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Evaluation of Secondary Metabolites and Antioxidant Activity of Water, Ethyl Acetate and Hexane Fractions from the Mangrove Young Leaves *Sonneratia alba*

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Authors' contributions

This work was carried out in collaboration among all authors. Author VD designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DW and LADYM managed the analyses of the study. Author LADYM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The fractions from young leaves of mangrove *Sonneratia alba* was studied for its associated secondary metabolites and antioxidant activities. The objective of this study was to determine the secondary metabolite components and antioxidant activity of water, ethyl acetate, and hexane fractions of the young leaves of mangrove *S. alba*. The fraction was obtained from dry powder of young leaf *S.alba* using continuous fractionation of crude extracts. The crude extract was attained by 2 extraction methods (soxhlet and maceration) and 2 extraction solvents (methanol and ethanol). Secondary metabolites analyses were qualitatively conducted to detect the presence or absence of phenols, flavonoid, tannin, steroid, triterpenoid and alkaloid. Total phenols were measured using Folin Ciocalteau reagents and gallic acid standard curves whereas antioxidant activity were analyzed using DPPH method (1- 1-diphenil-2-picrihydrasil). Results showed that all fractions contained secondary metabolite components tested. The highest rendement was found in

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the water fraction fromsoxhletation extract with methanol (6.36±0.29%). The total phenol values were found the highest in the ethylacetatefraction from macerated extract with ethanol (352±9.77 mgGAE/g). Stronger antioxidant activity was also found in ethylacetate fraction as indicated by the small value of IC50 DPPH namely the ethylacetate fraction with soxhletation extract with ethanol (3.43±0.25 µg / mL). The results of this study indicate that the semipolar fraction (ethylacetate fraction) has more potential as a source of natural antioxidants.

Keywords: Sonneratia alba; secondary metabolites; total phenols; antioxidants.

1. INTRODUCTION

Some historic studies informed that during the Japanese occupation many people on the islands of Sumatra, Java (Cilacap, Pantura, and East Java) and Kalimantan utilized mangrove plants as food. Various types of processed foods have been made from mangrove by several community i.e. 39 types of snacks have been made from fire mangrove fruit (*Avicenia marina*) and Brayo (*Avicenia offien*); 6 types of snack (syrup, juice, candy, wajid, dodol, fruit crackers) have been made from Pidada mangrove types (*S. alba* and *S. caseolaris*) [1]. Moreover, [2] stated that ripe *S. alba* fruit is eaten by people from Africa, Malay and Javanese. They confirmed stating that this mangrove fruit tastes like cheese. Furthermore, in several regions in Indonesia such as Java, Sulawesi and Maluku, mangrove plants have been traditionally used as medicine, drinks and as raw materials for various kinds of cakes. Nevertheless, it is not yet developed because there is not enough knowledge about the potential and benefits of mangrove plants as a source of functional food [3].

In Indonesia young *S.alba* leaves are usually consumed as vegetables. Picking young leaves does not damage the mangrove plants. Instead, new shoots will grow and leaves will increasingly lush. Nowdays,the study of antioxidant from mangrove plant leaves is still limited. However, all parts of mangrove plants have been traditionally used to cure various diseases caused by free radicals [4]. This indicates that mangroves contain bioactive compound, one of which is antioxidant.

There is a growing demand for the use of natural antioxidants rather than synthetic antioxidants. Some studies demonstrated that mangroves are a very strong source of natural antioxidants. The methanol extract of mangrove young leaves of *Heritiera formes* has a very strong antioxidant activity with IC50 DPPH values of 13.0 ppm [5], ethanol extract of mangrove bark *E.agollocha* has IC50 DPPH of 4.8 ppm [6], and ethanol extract of *R.apiculata* root has IC50 DPPH value of 11.4 ppm [7].

Several studies also reported that mangroves from *S. alba*are sources of natural antioxidants. The methanol extract on *S. alba* stem has an IC50 DPPH value of 12.2 ùg / mL [8], the methanol extract of *S. alba* flour has an IC50 DPPH value of 4.65 ùg / mL [9], the extract of leafof *S. alba* using soxhletation method with 80% methanol-water solvent has IC50 DPPH of 62.5 µg/ml [10], the ethanol extract of young leaves of *S. alba* by maceration method has IC50 DPPH of 5.01 µg/mL and the methanol extract of young leaves of *S. alba*by soxhletation method has IC50 DPPH of 5.16 µg / mL [11]. Some results of the study mentioned above hasIC50 DPPH in which are better than the IC50 DPPH vitamin C value of 5.21 µg/mL. This signify that *S. alba* stems, fruits and young leaves are very potential sources of natural antioxidants.

