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Antioxidant Activities of Mangrove Fruit (*Sonneratia alba*) taken from Wori Village, North Sulawesi, Indonesia

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Abstract : Mangrove fruits of Sonerratia alba has beenused licallyas food ingredients. However, it has not been developed yetdue to the lack of information about the potential and benefits of thefruit, in particular as a source of antioxidants. There is no research has been revealed about the antioxidant activity of methanol extract or fractions of S. albafruit. The aims of this study are to know the antioxidant activitiesthe extract of menthanol and also the fractions of n-hexane, ethyl acetae, and water obtained from fruit flour of S. alba. The method used in achieving the specific aims consists of two steps. First stepwasextraction with methanol, and then the extract was tested for total phenol and antioxidant activities. Second step was fractionated based on solventpolarity, namely:n-hexane, ethyl acetate and water. The solvents were then tested for total phenol, antioxidant activities of DPPH and Frap, and phytochemical testto obtain the most active fraction. The result showed that, the highest content of frap was in ethyl-acetate fraction i.e. 6.329 μ MFe^{2+}/mg of sample and the lowest of that was in n-hexane fraction i.e. 0.413 μ MFe^{2+}/mg of samples. The highest content of total phenol was in ethylacetate fraction, i.e. 3119 mg GAF/kg of sample and the lowest of that was in the n-hexane fraction i.e. 540 mg GAE/kg of sample. Phytochemical test showed that the ethyl acetate and water fraction gave a positive indicator to most of the test except for the steroid and saponin for ethyl acetate fraction, and triterpenoid and saponinfor water fraction, while the n-hexane fraction gave a lowest positive in indicator. Ethylacetate fraction has the most powerful antioxidant activity with IC_{50}=3.55 ppm followed by methanol extract IC_{50}=4.65ppm, the water fraction IC_{50}= 6.95 ppm, and n-hexane fraction IC_{50}= 162.79 ppm.
Keywords : *Sonneratia alba*, antioxidant, phenols, FRAP, phytochemicals.

Introduction

Study of mangrove forest communities in North Sulawesi indicated that the dominant species were *Rhizopora, Bunguiera* and *Sonneratia*. Dominant mangrove species in Wori village was *Sonneratia alba*[1] . In several regions of Indonesia such as Java, Sulawesi and Maluku, mangroves have been used traditionallyas medicines, beverages and raw material for a wide variety of cakes. However, cannot be developed because lack of knowledge about the potential and benefits of mangrove plants as food and functional food ingredient. [2]

According to Goldberg [3], plants that contain antioxidants can be formulated into functional foods, antioxidants and into daily menu. Based on recently research, formulation of functional food have benefits

such as increase endurance, increase appetite, accelerate the growth of the body, and can indirectly improve various.

Mangrove plants are usually in a state of stress, because of a harsh environment, extreme tidal, high content of organic matter, high minerals,and abundant living organismssuch as microorganisms and insects [4]. Plants that can survive in such an environment are necessarily contained compounds that can protect itself from destruction [5], Mangrove grown in tropical climates, can dometabolism throughout the year to produce a variety of the bioactive compounds as important secondary metabolite.The condition of the tropical environment trigger plants of one species, to generate new compound, and will always change and devolopment, along the times, produced many combinations and derivative products[13].

This study was conducted to determine the effect of extraction with methanol, and multilayer fractionation to methanol extract with different solvents based on the level of polarity (water, ethyl acetate and *n*-hexane) toantioxidant activity of fruit mangrove *Sonneratia alba*.The antioxidant activity of phytochemicalswere assessed for: total phenols, ability to capture free radicals 1,1-diphenyl-2-pikrihidrazil (DPPH), and Ferric reducing antioxidant power (FRAP).

