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Total Phenolic and Coumarin Content, Antioxidant Activity of Leaves, Fruits, and Stem Barks of Grey Mangrove (*Avicennia marina*)

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Abstract

Avicennia marina is one of the mangrove species used for traditional medicines. The leaves, fruits, and stem barks of *A. marina* are used for treating skin diseases. The stem barks are used for rheumatism, smallpox, and ulcers. The extract of *A. marina* was also reported to have antioxidant activity and indicates the presence of alkaloid, saponin, flavonoid, tannin, sterol/triterpenoid, and coumarin. However, the comparison of the antioxidant activity of leaves, fruits, and stem barks is not evaluated yet. The purpose of this study is to compare the antioxidant activity, total phenolic and coumarin content of leaves, fruits, and stem barks of *A. marina*. The antioxidant activity was determined using DPPH radical scavenging assay and was evaluated by spectrophotometric method at 515 nm. Quercetin was used for comparison. The fruits had the highest antioxidant activity with an IC_{50} value of 85.246 ppm, followed by stem barks and leaves with IC_{50} of 205.281 ppm and 307.037 ppm, respectively. Although the antioxidant activity of *A. marina* fruits was far from quercetin (IC_{50} of 3.789 ppm), it still categorized as a strong antioxidant. The strong antioxidant activity of fruits was followed by higher total phenolic and coumarin content than the stem barks and leaves part. Total phenolic and coumarin content of fruits were 49.119 mg GAE/ g and 8.894 x 10^{-3} mg CE/g, respectively. The leaves part had total coumarin content of 8.418 x 10^{-3} mg CE/g, but it had low IC_{50}. It may be caused by the other secondary metabolite compounds that could reduce the antioxidant activity of coumarin.

1. Introduction

Avicennia marina is a mangrove species commonly known as grey or white mangrove. The name may come from their greenish-grey colored stem bark. The leaves are green but more like the greyish-green color on the lower surface. It has yellow-orange flowers and heart-shaped propagules in a pale green color [1]. In Indonesia, we called it "API API Putih" or white API API. *A. marina* is one of the major mangroves so it can easily found in every mangrove ecosystem [2]. It spreads in India, Bangladesh, Vietnam, Indonesia, New Guinea, and Australia [3].

Mangrove has known traditionally to have medicinal properties. The leaves, fruits, and barks parts of *A.marina* are known to treat skin diseases [4]. People also tend to use the stem barks part for treating rheumatism, smallpox, and ulcers [5]. Ethyl acetate extract of *A. marina* leaves showed the presence of alkaloid, saponin, flavonoid, tannin, sterol/triterpenoid, and coumarin [6]. The fruits of *A. marina* was reported to have antioxidant activity [7, 8]. Mukherjee *et al.* [9] were also reported DPPH radical inhibition percentage of stem barks and leaves of *A. marina*.

Phenolic compounds such as flavonoids, phenylpropanoids, and phenolic acids in fruits, vegetables, or foods have a potential role as an antioxidant [10]. The phenolic hydroxy groups in their chemical structures are responsible for their antioxidant activity [11]. Coumarin is one of phenylpropanoid group [12], that also poses antioxidant activity [13], anticancer[14, 15] antiviral[16, 17] and antiproliferative [18]. Despite many bioactivities of coumarins,

antioxidant, and antiproliferative were the most dominant effects [19].

In the previous study, the antioxidant activity of leaves, fruits, and stem barks of *A. marina* is not evaluated yet. So, this study aims to compare the correlation between total phenolic content, total coumarin content, and antioxidant activity of leaves, fruits, and stem barks of *A. marina*.

2. Methodology

2.1. Equipment and Material

The equipment used in this study were reflux apparatus, graduated cylinder, volumetric flask, dropper, micropipette (Microlit SLP series), beaker glass, erlenmeyer, funnel, spoon, ring stand and clamp, water bath, vial, digital scale(Mettler Toledo), TLC chamber, capillary pipe, filter paper, electric stove, magnetic stirrer, UV-Vis lamp (CAMAG), UV-Vis spectrophotometer (PG Instrument Limited Model T60U).