Methanol and ethanol are organic solvents that are most widely used in the process of isolating organic compounds of natural materials because they can dissolve almost all classes of secondary metabolites [12]. In terms of polarity compounds, bioactives in plants are non-polar, semi-polar and polar. Separation of bioactive components based on the nature of polarity can be done by stratified fractionation using non-polar hexane solvents, semi-polar ethylacetate and polar water [13]. [9] reported that stratified fractionation of methanol extract in *S. alba* fruit flour exhibited various IC50 DPPH value i.e. 162.79 µg/mL, 3.55 µg/mL, 6.95 µg/mL in hexane, ethyl acetate, and water fractions respectively. As regards to the research report mentioned above, it can be seen that the extraction technique by stratified fractionation based on the polarity of the solvent in the crude extract can reveal different antioxidant activity in a sample. Therefore, the present study aimed to investigate the antioxidant activity of the hexane, ethylacetate, water fractions resulting from

stratified fractionation of methanol and ethanol extracts of young leaves from *S. alba.* This research is continuation of our investigations on exploiting *S. alba* mangrove leaves as a source of natural antioxidants [11].

2. MATERIALS AND METHODS

2.1 Plant Materials

Young leaves of mangrove (*S. alba)* were collected from the coastal area of Wori, North Minahasa Regency, North Sulawesi Province, Indonesia in March 2019. We picked up 3 – 4 shoot leaves from each tree. In total, we collected 1 (one) kg of *S. alba* young leaves. Previously, plant materials were identified in Jatinangor Herbarium, Plant Taxonomy Laboratory, Department of Biology FMIPA UNPAD Bandung.

2.2 Sample Preparation

S. alba young leaveswere washed thoroughly with clean water, dried with a tissue paper, then weighed to obtain initial weight. Furthermore, leaves were dried under the sun so that it will have final moisture content at 15%. Dried and ground samples were weighed and extracted using soxhlet extraction at 60^{o}C and maceration at room temperature in methanol and ethanol solvents. The maceration and soxhletation solutions were then filtered and evaporated using a rotary vacuum evaporator to obtain coarse extract. Afterwards, the coarse were fractioned with hexane, ethyl acetate, and water (Fig. 1).