Materials and Methods

Samples

The fruit of mangrove *Sonneratia alba*, which was taken from village of Wori Minahasa Utara Regency, North Sulawesi Province, Indonesia. Fruit picked and sorted to get homogen diameter of 3-5 cm,. Fruits were then washed and sliced thinly,and allow to driedatroom temperature. The drying time takes 10-14 days. The driedfruit were then blendered into the powder.

Chemical compund and reagent

Solvent used was methanol, sodium phosphate, disodium phosphate, trichloroacetic acid(TCA), $FeCl_3$ obtained from Merk. Folin-Ciocalteu,1,1-diphenyl-2-picrihidrazyl, ferrozin and gallic acid were obtained from Sigma.

Preparation of sample Extracts

Two hundred grams of dried powder samples were macerated with two liters of methanol for 48 hours, filtered, and separated from the pulp using What manno1 filter paper. The pulp were then macerated again in the same way as above for-2 times. All yields collected and evaporated with a rotary vacuum evaporator at temperature of 40°C, resulted a semi-solid extract (methanol crude extract). Methanol crude extract was further partitioned with each 200ml of a mixture of *n*-hexane-methanol (1:1), *n*-hexane section separated from the water and placed in thee vaporation flask, it is repeated until the part of *n*-hexane was colorless, subsequent sections were water spartitioned with 200 ml of ethylacetate according to the procedure in *n*-hexane to obtain ethyl acetate part. The final remaining part of the partitioning process was part of the water, then the third part is evaporated by rotary vacuume vaporator at 40°C and the*n*-hexane, ethylacetate and water fraction. The third fraction is packaged in dark glass containers and storedat-20° Cuntil used for analysis.

Phytochemical test

The methanol extracts and fractions, namely fraction of *n*-hexane, ethyl acetate and water, were analyzed quantitatively using phytochemical test [7-8].

Total Phenol Content

Total phenols in methanol extract and in each fractions i.e. *n*-hexane, water and ethyl acetate fractions, from the fruit of mangrove *S.alba*, can be obtained by determining the concentration of the extract and the concentrations of each fraction and measuring the absorbance at each level of concentration at a wavelength of 760 nm. The data is then included in the regression line, where the regression equation was obtained by measuring the absorbance of gallic acid concentrations.

Total phenol content was measured using Folin-Ciocalteu reagent by modifying methods of Devi *etal.*[9], Ganesan *etal.*[10], and Anarwulan *e tal.*[11]. As much as 0.1 gram of extract was dissolved in 10 ml methanol in a flask,and then of 0.1 ml extract solution was taken an added1mlof1:2Folin- Ciocalteu-aquadest,and left for5 min.It was then added 1ml sodium carbonate 7%, homogenized and incubated at room temperature for 30 minutes in dark condition.The mixture absorbence was measured at 750 nm.Total phenol content was interpreted as mg gallic acid equivalents(GAE)/kg sample.The regression equation ofgallic acid was y=1,0059X – 0,0013 (R^2= 0,9991).

The Antioxidant Activity.

Ferric Reducing Antioxidant Power(FRAP).

Value of FRAP in the methanol extract and fractions of *n*-hexane, water and ethyl acetate, from the fruit of *S. alba*, can be obtained by determining the concentration level of the extract and concentration levels of each fraction. Each concentration was then measured it absorbance at a wavelength of 593 nm. The data then included in the regression equation, which is obtained from the concentration of Fe^{2+} levels by measuring the absorbance at each level of concentration.

Measurements performed using the method of ferric reducing antioxidant power Khumar, *et al.*[12],, Chew, *et al.*[13], Andarwulan ,*et al.*[11], were modified as follows: extract were prefored with a concentration of 2000 ppm in methanol, furher more taken 1ml and was mixed with 1ml of phosphate buffer(0.2M, pH6.6) and 1ml of potassium ferrici-anida[$K_2Fe(CN)_6$]1%. Mixture was homogenized and incubated at 50°C for 30 minutes (mixtureA). One ml of trichloroacetic acid (10%) was added to a mixture (mixture B), a mixture of B and centrifuged (10min,3000 rpm). Subsequently 1 ml was taken on the to player of mixture B was added to 1 ml of water and 5 ml of distillate $FeCl_3$ 0.1% and the mixture homogenized, mixed absorbance was measured at 700nm. Reducing power values are interpreted as µM ion Feroequivalent (Fe^{2+}µM)/mg sample.µM ion Fero calibration curve was y=0.1994x+0.027(R^2=0.9975).

