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Total Fenol dan Aktivitas Antioksidan dari *Sonneratia alba* Bark

Total Phenolic and Antioxidant Activity of Sonneratia alba Bark

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ABSTRAK

Tujuan penelitian ini adalah untuk menguji kandungan total fenol dan aktivitas penangkapan radikal bebas yang berkaitan dengan potensi antioksidan dalam berapa fraksi (n-heksana, kloroform, dan etil asetat) dari ekstrak metanol kulit batang tumbuhan mangrove, *Sonneratia alba.* Kandungan fenol total ditentukan dengan metode Folin-Ciocalteau sedangkan potensi antioksidan ditetapkan sebagai kemampuan penangkapan radikal menggunakan radikal stabil diphenyl-picryl-hydrazyl (DPPH). Kandungan fenol total ketiga fraksi dan ekstrak metanol adalah 217.84 ±0.974, 156.310 ±0.703, 226,89 ±0.605, 249.56 ±0942 GAE μg/g, berturut turut untuk fraksi n-heksan, kloroform, etil asetat, dan ekstrak metanol. Ekstrak metanol dan fraksi etil asetat menunjukkan aktivitas penangkapan radikal bebas lebih tinggi dari asam askorbat sebagai control positif dengan nilai IC_{50} berturut-turut sebesar 9,28, 10.27, 17,64 μg/ml, kloroform menunjukkan aktivitas yang sedang (27,34 μg/ml) dan n-heksan tergolong lemah (147 μg/ml). Terdapat korelasi linier antara aktivitas antioksidan dan kandungan fenol total dalam kulit batang *S.alba* (r^2=0.918). Data ini mengindikasikan bahwa aktivitas antioksidan disebabkan oleh senyawa fenol yang terkandung dalam kulit batang *S. alba.* Hasil penelitian ini menunjukkan bahwa *S.alba* dapat menjadi sumber antioksidan alami yang penting.

Kata kunci: *Sonneratia alba*, total phenolics, aktivitas penangkapan radikal bebas, antioksidan, DPPH

ABSTRACT

The aim of this research was to examine the total phenolic content and radical scavenging capacity related to antioxidant potential in different fractions of methanolic extract of mangroves trees, S*onneratia alba* bark. Total phenolic content was determined by the Folin-Ciocalteau method. Antioxidant potential has been determined as the free

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radical scavenging ability using a stable radical, diphenyl-picryl-hydrazyl (DPPH). Three fractions of methanol extracts of *S. alba* were subjected to free radical scavenging activity in order to evaluate their antioxidant activity. The free radical scavenging activity of different fractions obtained from successive fractionation of methanol extracts with organic solvents of different polarities; hexane, chloroform, and ethyl acetate. Total phenolics content of three fraction and crude extract are 17.84 ±0.974, 156.310 ±0.703, 226.89 ±0.605, 249.56 ±0942 (GAE μg/g) respectively for hexane, chloroform, ethyl acetate, and crude extracts (methanol extract). Antioxidant assay showed that the ethyl acetate and chloroform fractions have the high activity with IC_{50} 10.27 μg/ml and 27.34 μg/ml, respectively, while a weak activity was showed by hexane fraction with 147 μg/ml. Linear correlation between the total antioxidant capacity of the fractions and the total phenolic contents was observed (r^2=0.918). This data indicated that the antioxidant activity mainly due to phenolic compound contain in those fraction. The results indicate promising *S.alba* or the utilization as significant source of natural antioxidant.

Key words: *Sonneratia alba*, total phenolics, radical scavenging activity, antioxidant, DPPH

INTRODUCTION

A mangrove usually grows in estuarine swamps; have unique adaptations to combat environmental stress conditions e.g. high salinity, high temperature, low nutrient and excessive radiation. An inevitable consequence of this process results in the production of ROS (reactive oxygen species) and accordingly the antioxidant enzymes were upregulated due to altered expression of these antioxidant genes (Banerjee, *et al.,* 2008; Jitesh, *et al.,* 2006). Moreover, mangroves are good source of polyphenols like tannins (Nashkar and Guha, 1995). Phenolics have been considered classic defense compounds for protecting plants from herbivores, ever since plant secondary metabolites were suggested to have evolved for that reason. In contrast to these concepts, it has been suggested that the main role of many plant phenolics may be to protect leaves from photo damage, not herbivores; they can achieve this by acting as antioxidants; and their levels may vary with environmental conditions in order to counteract this potential photo damage. The phenolics especially flavonoids were shown to protect mangroves from UV radiation (Agati, *et al.,* 2007).