The materials used in this study were a sample of leaves, fruits, and stem barks of *A. marina* from Kaliwlingi village, Brebes, Central Java, Indonesia that collected in October 2018, coumarin (1,2 benzopyrone) (Merck), potassium hydroxide, methanol, ethanol, n-butanol, glacial acetic acid, distilled water, lead acetate, Folin–Ciocalteu reagent, gallic acid, Na_2CO_3 (Merck), DPPH (Merck), quercetin (Merck), and TLC plat 60 F254. All reagents used are of analytical grade and without further purification

2.2. Coumarin Identification

Identification of coumarin in leaves and fruits using a thin-layer chromatography method were made in Butanol: Acetic Acid: Water (4:1:5) mobile phase system. Chloroform: ethyl acetate (4:1) system was used for stem barks identification. The sample was prepared in the methanolic extract of leaves, fruits, and stem bark of *A. marina*. The methanolic extract of each part was applied several times on the spots marked on the line of the plate. The plate prepared with the sample spot was placed in a TLC chamber that already filled with the mobile phase and filter paper. Coumarin can be identified under UV 365 nm with blue and blue-green fluorescence and distinct fluorescence quenching for all coumarin under 254 nm UV light and intensified by spraying 5-10% (w/v) methanolic KOH [20].

2.3. Determination of Total Coumarin Content

Total Coumarin Content was determined using Osório and Martins [21] method with needed modifications. Each sample powder was weighed for 6.25 gr and extracted in methanol (80% v/v) under reflux for 30 minutes. The extract was filtered, and the filtrate was stored in a vial for analysis. Coumarin (1, 2 benzopyrone) was used as a standard. The extract (500 µL) and lead acetate solution (5%, w/v, 500 µL) were prepared in 10 mL of volumetric flask. The mixture was shaken by adding 2 mL of distilled water. Then 7 ml of distilled water were added to make 10 ml of mixture volume. The sample was prepared by taking 2 mL of the mixture and 8 mL of HCL solution (0.1 M v/v) into a 10 mL volumetric flask. The sample was shaken and kept at room temperature before the triplicate measurement at 274 nm. Total coumarin content was quantified using a calibration standard curve of coumarin (1,2 benzopyrone) varied from 3–12 ppm concentration. Total coumarin content was expressed as mg CE/g sample.

2.4. Determination of Total Phenolic Content

Lim and Murtijaya [22] method was used for total phenolic content determination of leaves, fruits, and stem barks of *A. marina*. The powder was weighed for 20 gr and extracted with ethanol in a hotplate for 30 minutes. After extraction, each extract was filtered and stored in a vial for analysis. It was quantified by the Folin–Ciocalteu method with gallic acid as a standard. The sample was made by taking 300 µL of methanol: water extract (1:1) (and 1.5 mL of Folin–Ciocalteu reagent (1:10). The mixture was shaken and waited for 3 minutes before adding 1.2 mL of Na_2CO_3 7.5%. The sample was ready after kept in the dark place for 30 minutes. The absorbance was measured triplicate at 575 nm (measured maximum wavelength). Quantification was done on a standard curve of gallic acid, and the result was expressed as mg GAE/g sample extract.

2.5. Determination of Antioxidant Activity

Antioxidant activity was measured following the method of Brand-Williams *et al.* [23] with some modifications. Each of the leaves, fruits, and stem barks powder were weighed 20 gr and extracted in 100 ml ethanol in a hotplate for 30 minutes. The extract was filtered using filter paper. The filtrate was evaporated. The solid extract of leaves and stem barks were made into various concentration (50, 100, 150, 200, and 250 ppm). The solid extract of fruit was varied into 50, 75, 100, 125, 150 ppm. Various concentration of the extract was mixed with DPPH solution (0.1 mM) by 1:3. The mixture was shaken and kept in the dark place at room temperature for 30 minutes. The sample was measured at 515 nm wavelength (measured maximum wavelength of DPPH). The reduction of DPPH radical was calculated by the equation of %inhibition of DPPH [24]. The reduction result was used for calculating IC_{50} value by using the equation from concentration versus %reduction of DPPH curve, and quercetin was used as a standard.