DPPH ScavengingAntioxidantActivity.

Percent of inhibition and concentration of each extract and fraction, were fixed to alinear equation, respectively, and then IC_{50} value of each sample was obtained by replacing Y = 50 of each regression equation.

DPPH radical scavengers were measured using the modified method of Khumar, *et al.*[19], two ml of DPPH93 µM were addedto0.5 ml of extract (2000 ppm in methanol). The extract were shaken and incubated at37°C for 30 min, absorbance was measured at a wavelength of 517 nm. Yuan and Walsh,[14]; Devi,*et al.*[9]: states that the activity of DPPH radical scavenging determined as percent inhibition and calculated by the equation: % inhibitory={(Absorbance of control-Absorbance of the sample) /(Absorbance of control)}x100.

Result and Discussion.

Phytochemical.

Phytochemical test results of mangrove *A. Alba*, can be seen in Table 1.

Table 1. PhytochemicalsTest of Mangrove*S. Alba*Fruit Extract and Fractions

No.	Metabolite Secondary	Phytochemical Test	Result test			
			***n*-Hexana**	**EtOAc**	**Water**	**MeOH**
1	Phenolic	Reagent $FeCl_3$ 5%	+	+	+	+
2	Flavonoid	a. Reagent HCl + Mg	-	+	+	+
		b. Reagent H_2SO_4 2N	-	+	+	-
		c. Reagent NaOH 10%	+	+	+	+
3	Steroid	Reagent Lieberman-Burchard	+	-	+	+
4	Triterpenoid		+	+	-	-
5	Saponin	Reagent HCl + H_2O	-	-	-	-
6	Tanin	Reagent $FeCl_3$ 1%	-	+	+	+

Data of phytochemical test extract *S. alba* in Table 1showed that the ethyl acetate and water fractions gave a positive indicator to most of thephytochemical test except for the steroidand saponin fractions for ethyl acetate, and saponins and triterpenoid for water fraction, while the *n*-hexane fraction gave a lowest positive indicator. According to Jacoeb *etal.*[15], the bioactive components were detected on the leaves of Api-api are flavonoid, steroid and sugar reduction, while the young and old leaves and the fruit of mangrove *S.alba* contain flavonoid, tannin, saponin, and the steroid[16].

Total Phenolic.

Total phenol histograms of methanol extract and fractions, can be seen in Figure 1.

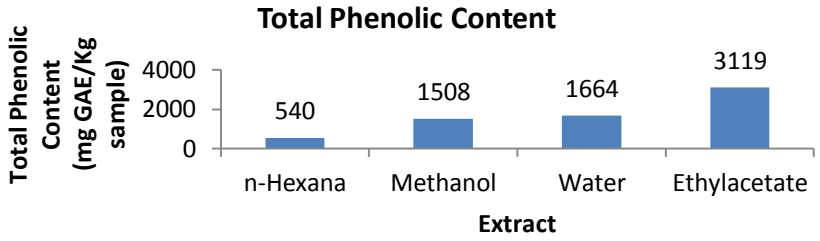


Figure 1. Total PhenolContent of *S.alba*

Figure 1 showed that, the highest total phenols wasin ethylacetate fraction i.e. 3119 mg GAE/kg sample, whereas the lowest was in *n*-hexane fraction i.e. 540 mg GAE/kg. Total phenol in methanol extract is 1508 mg GAE/kg and in fraction of water is 1664 mg GAE /kg. Different levels of total phenols in *S. alba* are: ethyl acetate> water> methanol>*n*-hexane. The number of -OH groups on the core of flavonoid antioxidant phenol compounds affecting the activity, the more the -OH group, the higher the antioxidant activity [17]. According to Pratt and Hudson [18], phenolic compounds that have a function as antioxidants, play a role in the process of lymphocyte cell membrane protection from oxidation, caused by free radicals and stimulates cell proliferation of lymphocytes.

