic acid showed an excellent scavenging activity (IC_{50}= 0.23 mg ml^{-1}), while the IC_{50} value of PME was 1.88 mg ml^{-1}.

Table 1: Phytochemical Analysis of PME

Phytochemicals	Plant Extracts
Saponin	+
Carbohydrate	-
Tannin	+
Alkaloid	-
Steroid	-
Triterpenoid	-
Flavanoid	-

(+) Present and (-) Absent

The scavenging ability of methanolic extract of *Polyscias guilfoylei* on hydrogen peroxide is shown in Figure 3. At a concentration of 6.0 mg/ml, for ascorbic acid showed an excellent scavenging activity (97.77 %). It was observed that PME also had strong scavenging activity (93.26 %). Hence, the PME can be considered as a good scavenger of hydrogen peroxide.

At a concentration of 0.5 mg ml^{-1}, the scavenging activity of PME and ascorbic acid was found to be 16.44 % and 99.01 % respectively. Activity of the PME on hydroxyl radical has been shown Figure 4. The IC_{50} value of PME was 2.70 mg ml^{-1}, while the IC_{50} value of ascorbic acid was 0.23 mg ml^{-1}.

Chelating activity of PME was compared to chelating standard, EDTA-2Na (Figure 5). With this assay, EDTA-2Na showed strong Fe^{2+}-chelating. Even at the minimal concentration of 0.5 mg ml^{-1}, its chelating rate was 99.28 %. However, chelating activity for PME at a concentration of 4 mg ml^{-1} was 98.11 %. The result showed that PME exhibited strong Fe^{2+}-chelating activities at high concentration and can be considered as a good chelating agent.

In the reducing power assay, the more antioxidant compounds convert the oxidation form of iron (Fe^{3+}) in ferric chloride to ferrous (Fe^{2+}). As showed in Figure 6, the reducing power of PME was 0.500 at 3.65 mg ml^{-1} and 0.600 at 5.35 mg ml^{-1}. However, ascorbic acid showed higher activity with a reducing power of 0.500 and 0.600 at 0.25 mg ml^{-1} and 0.30 mg ml^{-1}, respectively.

Figure 1. The leaf of *Polyscias guilfoylei*.

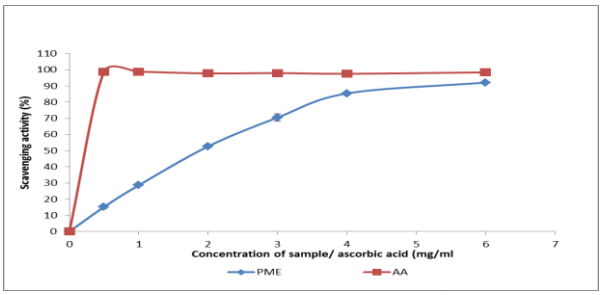

Figure 2. The DPPH radical-scavenging activity of methanol extract from *Polyscias guilfoylei* (PME) and ascorbic acid (AA) as positive control.

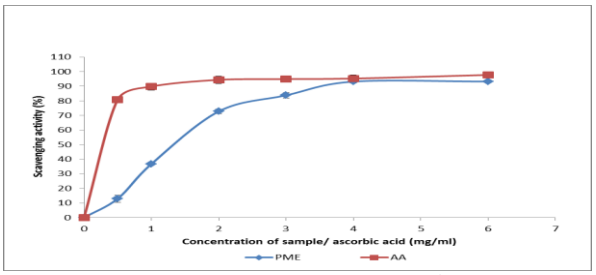

Figure 3. The H_2O_2 radical-scavenging activity of methanol extract from *Polyscias guilfoylei* (PME) and ascorbic acid (AA) as positive control.

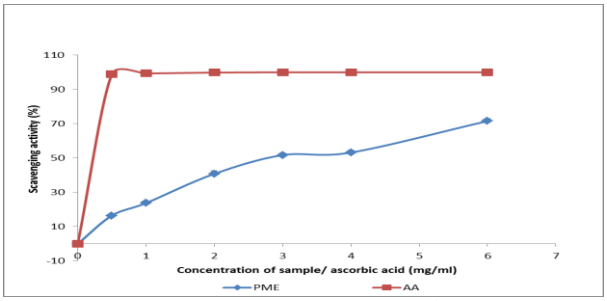

Figure 4. The OH radical-scavenging activity of methanol extract from *Polyscias guilfoylei* (PME) and ascorbic acid (AA) as positive control.

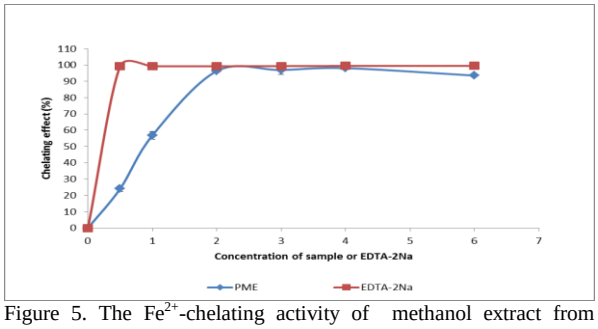

Figure 5. The Fe^{2+}-chelating activity of methanol extract from *Polyscias guilfoylei* (PME) and EDTA-2Na as positive control.

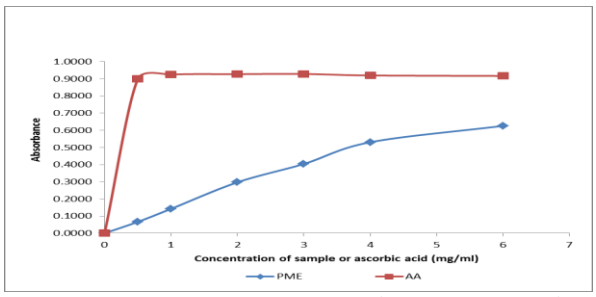

Figure 6. The reducing power activity of methanol extract from *Polyscias guilfoylei* (PME) and ascorbic acid (AA) as positive control.

Preliminary screening showed variation in the antimicrobial properties of plant extract (Table 2). The PME had 48.0 % (10 mm ± 0.0), 36.0 % (9.3 mm ± 0.7) and 43.1 % (9.0 mm ± 0.2) activity of *Salmonella thypii*, *Propionibacterium acne* and *Bacillus cereus*, respectively. While chloramphenicol was used as a positive control with the size of inhibition zone of 21.0 mm ± 0.6; 29.0 mm ± 1.4 and 20.0 mm ± 1.2 at the same concentration, respectively. The antimicrobial assay revealed that PME exhibited broad spectrum activity against the *Salmonella thypii* as compared to other bacteria.

Table 2: Antimicrobial Activity of PME

Bacteria	Inhibition Zone (mm)	
	PME	Chloramphenicol
S. thypii	10.0 ± 0.0	21.0 ± 0.6
P. acne	9.3 ± 0.7	29.0 ± 1.4
B. cereus	9.0 ± 0.2	20.0 ± 1.2

Diameter of well 7 mm; Extract and chloramphenicol conc. 5 mg/ml; Result are average of three readings.
Chloramphenicol was used as standard against bacteria.

4. Conclusions

To the best our knowledge, this is the first report of antioxidant and antimicrobial activity of *Polyscias guilfoylei* leaves. On the basis of results obtained from above investigation, we can conclude that the methanolic extract of *Polyscias guilfoylei* is a promising source of natural antioxidant as well as antimicrobial agents. Isolation and identification of active compound in the extract are needed in further research which could be used for pharmaceutical use.

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