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Phytochemical Content, Total Phenols, and Antioxidant Activity of Mangrove*Sonneratia alba*Young Leaf Through Different Extraction Methods and Solvents

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Abstract : This study was aimed at finding out the antioxidant potential of mangrove *Sonneratia alba*young leaf collected from Wori village, Wori district, North Sulawesi. The extract was obtained from dry powder of young leaf of *S.alba*using 2 extration methods (soxhlet and maceration), and2 solvents (methanol and ethanol). Phytochemical analyses were qualitatively done to detect the presence of phenols, flavonoid, tanin, steroid, triterpenoid,and alkaloid, total phenols using Folin Ciocalteau and antioxidants using DPPH (1-1-diphenil-2-pikrihidrasil) method. Results found that the extract rendement was higher in soxhlet extraction using 9.77% methanol or 9.18% ethanol than in maceration method using 2.61% methanol and2.51% ethanol.Phytochemical analyses found that soxhlet extraction with either methanol or ethanol detected all phytochemical components tested, while maceration extraction did not detec the presence of alkaloid**.** The highest total phenol was recorded in the maceration extract with ethanol (34.2 mgGAE/g extract) followed by soxhlet extraction with methanol (33.6 mgGAE/g), metanol maceration (31.7 mgGAE/g), and ethanol maceration (28.6 mgGAE/g). Higher antioxidant activity was found in 2 samples macerated with ethanol (IC_{50} DPPH=5.01µg/mL) and sokhlet with methanol (IC_{50} DPPH=5.16µg/mL) than that of vitamin C (IC_{50} DPPH=5.21µg/mL), while 2 other samples had lower antioxidant activity than that of vitamin C, soxhlet ethanol extract (IC_{50} DPPH=6.23µg/mL) and methanol maceration (IC_{50}=7.45µg/mL). As a whole, this study concluded that young leaf extract of *S.alba*is potential as natural antioxidant source.
Keywords : young leaf, *S.alba*, phytochemicals, total phenol, antioxidant.

Introduction

Bioactive antioxidant is active chemical compound that can ward off free radicals source of various diseases, such as cancer, stroke, rheumatic, and cardiovascular, from aterosklerosis and haemolysis of the erythrocytes[1,2].

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The use of synthetic antioxidant nowadays starts to be limited because it is carcinogenic so that the use of natural antioxidant is developing. Natural antioxidants mostly originate from plants, including mangrove, and they are phenolic compounds distributed in the entire body of the plant, i.e. root, wood, wood skin, seed, flower, pollen, and fruit[3].

Indonesia is a country that possesses mangrove forest with the highest biodiversity and the most varied structure,and mangrove plant has been traditionally utilized as drug, drink, and raw materials of various cakes in Indonesia, such asJava, Sulawesi, and Moluccas. However, it cannot have been developed yet because there is not much knowledge available about the potential and the benefit of mangrove plants as source of functional food and food materials[4].

Sonneratia alba fruit is not toxic, in which the unripe fruits can be directly eaten and processed as syrup, while the ripe ones can be used as raw material of cookies production[5], and its leaves can be taken as vegetables. Extracts of *S.alba* stem and leaf obtained using soxhlet method with 80%methanol-water have 50%inhibitionconcentration of diphenyl picryhidrazil(IC_{50} DPPH) as much as62.5 and 87.5ppm [6].It was found [7] that DPPH free radical inhibition of methanol extract of *S. alba*stem skin was 40.71% at 10 ppm.Methanol maceration extract of *S. alba* stem has IC50 of 14µg/ml[8]. Also, Kusyana [9] found that methanol maceration extract of *S.alba*leaf has IC_{50} DPPHof 37.43 ppm. Methanol extract of*S.alba*fruit had antioxidant activity IC_{50} DPPH of 4.65 ppm. All these reports indicate that this plant has very strong antioxidant activity [10]. They alsoreported that methanol extract of *S.alba*collected from Wori, North Minahasa regency, North Sulawesi, Indonesia, contained phenols, flavonoid, steroid and tanin, with total phenol of 1.51 mg GAE/g sample. All these are positively correlated with antioxidant of *S.alba*fruit.