Sonneratia alba, one of mangrove species, has been used as preservative in production of alcoholic traditional beverage from palm trees. Firdaus and Sinda (2003) have reported that *S. alba* bark played an important role to prevent the formation of acetate acid generated from oxidation of ethanol. We suggest that preservative property of this plant due to the presence of antioxidant or antibacterial compound. In addition, It is reported that *S.alba* was traditionally used as astringent and antiseptic, sprain poultices, in treating piles and in arresting hemorrhage (Bandanarayake, 1996). However, the in vivo safety of extracts needs to be thoroughly investigated in experimental rodent models prior to its possible application as an antioxidant ingredient, either in animal feeds or in human health

foods. Currently, relevance of in vitro and in vivo tests of measuring antioxidant activity is also vital before launching human clinical trials. It is not desirable to assay any biological activities through in vivo methods directly without conducting in vitro tests using chemical methods or cell lines. Hence, the aim of the present study was to examine the total phenol content and radical scavenging capacity related to antioxidant potential in different fractions of methanol extract of mangroves trees, *S.alba* bark.

MATERIALS AND METHODS

1. Plant Material

Sample of bark of *S.alba* were collected within the Province of South Sulawesi (Indonesia) in February 2009. Bark was air dried, grounded to fine powder, and stored on tight-seal dark plastics container until needed.

2. Chemicals

All chemical and reagent were analytical grade purchased from Merck (Darmstard-Germany), DPPH , butylated hydroxyltoluene (BHT), folin–ciocaleteau reagent; L(+) ascorbic acid, etylenediamine tetraacetic acid (EDTA) , trichloroacetic acid (TCA), $FeCl_2$, $FeCl_3$, potassium ferricyanide, phosphate buffer saline, methanol, hexane, etil acetate, chloroform.

3. Extract Preparation

The air-dried bark of *S.alba* (10 kg) was extracted with MeOH. The MeOH extract, on removal of solvent under reduced pressure, gave a dark brown residue. The residue (total extract) was partitioned with n-hexane, chloroform and ethyl acetate.

4. Determination of DPPH free Radical-Scavenging Activity

The method of Blois (Borneo *et al.,* 2009) was adopted. The free radical scavenging activity of different fractions and crude extract were determined using the stable radical DPPH. Aliquots of various concentrations of the tested sample were placed in test tubes and 3.9 ml of freshly prepared DPPH solution (25 µg/L) in methanol was added in each test tube and mixed. 30 min later, the absorbance was measured at 517 nm. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenged (\%)} = \frac{(Ac - At)}{Ac} \times 100\%$$

Where Ac is the absorbance of the control reaction and at is the absorbance in presence of the sample of the fractions. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration in mg of dry material per ml that inhibits the formation of DPPH radicals by 50%. Each value was determined from regression equation.

5. Statistical Analysis

Data were generated for each assay from an extract and three separate fractions in duplicate. A one-way ANOVA test was performed on the antioxidant activity results to investigate significant differences between the extracts. The method used to discriminate among the means was Duncan's multiple range tests. Simple regression analysis was performed to look

for relationships between GAE for different extracts.

RESULTS AND DISCUSSIONS

1. Total Phenolic Content

The crude methanolic extract showed a high content of total phenolic (Table 1). Phenolics content in all fractions were found to have significant differences ($P<0.01$) with ethyl acetate fraction was higher than those of the hexane and chloroform. Those data showed that most of phenolic compound in crude extract exist in ethyl acetate fraction after fractionation. It can be conclude that phenolic compounds in *S. alba* bark are semi polar for their high solubility in ethyl acetate solution.

Table 1. Total phenolics content and radical scanvenging activities of crude extract and fractions.