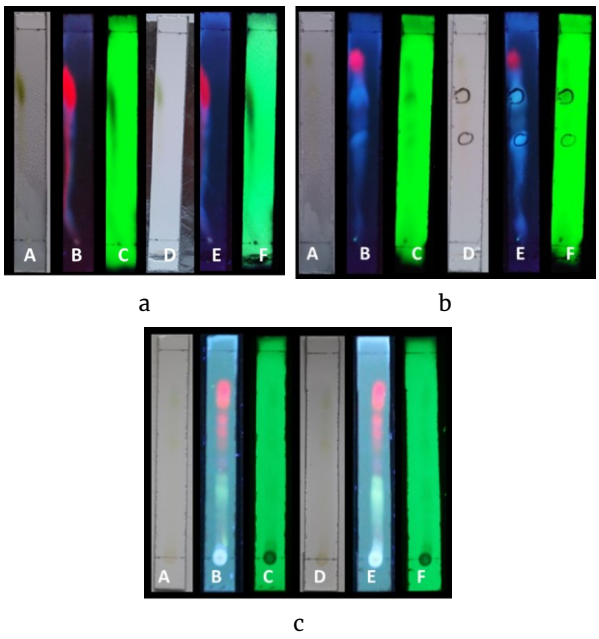
$$\%inhibition\ of\ DPPH = \frac{(A_{con} - A_{test})}{A_{con}} \times 100\%$$

A_{con}: the absorbance of control reaction
A_{rtist}: the absorbance of the sample extract

3. Result and Discussion

3.1. Coumarin Identification

Coumarin identification result was positive in the methanolic extract of leaves, fruits, and stem barks of *A. marina*. Two blue spots (leaves and fruits) and blue-green spots (stem barks) appeared in UV 365 nm. The color was intensified with methanolic KOH (5%, w/v) [20].



a b c

Figure 1. Coumarin identification in methanolic extract of a) leaves, b) fruits, and c) stem barks of *A. marina* in butanol:acetic acid: water (4:1:5) system for leaves and fruits, chloroform: ethyl acetate (4:1) system for stem barks, using silica gel F_{254} as stationary phase; (A) after elution; (B) under UV 365 nm; (C) under UV 254 nm; (D) after sprayed with methanolic KOH (5%, w/v) and heating; (E) under UV 365 nm after spraying and heating; (F) under UV 254 nm after spraying and heating.

3.2. Total Coumarin Content (TCC)

The 80% methanolic extract of fruits of *A. marina* contains the most coumarin followed by leaves and stem barks (Table 1). One of the most dominant bioactivities of coumarin was an antioxidant activity [19]. Coumarin was also present in *A. marina extract* [25, 26]. Of the previous study above, no study compares the antioxidant activity and total phenolic content of leaves, fruits, and stem barks of *A. marina* with adding the total coumarin content as one of the parameters that might influence the antioxidant activity.

3.3. Total Phenolic Content (TPC)

The highest TPC was obtained by the part of the fruits, followed by stem barks and leaves part (Table 1). In the previous study by Huang *et al.* [27] total phenolic content of leaves and fruits, the ethanolic extract of *A. marina* was 22.82±1.80 and 49.96±3.85 mg/g, respectively. The result is a bit similar to the leaves and fruits compared to our study. The use of a polar solvent in extraction might cause the similarity of both results. The study that uses methanol for the leaves extraction from different origins showed lower total phenol (1.6 g/100 g) [28]. For the stem barks part, there were not many studies about total phenol in *A. marina* stem barks extract. Another species of *Avicennia* (*Avicennia officinalis L.*) reported higher total phenol in 90% (v/v) ethanolic extract of the bark, which was 48.22±0.51 mg/g GAE [29]. In aqueous methanol (20%, v/v) extract of *Avicennia rumphiana* bark had lower total phenol (0.9072 mg/g GAE) [30]. The total phenol in three different species, variety, and origin of *the Avicennia* genus showed different results. The use of a solvent with different polarities might cause different results of total phenol.