This study focuses on total phenol content, phytochemical content and antioxidant activity of *S.alba*leaf using two different extraction method (soxhlet and maceration) andtwo different solvents (methanol and ethanol).This study is important since *S. alba*is mangrove species dominantly growing in the coastal area of Wori village, North Minahasa regency, North Sulawesi, and the fruit is known containing very strong antioxidant, while the antioxidant of mangrove leaves consumed as vegetables needs to be studied.

Materials and Method

Young leaves of mangrove *S.alba*(3-4 sheets of the tops)were collected from the coastal area of Wori, NorthMinahasaregency, North Sulawesi,Indonesia, in November 2018and identified Herbarium Jatinangor, Plant Taxonomic Laboratory,Biology Department, FMIPA UNPAD Bandung.

Sample preparation

Samplesof*S.alba* leaves (3-4 sheets of the edge**)**were collected, washed in clean water, then wiped with tissue paper up to dry, and weighed, to obtain fresh sample weight (initial weight). The samples were then dried under the sunlight up to dry weight gained**,** and mashed into powder. It was weighed and extracted using soxhlet extraction at 50^{o}C and maceration at room temperature in methanol and ethanol solvents. The macerate was filtered and evaporated using a rotary vacum evaporator to obtain coarse extract. Extract rendement was estimated by comparing the extract weight and the fresh sample weight and multiplied by 100%. The coarse extract was analyzed to know the phytochemical content, total phenols.and antioxidant, thefree radical 1-1-diphenyl-2-picryhidrazil (DPPH)inhibition usingspectrophotometry method.

Phytochemical analysis

Phytochemical analysis followed Harborne [11]. The analysis was qualitatively done on the young leaves of*S.alba*. The secondary metabolite groups measured werephenols, flavonoid, tanin, triterpenoid, saponin, steroid, and alkaloid responsible for its antioxidant feature.

Total phenol

Total phenol content was measured with a spectrophotometer using *Folin-Ciocalteau*[12]with some modification. As much as 0.1 g of dry extract was dissolved in 10 ml of methanol andcentrifuged at 5900 rpm to obtain a supernatant.As much as 50 µL of supernatant was taken and added with 2.5 mL of Folin-Ciocalteau (1/10 dilution of the initial concentration), then added with 2 mL of 7.5% Na_2CO_3, incubated at 45°C for 15

min., and the absorbancerecorded at 765 nm wavelength. A standard curve of galic acid was made using the procedure above, but the sample was substituted with galic acid. Total phenol content was expressed as mg galic acid equivalence/g extract (mg GAE/g extract).

DPPH radical scavenging activity

Antioxidant activity analysis used 1,1-diphenyl-2-picrylhydrazyl (DPPH) method based on[13]with some modification. This analysis was based on the sample ability to reduce DPPH free radicals. As much as 2 ml of 2-12 ppm sample was put in a flask and added with 1 ml of DPPH solution (1 x 10^{-4}M), homogenized and incubated at room temperature for 30 min.The absorbance was measured at 517 nm wavelength. Control was prepared as well following the procedure above, but the sample was replaced with methanol. The antioxidant activity of DPPH free radical scavenger was expressed as percent inhibition calculated as follows

$$\% \text{ inhibition} = \frac{control\ absorbance - sample\ absorbance}{control\ absorbance}\ x\ 100 \quad (1)$$

The absorbance value of each concentration variation was plotted to inhibitory curve and IC_{50} was then determined.As comparison, the inhibitory activity of DPPH free radicals of vitamin C was used.

Data analysis

This study used two replications, and data were presented in figure and analyzed using mocrosoft exel 2010. Phytochemical tests were qualitatively done and presented in table.

Results and Discussion

Vegetation in mangrove is under stressful conditions, such as severe environment, seawater tides, high organic matters, high minerals, and high abundance of living organisms, such microorganisms and insects[14].Plants that can live in such an environment usually hold compounds assisting them protect themselves from destructions[15]. Phenolic compounds, such as flavonoid, can be found in nearly all types of plants and function as protection from environmental pressures [16]. These compounds have chemical and biological activity spectra including free radical scavenger as antioxidant.