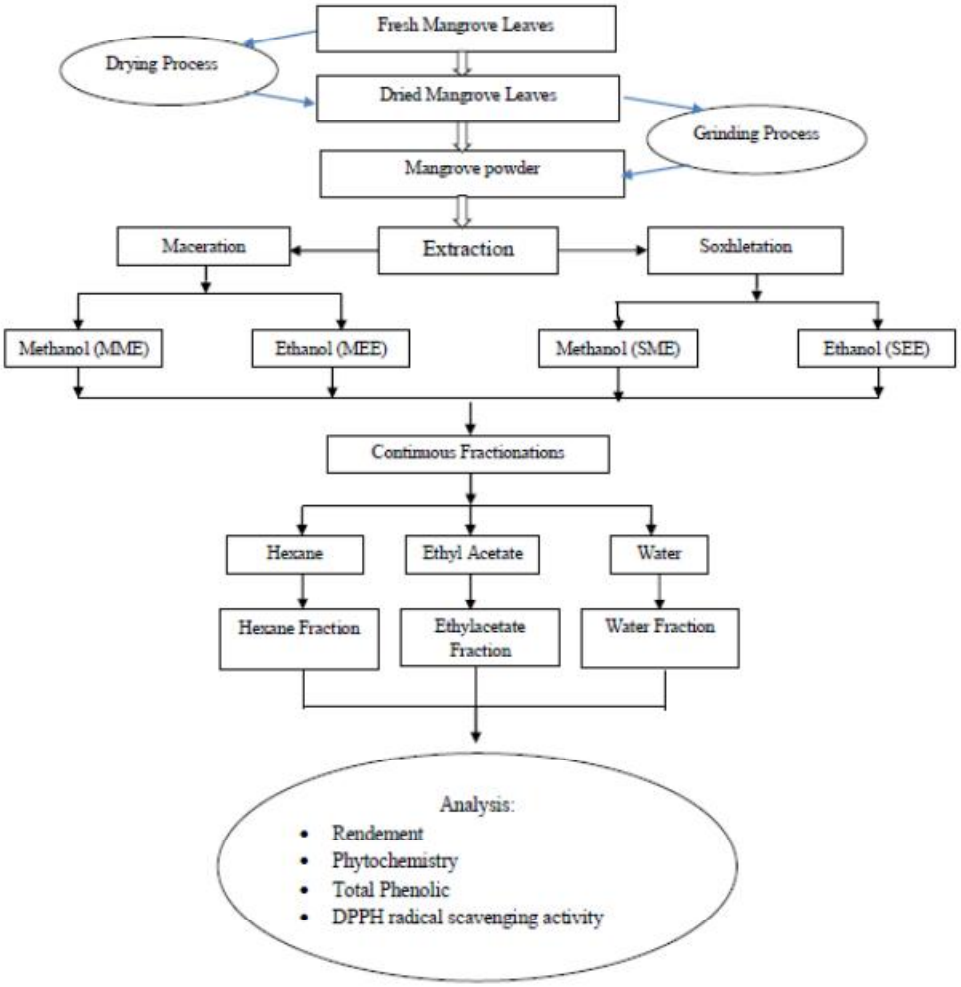

Fig. 1. Sample preparation

2.3 Rendement Measurement

Rendement of the extract was measured by comparing the extract weight and the fresh sample weight and multiplied by 100%. The coarse extract was then fractionated continuously with hexane, ethylacetate and water using the separating funnel method, hereafter the fractionated solvent was evaporated using a rotary vacuum evaporator. The rendement of dry hexane, ethylacetate and water was then estimated and further analyzed.

2.4 Secondary Metabolite Analysis

Secondary metabolite analysis was determine according to [14]. The secondary metabolites were measured qualitatively i.e. phenols, flavonoid, tanin, triterpenoid, saponin, steroid, and alkaloid.

2.5 Total Phenol

The total phenol content was measured by a spectrophotometer using Folin-Ciocalteau reagents. The methods follow protocols from [15] with some modification. The supernatant was obtained by dissolving 0.1 g of dry extract in 10 ml of methanol and centrifuged at 5900 rpm. Subsequently, 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 absorbance recorded at 760 nm wavelength. The total phenol content was measured by a spectrophotometer using Folin-Ciocalteaureagents.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).

2.6 DPPH Radical Scavenging Activity

The method described by [16] were followed to measure the DPPH radical scavenging activitywith some modifications. The principle of this analysis was the sample ability to reduce DPPH free radicals. 2 mL from a various concentration of sample (2 – 12 ppm) 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 minutes.Afterwards, using spectrophometer, the absorbance of sample was measured at 517 nm wavelength. Control was prepared following the above procedure. However, the sample was replaced with methanol.The antioxidant activity of DPPH free radical scavenger was expressed as percent inhibition calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} x\ 100$$

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.

2.7 Data Analysis

All the samples were analyzed descriptively in triplicate and the results were averaged. The datawere presented in figures and analyzed using Microsoft Excel 2010. Secondary metabolites tests were qualitatively done and presented in tables.

3. RESULTS AND DISCUSSION

3.1 Rendement

Fig. 2 shows that the water fraction of *S. alba* leaves generally has the highest rendement compared to ethylacetate and hexane fractions. Some studies also reported that the rendement of *R. mucronata* extracted with water temperatures of 30, 45, and 60^0C respectively were 6.05, 4.47 and 3.16% [17], the rendement extract of lindur leaf (*Bruguierra gymnorrhiza*) were 2.85% and 1.53% [18], and the rendement extract of *R. stylosa* fruit were 0.32% and 0.14% [19].

This results is an agreement with [20] which obtained higher yield value in water extracts of S.alba mangrove fruit taken at the same location as mangrove leaves in this study. They study reported that yield in water, ethyl acetate, and hexane extracts were 2,308%, 0.602%, and 0.467%, respectively. The high yield of mangrove leaves in the water fraction indicates that the bioactive compounds in mangrove leaves are more polar in nature. This is very beneficial for the utilization of bioactive compounds from *S.alba* mangrove leaves since it does not need special solvents if we want to use daily. Previous study of [21], reported that 30 minutes boiling water extract from *S.alba* mangrove leaves has a very strong antioxidant activity (IC50DPPH = 9.64 µg / mL). Thus, in the future it can be used as an functional beverages.