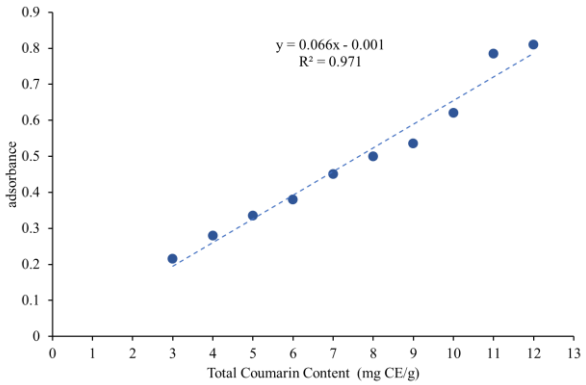


Figure 2. Standard Curve of Coumarin for Total Coumarin Content Calculation

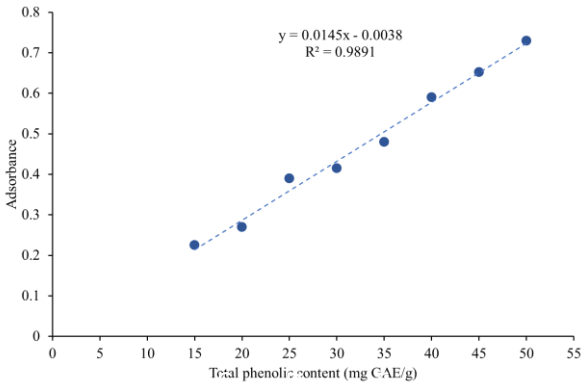


Figure 3. Standard curve of gallic acid for total phenolic content calculation

Table 1. Total Coumarin Content (TCC), Total Phenolic Content (TPC), and Antioxidant Activity (AA) of Leaves, Fruits, and Stem Bark of *A. Marina*

Analysis	Plant part		
	Leaves	Fruits	Stem barks
TCC (mg CE/g)	8.418×10^{-3}	8.894×10^{-3}	4.275×10^{-3}
TPC (mg GAE/g)	23.024	49.119	33.738
AA (ppm)	307.037	85.246	205.281

3.4. DPPH Radical Scavenging Assay

The methanolic extract of fruits of *A. marina* was found to be the strongest antioxidant agent followed by the stem barks part and the leaves part (Table 1). According to Jun *et al.* [31], it considered a strong antioxidant, although it was far from the standard quercetin (3.789 ppm). In the previous study, two isolates (caffeic acid derivative, maricaffeolylide A (1), and a new megastigmane derivative, maricyclohexene A (2) from *A. marina* fruits were run into antioxidant assay with EC_{50} of isolate 1 was 24±0.3 µm that was good for antioxidant agent [32]. Both results showed different antioxidant activity because the present study uses the crude extract for the assay. For the other studies, many of them studied the antioxidant activity of the isolates obtained from the isolates of the fraction of *A. marina* fruits. No article

performs antioxidant assay of crude extract of *A. marina* fruits.

The stem barks were the second-highest for antioxidant activity with medium type antioxidant followed by the leaves as the lowest IC_{50} (weak antioxidant) [31]. These results were supported by the previous study [9] that reported the stem barks n-hexane extract of *A. marina* showed higher inhibition of DPPH radical (about 80%) than the leaves part (about 76%). The detailed results cannot be compared because the previous study did not calculate the IC_{50} of antioxidants. The IC_{50} of leaves ethanolic extract with 48 hours maceration showed vigorous antioxidant activity (82.2792 ppm) [33]. The result was higher compared to the present study. The extraction time of the present study was only 30 minutes, so 48 hours extraction was higher in IC_{50}. Besides that, the origin, variety of plants, homogeneity of the young, and the old leaves of the mangrove that use in the experiment will make a different result. In other studies, the antioxidant activity of the leaves of *A. marina* showed the lowest result in polar and semi-polar solvent (methanol and ethyl acetate) compared to nonpolar solvent (n-hexane). In other studies, an excellent antioxidant activity was achieved by the use of a nonpolar solvent (acetone) for extraction [34].

4. Conclusion

The result of total phenol from the highest was fruits>stem barks>leaves. The antioxidant activity showed the same result. Potent antioxidant was started from fruits>stem barks>leaves. For the total coumarin content, the leaves were the second-highest close to the fruits, but it had low IC_{50}. It was possible because there might be other secondary metabolites on the leaves that decrease the antioxidant activity. For further study, analysis of major and minor phytoconstituents of leaves, fruits, and stem barks extract needs to be done in the future.

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