Rendement

Extract rendement is obtained through sample extraction using solvent. This rendement is used to know the active chemical components contained in the sample. It is estimated based on produced extract weight and sample weight comparison multiplied by 100% (17].

Extract rendement data of *S.alba*young leaves used two extraction methods (soxletsi and maceration) with 2 solvents (methanol and ethanol) are presented in Figure1.These data reveal that rendement obtained through soxhlet extraction with methanol (9.77%) and ethanol (9.18%) is higher than maceration method with methanol (2.61%) and ethanol (2.51%). The present study found higher rendement than that previously reported [18]. Other studies found 2.45% rendement from *S. alba*leaf [19] and 2.13% from mangrove leaf of *Bruguiera gymnorrhiza* through maceration in methanol [20]. It was also reported [18] that soxhlet extraction of palm *Areca vestiaria* Giseke yielded 3.9% rendement and maceration method gave 3.3% rendement.Higher rendement obtained in soxhlet extraction than in maceration could result from the use of higher temperature in soxhlet method that could increase the ability to extract compounds insoluble at room temperature so that compound withdrawal could be more maximal [11].

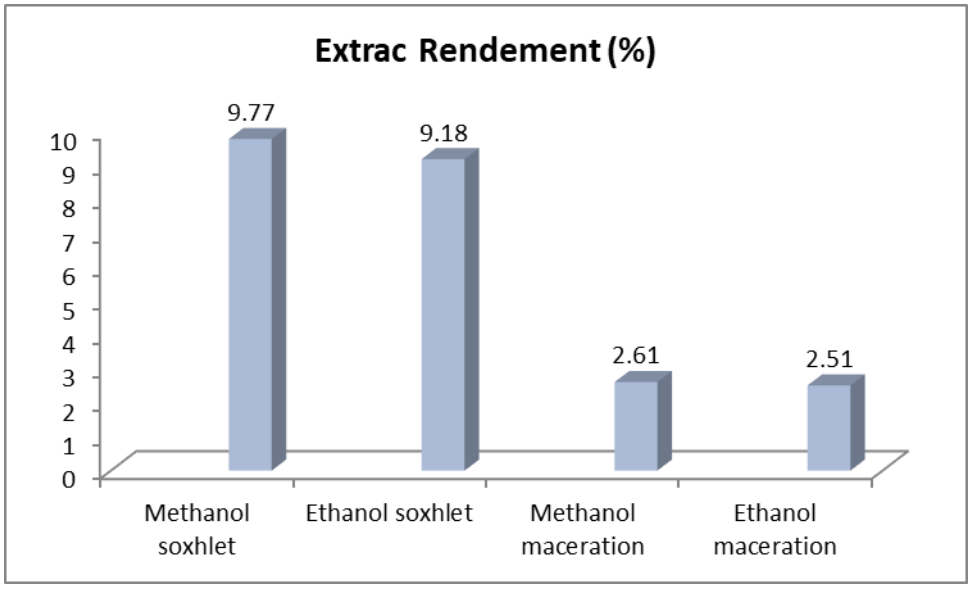

Figure 1. Young leaf extract rendement of *S. alba*.

Phytochemical content

Phytochemical content (secondary metabolite) of young leaf of*S.alba*obtained through sokhlet and maceration method with methanol and ethanol can be seen in Table 1. These data reveal that all young leaf extracts of *S.alba*positivelyhold secondary metabolites functioning as antioxidant, phenol, flavonoid, steroid, saponin, triterpenoid, and tanin.Mangroves contained flavonoid, isoflavon, flavon, anthocyanine, coumarine, lignin, kaletine, isocathein, and others [21]**.** Compared with marine plants, such as red seaweed*Laurencia* sp, fewer phytochemical component was detected, and maceration in methanoldoes not detect alkaloid and steroid [22].