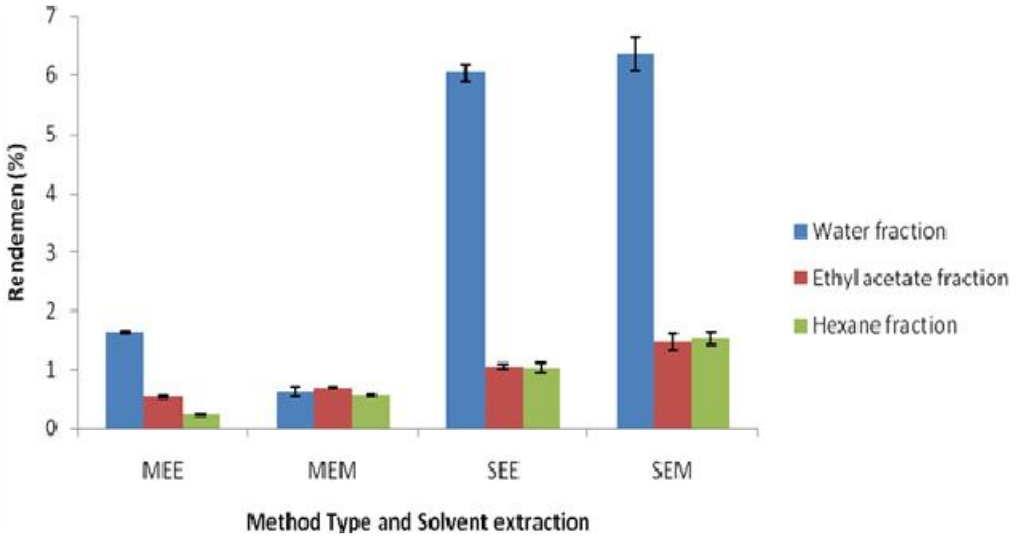

Fig. 2. Rendement of water fraction, ethylacetate fraction, and hexane fraction as a result of stratified fractionation derived from methanol and ethanol extract of *S. alba* young leaves

MEE = Maceration Extract with Ethanol
MEM = Maceration Extract with Methanol
SEE = Soxhletation Extract with Ethanol
SEM = Soxhletation Extract with Methanol

3.2 Secondary Metabolite Content

Secondary metabolite data from stratified fractionation based on polarity of the solvent, i.e. water, ethylacetate and hexane from *S. alba* young leaves extracted with methanol and ethanol using two extraction methods (maceration and soxhletation) are presented in Table 1.

Table 1 describes that the water fraction (polar fraction) contains all secondary metabolite components tested except for steroids. Table 1 shows that the ethylacetate fraction (semipolar fraction) contained all phytochemical components tested except for saponin, and the hexane fraction (nonpolar fraction) contained all phytochemical components tested except for triterpenoids. It can be concluded that both the water, ethylacetate and hexane fractions contain important chemical components that function as antioxidants, i.e. phenolic compounds, flavonoids and tannins.

Hydroxyl groups in phenol compounds are capable to capture free radicals and to reduce the radical nature of reactive oxygen compounds such as superoxide, peroxide radicals, hydroxyl radicals and feroxinitrites [22]. Furthermore, the main function of most phenolic compounds was to protect plants from damage through excessive light by acting as antioxidants. Likewise, [23] proved that phenolic compounds can protect mangroves from damage caused by ultraviolet radiation. Besides, mangroves tend to increase the production of phenolic compounds as the plants grow and survive under stress condition [24].

3.3 Total Phenol Content

Fig. 3 describes that the highest total phenol content is obtained in the ethylacetate fraction. The order of total phenol levels in *S. alba* is: ethylacetate fraction> water fraction> n-hexane fraction. It is in accordance with the study of [9] who did the research on *S. alba* fruit taken at the same location. This shows that the phenolic compounds found in *S. alba* leaves are more semipolar. That is why, they dissolve in a semipolar ethylacetate solvent. As it is known, flavonoids are one of the phenolic compounds. The number of OH groups in the flavonoid nucleus in phenol compounds affects antioxidant activity, the antioxidant activity will be higher when the OH groups are more numerous [25]. Moreover, [18] reported that the total phenol of lindur leaf (*Bruguiera gymnorrhiza*) in ethylacetate extract was 97.57 and hexane extract was 12.41 mg GAE/g extract.