Fractions	Total phenolics content (GAE mg/g dry extract)	IC_{50} (µg/ml)
Hexane	17.84 ± 0.974^{D}	147^{c}
Chloroform	156.310 ± 0.703^{C}	27.34^{b}
Etil acetate	226.89 ± 0.605^{B}	10.27^{a}
Crude Extract	249.56 ± 0942^{A}	9.28^{a}
Ascorbic acid		17.64

Means with different letters within a column were significantly different at P,0.01[a]Each values was presented as the mean ± SD (n=2)

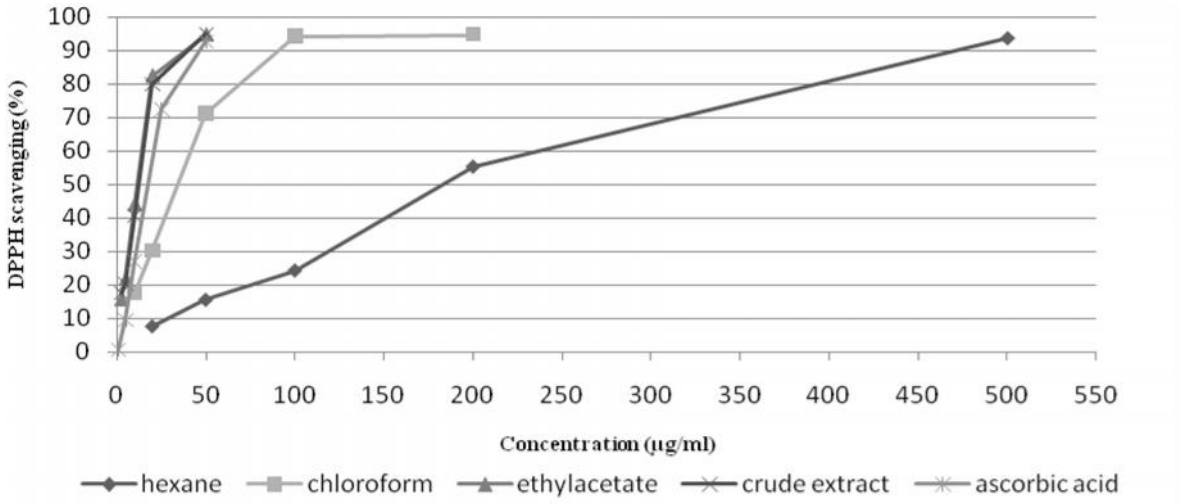

Figure 1. DPPH radical scavenging activities of crude extract and fractions

2. Radical Scavenging Activity Using DPPH Method

DPPH radical scavenging activity was expressed as inhibitory concentration (IC_{50} in µg/ml), the lower the IC_{50} value is the greater the free radical scavenging activity. All fractions evaluated had substantial DPPH scavenging activity ranging from 12,45 to 177,05 µg/ml (table 1). Scavenging activity increased as the fractions concentration increased and the order of scavenging activity was: hexane<chloroform<ascorbic acid (control) <ethyl acetate. IC_{50} value was obtained from regression linier (figure 1).

The highest free radical scavenging activity was recorded in ethyl acetate fraction *i.e.*11,66 µg/ml, which is much

higher than ascorbic acid as positive control followed by crude extract, ascorbic acid, chloroform and hexane fraction (12,45, 17,64, 41,09, 177,05 µg/ml) as shown in table 1. Base on the antioxidant data obtained, the activity of crude extract, ethyl acetate, and chloroform fraction were categories high antioxidant power. Any molecule that can donate an electron or hydrogen to DPPH can react with DPPH and thereby bleach the DPPH absorption.

Antioxidant activity of the sequential extracts/fractions are presented in fig. 1. The results indicate the choice of the solvent for obtaining the extract with high antioxidant activity. A high radical scavenging activity was observed in each fraction in a concentration dependent manner. Proton-radical scavenging action is an important attribute of antioxidants, which is measured by DPPH radical scavenging assay. DPPH, a protonated radical, has characteristic absorbance maxima at 517 nm which decreases with the scavenging of the proton radical (Srivastava *et al.,*2006; Yamaguci, 1998).