Production of secondary metabolite in plants is affected by their living environment. Phenolic compounds in mangrove plants can protect the plant from damage of ultraviolet radiation[23]. Other authors [24] added thatthe tendency of increased phenolic compound content in mangroveoccurs when the plant could grow and survive in stressful condition.

The phytochemical components in plant extracts have certain biological function. Flavonoid in plants acts as protection against environmental pressures [16]. Flavonoid is the most important phenolic compound that possesses extensive chemical and biological activity spectrum including free radical scavenger as an antioxidant [25].Triterpenoid in plants functions as protection against insects and microbe [26]. Khairunnisa [27]reported that alkaloid in the stem of heart-leaved moonseed*Tinospora crisoa* L functioned as antioxidant (DPPH IC50 =46.96µg/ml). According to [10], thefruit of*S.alba*collected from Wori coast,Wori district, North Minahasa regency, North Sulawesi, Indonesia, contains phenols, flavonoid, steroid, and tanin, and has antioxidant (DPPH IC50 DPPH) activity of 12.5 ppm.

Table 1. Phytochemical content of *S.Alba*young leaf.

No	Phytochemical component	Method	Extract obtained using differentextraction method and solvents			
			Soxhlet Methanol	Soxhlet Ethanol	Maceration Methanol	Maceration Ethanol
1	Phenol	5% $FeCl_3$ reagent	+	+	+	+
2	Flavonoid	a. HCl conc. + Mg	+	+	+	+
		b. H_2SO_4 2N	+	+	+	+
		c. 10% NaOH	+	+	+	+
3	Steroid	Lieberman-Burchard	+	+	+	+
4	Triterpenoid		+	+	+	+

5	Saponin	$HCl + H_2O$	+	+	+	+
6	Tanin	1% $FeCl_3$	+	+	+	+
7	alkaloid	a. Dragendorf	+	+	-	-
		b. Wagner	-	+	-	-
		c. Mayer	-	+	-	-
		d. Hager	-	+	-	-

Total phenol

Total phenol of young leaf extract of *S. Alba* is presented in Figure2. The highest was found in the leaf extract of*S.alba* obtained through maceration with ethanol (34.2 mg GAE/g), followed by that of soxhlet method with methanol (33.6mg GAE/g), maceration with methanol (31.7mg GAE/g), and then soxhlet method with ethanol (28.6mg GAE/g). These findings indicate that total phenols vary with extraction method and the solvent used.Total phenol in *S. alba* root obtained through maceration in methanol is 216.53±3.09 mgGAE/g andin ethanol 205.93±4.27 mgGAE/g [28].However, total phenol in plants could vary with species, part of plant and extraction solvent used [29].

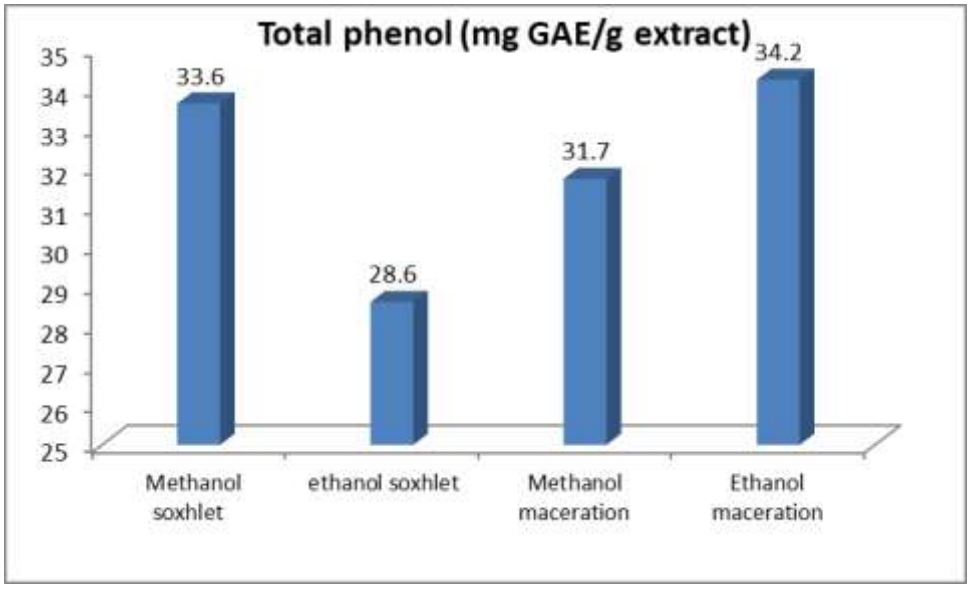

Figure 2. Total phenol (mg GAE/g) of *S. alba* young leaf.