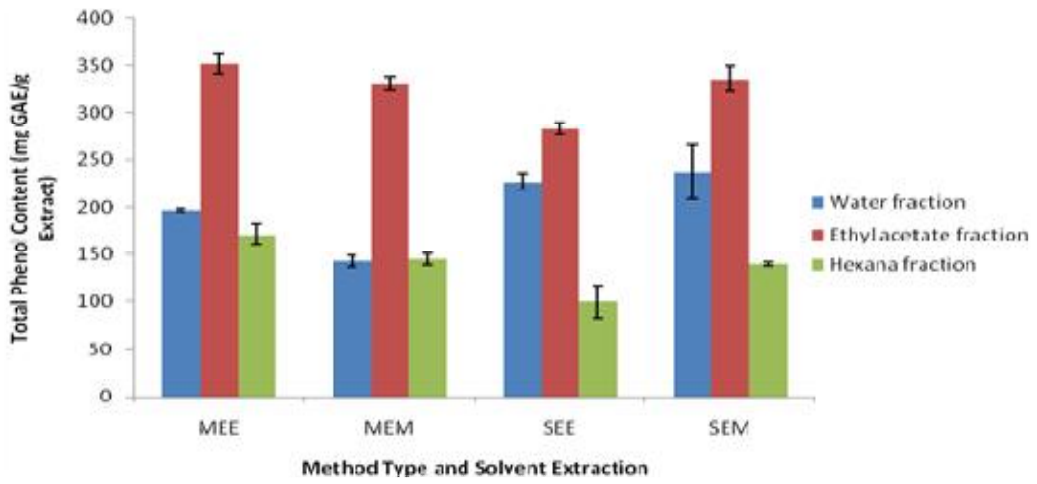

Fig. 3. Total phenol of water fraction, ethylacetate fraction, and hexane fraction as a result of stratified fractionation derived from methanol and ethanol extract of *S. alba* young leaves

MEE = Maceration Extract with Ethanol
MEM = Maceration Extract with Methanol
SEE = Soxhletation Extract with Ethanol
SEM = Soxhletation Extract with Methanol

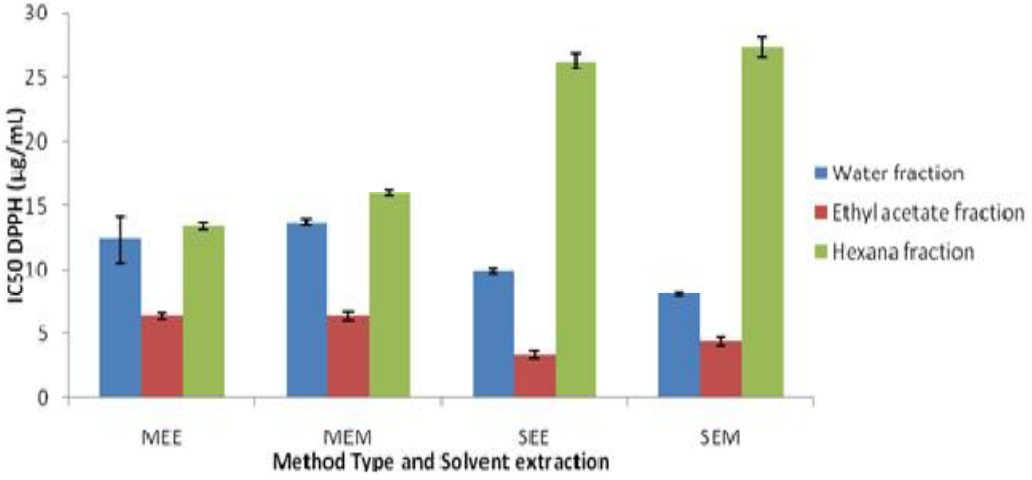

Fig. 4. IC50 DPPH of water fraction, ethylacetate fraction, and hexane fraction as a result of stratified fractionation derived from methanol and ethanol extract of *S. alba* young leaves

MEE = Maceration Extract with Ethanol
MEM = Maceration Extract with Methanol
SEE = Soxhletation Extract with Ethanol
SEM = Soxhletation Extract with Methanol