Hydrogen-donating ability of the antioxidant molecule contributes to its free radical scavenging nature (Srivastapa *et al.,*2006; Chen and Ho, 1992). It is known that free radicals cause auto oxidation of unsaturated lipids in food (Jayaprakhash *et al.,* 2007; Kaur and Perkins , 1991). On the other hand, antioxidants are believed to intercept the free radical chain of oxidation and to donate hydrogen from the phenolic hydroxyl groups, thereby forming stable end product, which does not initiate or propagate further oxidation of lipid (Jayaprakasha *et al.,* 2007). The data obtained revealed that all fractions isolated from *S.alba bark* are free radical scavengers and primary antioxidants that react with DPPH radical, which may be attributed to its proton donating ability.

3. Correlation Between *S.alba* Bark Total Phenolics Content of Extract and Their Radical Scavenging Activity

Antioxidant activity of the plant extract is often associated with the phenolic compounds present in them. Hydrogen donating property of the polyphenolic compounds is responsible for the inhibition of free radical induced LPO (Liu *et al.,* 2008; Yen *et al.,* 1993). Correlation between the content of the total phenolics and radical scavenging activity of extracts and fractions were studied and the results are presented in table 1 and figure 2.

Some of the studies reported no correlations between the total phenolic content and the radical scavenging activity (Yu *et al.,* 2002), but in our study, there seemed to be high correlation between the phenolic content and antioxidant activity of the extracts (r^2=0.91). The high correlation between total phenolic and radical scavenging activity indicate that phenols compound is mayor contributor to antioxidant activity of both crude extract and fraction. While, hexane fractions with lower phenolic content still showed a weak antioxidant activity. However, it is known that nonphenolic antioxidants could also contribute to the antioxidant activity of an extract (Haris and Shivanandappa, 2005).
The results of this study strongly suggest that phenolics are important components of this plant, and some of its pharmacological effects could be attributed to the presence of these valuable constituents.

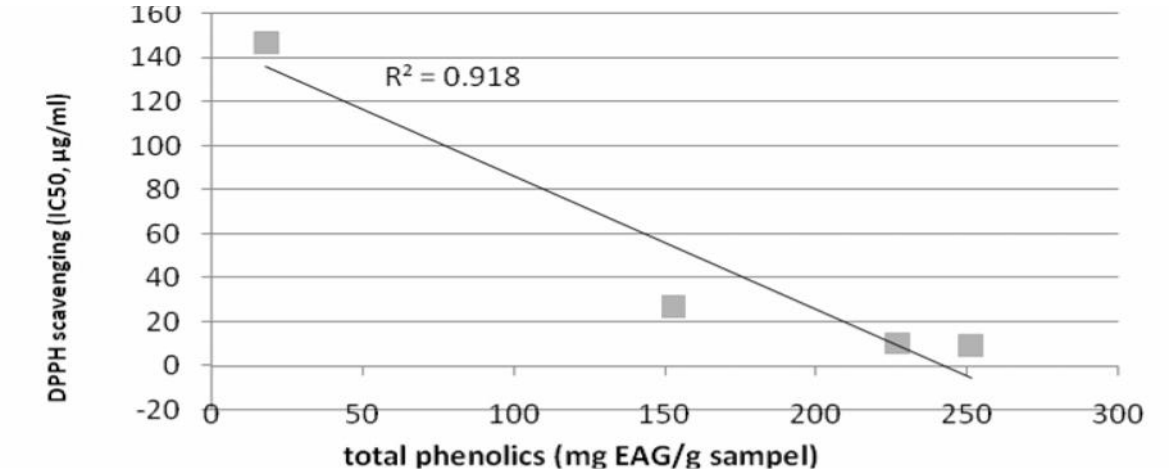

Figure 2. Correlation between *S.alba* total phenolics content and their radical scavenging activity by DPPH method of hexane , chloroform, ethyl acetate, and crude extract.

CONCLUSION

The results obtained in the present study demonstrate that the bark of *S.alba* contain a high total phenolic content. A good correlation between total phenols content and the radical scavenging activity support the idea that phenols may be the principal contributor of the antioxidant power of *S. alba* bark. The antioxidant activity of fractions demonstrated in this study clearly indicates the potential application value of *S.alba*. Further studies are needed on the isolation and characterization of individual compounds to elucidate their different antioxidant mechanisms.

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