Total phenol data above also reflect correlation between total phenolof young leaf of*S.alba* antioxidant activity (DPPH IC_{50}).Young leaf extract of S.*alba* obtained through maceration with ethanol had the highest total phenol,34.2 mg GAE/g, and through soxhlet method with methanol the extract had total phenol of33.6mg GAE/g),each of which had DPPH $IC_{50}$of 5.01µg/mL and 5.16µg/mL, respectively, and higher than that of vitamin C, 5.21µg/mL.The presence of correlation between total phenol and antioxidant activity was also reported in [28] that *S.alba*root extracted with methanol and ethanol had the highest total phenol and high DPPH IC50 nearly similar to DPPH IC50 of BHTcompound as control.

According to[16], phenolic compounds, such as flavonoid, can be found in almost all plant species. Plant flavonoid functions as protection against environmental pressures.Flavonoid is one of the most diverse natural compound groups and widespread as the most important phenolic compound.

DPPH radical scavenging activity assay

DPPH possesses unpaired electrons and gives strong absorbance at 515 nm wavelength.When the electrons are paired due to addition of an electron or hydrogen atom from antioxidant compounds, color change will occur from purple to yellow, and the maximum absorbance occurs at 517 nm wavelength.Decline in free radical scavenger activity could be indicated with percent decline of DPPH purple color, and this method is

often utilized to detect the antioxidant ability of the compound, since it is, in fact, accurate, relatively fast and practical[30]. DPPH free radical inhibition value is determined asIC_{50}(50% inhibitory concentration), in which the value is a measure of compound effectivity in inhibiting biological or biochemical functions[6].

DPPH free radical-inhibiting antioxidant activity data of *S. alba*young leaf employed two extraction methods (soxhlet and maceration) and two extraction solvents (methanol and ethanol) are given in Figure3.These demonstrate that the DPPH $IC_{50}$of *S. alba* young leaf extract through maceration with ethanol (5.01µg/mL)andsoxhlet method with methanol(5.16µg/mL)is better than that of vitamin C (5.21µg/mL) as positive control, meaning that both extracts of *S.alba* young leaf are highly potential as natural antioxidant source. DPPH$IC_{50}$of *S.alba*young leaf through soxhlet method with ethanol (6.23 µg/mL) and maceration in methanol (7.45 µg/mL)is also potential as natural antioxidant source, because it is slightly different from the DPPH IC_{50}ofvitamin C (5.21µg/mL) as control.