FRAP.

Histogram of FRAP value, can be seen in Figure 2.

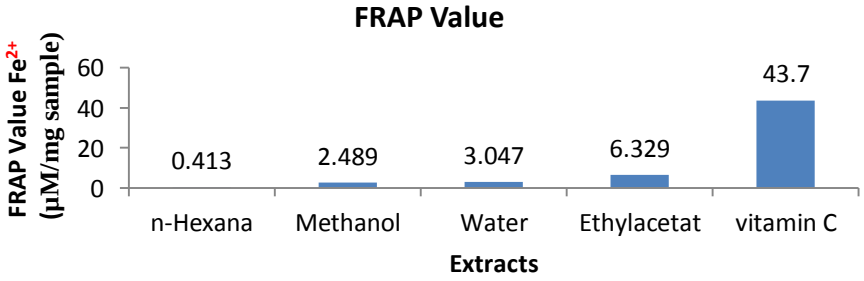
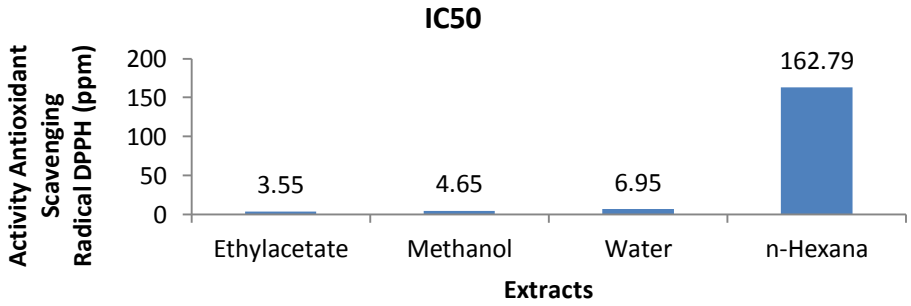


Figure 2. FRAP value of *S. alba*

Figure 2 shows that extract containing the highest FRAP value is ethyl acetate fraction i.e. 6.329 µMFe^{2+} /mg sample. FRAP of Vitamin C just as a comparison. The lowest FRAP was 0.413 Fe^{2+}*n*-hexane fraction (µM/mg sample), FRAP of methanol extract was 2.489 Fe^{2+} (µM/mg sample), and water extract was 3,047 Fe^{2+} (µM/mg sample). Different levels FRAP in *S. alba* are: ethyl acetate> water> methanol>*n*-hexane.Data of total phenol and FRAP positively correlated. The FRAP valueswas highest in leaf methanol extracts of both *C.manghas* and*E. agollacha* is4,75 ±0,08 mM AAE/g dry wt and lowest was in *B.Parviflora* is 1,02 ±0,015 mM AAE/g dry wt. In the present study *C. manghas* and *E. agollacha* was higher reducing power than other species.The highest FRAP value of root sample was recorded in *C.iripa* is 5,19 ±0,40 mM AAE/g dry wt. followed by *A.marina* is 4,84±0,21 mM AAE/g dry wt. Sample with lowest FRAP value was

observed in both *Heritierafomes* an *Heritieralittoralis* is1,46±0,23 mM AAE/g dry wt [19].The results of research value FRAP leaves and roots highest by Rout and Basak[19], lower than the value of FRAP fractions ethyl acetate mangrove *S. alba*.

DPPHScavenging antioxidant activity.



IC_{50} data from all the samples either extracts or fractions can be seen in Figure 3.

Figure 3: Antioxidant activity of DPPH Free Radical Scavinging *S. alba*.

Figure 3 shows that the ability of antioxidant to reduce free radicals DPPH is very strong in ethyl acetate fraction with IC_{50} = 3.45 ppm, followed by methanol extract with IC_{50} = 4.65 ppm, water extract with IC_{50} = 6.95 ppm, and the weakest is the fraction of *n*-hexane extract with IC_{50} = 162,79 ppm. Ordinal levels of antioxidant activity to reduce free radicals DPPH are: ethylacetate> methanol> water>*n*-hexane. Total phenol and FRAP were not positively correlated with antioxidant activity to reduce free radicals DPPH. Solvents used were based on the nature of polarity, solubility and mass transfer of compound extracted [20]. According to Samarakoon *et al*.[21], methanol extract of the leaves Phoenix paludosa showed the highest antioxidant activity compared to solvent *n*-hexane, chloroform and ethyl acetat. Extracts methanol, ethanol and chloroform on the leaf and bark *S. alba* showed good antioxidant activity with IC_{50} values betwen 0,019-0,37 mg/ml [22]. According to Gawali and Jadhav [23], the antioxidant activity of extract methanol 80% in soxhlet from stem and leaves *S. alba*was IC_{50} 62,5 and 87,5 ppm while from*B. Cylindrica* was 162,5 and 175 ppm. According to Banerjee *et al*.[24], the Antioxidant activity methanol extract 20% *Ceriops decandra* stem bark showed the lowest IC_{50}= 0,65 ppmand Suaeda matima was found to have the highest IC_{50}=119 ppm. Strong inhibition was observated for *C. decandra*IC_{50} root = 0,93 ppm and *Aegiceras corniculatum*IC_{50} stem = 0,96 ppm.A compound that have a very strong antioxidant activity if has IC_{50}< 50 ppm, strong if has IC_{50} 50-100 ppm, medium if has IC_{50} 100-150 ppm and weak if has IC_{50} 150-200 ppm [25].

The fruit *S. alba* taken from Wori village have IC_{50} = 3.45 ppm for ethyl acetate fraction, IC_{50} = 4,65 ppm for extract methanol and IC_{50} = 6.95 ppm for the water fraction, stronger Compared to the same species of mangrove *S.alba* but taken from different location, gave different results even with the same solvent, ie methanol extract IC_{50} = 39.30 ppm of fruit *S.alba*[16]. According to Sudirman et al.[26], old fruit of *Bruguieragymnorrhiza* has a more effective antioxidant activity IC_{50} = 13,47 ppm compared to young fruit IC_{50} = 81,60 ppm.The characteristics and composition of each mangrove species are influenced by weather factors, coastal land form, the distance between the tide, water availability and soil type [5].

Conclusion

Mangrove fruit *S. alba* extracted with methanol, ethyl acetate fraction and water fraction has potential to be source of natural antioxidant, based on assessment of phytochemicals, total phenols FRAP and IC_{50}. Phytochemicals detected were: phenolics, flavonoids, steroids, triterpenoids and tannins, while saponin was not detected in fruit mangrove *S. alba*. Data total phenol and FRAP positively correlated, but not positively correlated with antioxidant activity DPPH free radical absorbans.The highest total phenol was in ethyl acetate fraction i.e. 3119 mg GAE/L and the lowest was in hexane fraction i.e. 504mg GAE/L. The highest FRAP value was in the ethyl acetate fraction i.e. 6.329 Fe^{2+} (µM/mg sample) and the lowest was in the hexane fractioni.e.

0.413 Fe^{2+} (µM/mg sample). The strongest IC_{50} wasthe fraction of ethyl acetate i.e. 3.45 ppm and the weakest is the fraction of hexane i.e.162.79 ppm.

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