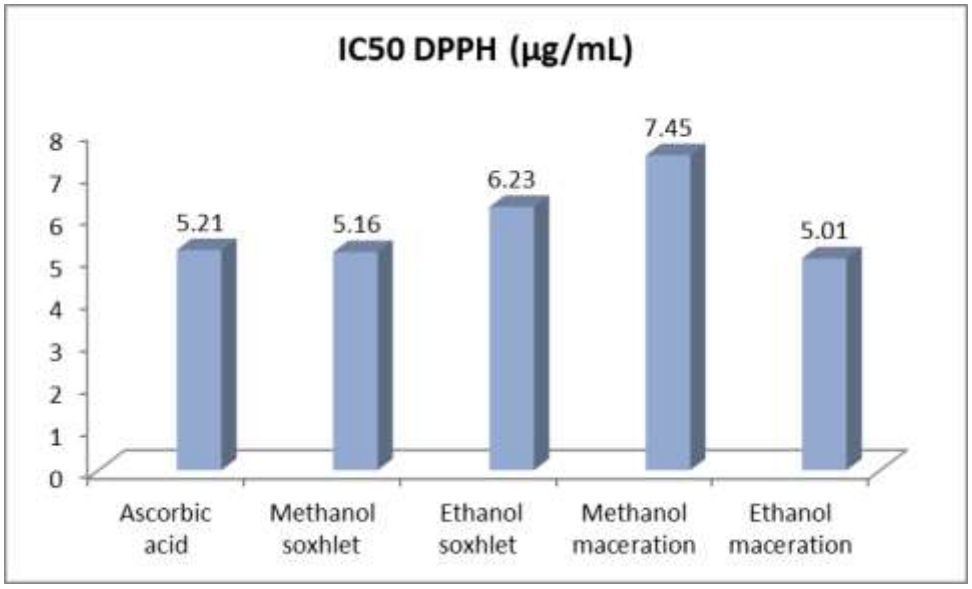

Figure 3. IC value of *S. alba* young leaf and ascorbic acid.

S.alba leaf extract through soxhlet method with 80% methanol-water had DPPH $IC_{50}$of 62.5µg/ml and *Bruguera cylindrica* had DPPH IC_{50}of175µg/mL, in which the antioxidant activity of both mangrove species is lower than DPPH $IC_{50}$of vitamin C,31.25µg/m [6].Antioxidant of several methanol extracts of mangrove leaves as follows: DPPH $IC_{50}$of 13 µg/mLfor*Heritiera formes* [31], 248.0 µg/mLfor *A. Marina* [32], and 141.56µg/mL for*E. agollocha*[33]. These exhibit that the antioxidant activity (DPPH IC_{50})of several methanol extracts of mangrove leaf is lower than that of methanol extract of *S. alba* young leaf(maceration method = 5.01 µg/mL and soxhlet method = 5.16µg/mL)**.** Moreover, the antioxidant activity of ethanol extract of several mangrove plants has also been reported as follows:DPPHIC_{50} of 58.33µg/mLin *R.mucronata* root[34], DPPH $IC_{50}$of 11.4 µg/mLin*R. apiculata*root [35], DPPH IC_{50} of 27.6µg/mL in*A. canthusillicifolius*root [36], and DPPH IC_{50} of32.7µg/mL in *Tinospora crisoa*leaf[37].Based on reports above, it was found that antioxidant activity (DPPH IC_{50}) of several ethanol extracts of mangrovesis lower than ethanol extract of *S. alba* young leaf (maceration method = 6.23µg/mL and soxhlet method = 7.45µg/mL).

It was reported [18] that methanol extract of palm fruit skin *Areca vestiaria* Gisekeusing soxhlet method had higher free radical scavenger activity at the dose of 50 ppm (85.16%) and 100 ppm(92.31%) than that in maceration method that possessed 74.61% free radical scavenger at the dose of 50 ppm and 81.32% at the dose of 100 ppm.Study on *S.alba*leaves indicates that soxhlet with methanol and maceration method with ethanol have higher antioxidant activity than that of vitamin C.

Conclusion

These results demonstrated higher extract rendement in soxhlet method with methanol (9.77%) or ethanol (9.18%). Soxhlet extract with ethanol contains all phytochemical components tested, phenol, flavonid, steroid,

saponin, triterpenoid, tanin and alkaloid, while three other samples did not contain alkaloid. The highest total phenol was recorded in maceration extract with ethanol (34.2 mgGAE/g extract) and the lowest in soxhlet extract with ethanol (28.6 mgGAE/g). The antioxidant activity found in the extract of ethanol maceration (DPPH IC_{50}=5.01µg/mL) and soxhlet method with methanol (DPPH IC_{50} =5.16µg/mL) was higher than that in vitamin C (DPPH IC_{50} =5.21µg/mL), while two other samples had slightly lower antioxidant activity than that in vitamin C, the extract of soxhlet method with ethanol (DPPH IC_{50}= 6.23µg/mL) and maceration in methanol (IC_{50}=7,45µg/mL). As a whole, this study concluded that young leaf extract of*S.alba*was potential as natural antioxidant source.

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