3.4 DPPH Radical Scavenging Activity Assay

The unpaired electrons in the DPPH free radical give a strong absorption at the wavelength of 515 nm. When the electrons become paired up because of an electron or hydrogen atom from an antioxidant compound, the color will change from purple to yellow, and the maximum absorption is at a wavelength of 517 nm. This method is often used to detect the antioxidant ability of a compound, because the results prove

Table 1. Secondary metabolite content in water fraction, ethyl acetate fraction, and hexane fraction of *S. alba* young leaves

No	secondary metabolite	Detection Method	Results		
			Water Fraction	Ethyl Acetate Fraction	Hexane Fraction
1	Phenolic	5% $FeCl_3$ reagent	+	+	+
2	Tannin	1% $FeCl_3$ reagent	+	+	+
3	Flavonoids	a. Concentrated HCl reagent + Mg	-	-	-
		b. H_2SO_4 2N reagent	-	-	-
		C. 10% NaOH reagent	+	+	+
4	Saponins	2N HCl reagent	+	-	+
5	Triterpenoids	Concentrated H_2SO_4 reagents + CH_3COOH anhydride	+	+	-
	Steroids		-	+	-
6	Alkaloids	a. Dragendorff reagent	+	+	+
		b. Wagner reagent	+	+	+

to be accurate, relatively fast and practical [26]. The DPPH free radical inhibition value is determined as IC50 (50% inhibitory concentration), where this value is a measure of the effectiveness of a compound in inhibiting biological or biochemical functions [10]. IC50 DPPH values in the water, ethylacetate and hexane fractions resulting from continuous fractionation of methanol and ethanol extracts using maceration and soxhletation extraction methods of *S. alba* leaves is presented in Fig. 4.

Fig. 4 illustrates that IC50 DPPH of ethylacetate fraction resulting from stratified fractionation of ethanol soxhletation extract (SEE) (3.43 ± 0.25 µg/mL) and methanol soxhletation extract (4.43 ± 0.31 µg/mL) have a stronger antioxidant. This figure also shows that the antioxidant activity in all ethylacetate fractions is better than the water and hexane fraction. This is supported by the data on total phenol content. The highest total phenol content was found in the ethylacetate fraction. Simple phenolic compounds, phenolic acids and flavonoids are hydroxycamic acid derivatives that are bioactive, and there are many in food plants [27]. Phenols are bioactive compounds that act as antioxidants [28]. Some studies on antioxidant activity (IC50DPPH values) of ethylacetate extract /fraction in several types of mangroves are as follows: 124.19 µg/mL in hypocotil *Kandela candel* [29], 182.33 µg/mL in *Avicenia marina* leaf [30], 26.81 µg/mL in nipah leaf (*Nypa fruticans*) [31], 460 µg/mL in *A. marin* leaf [32] and 3.55 µg/mL in *S. alba* fruit [9]. Study on *S. alba* leaves and fruit indicate that the ethylacetate fraction has stronger antioxidant activity than that of other mangrove species

4. CONCLUSION

All solvent fractions (hexane, ethyl acetate and water) of *S. alba* leaves contain almost all secondary metabolite components tested, i.e. phenolic, flavonid, steroid, saponin, triterpenoid, tannin and alkaloid. The highest rendement was recorded in water fraction of soxhletation ethanol extract (6.04±0.14%) and methanol soxhletation extract (6.36±0.29%). All ethylacetate fractions had higher total phenol values than water and hexane fractions.Ethylacetate fraction of MEE, SEM, MEM, and SEE was 352±9.77, 335.9±13.66, 331.4±7.49, and 283.9±5.72 mgGAE/g, respectively. Antioxidant activity (IC50 DPPH) of ethylacetate fraction resulting from stratified fractionation on ethanol and methanol soxhletation extract (SEE and SEM) was 3.43 ± 0.25 µg/mL and 4.43 ± 0.31 µg/mL have a stronger antioxidant, these data indicate that the soxhletation method is best for future use than the maceration method. To sum up, thestratified fractionation with solvents based on the nature of polarity is capable to reveal the antioxidant activity of *S. alba* leaves.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Santoso N, Nurcahya BC, Siregar AF, Farida I. Food recipes made from mangrove and utilization of nipah. LPP Mangrove; 2005.
   ISBN. 15: 979-3667. [In Indonesian]
2. Peter KL, Sivasothi N. Guide to the mangroves of Singapore I (Plant Diversity) Singapore Science Centre. 1999;60.
3. Purnomobasuki H. Potential of mangroves as medicinal plants. Biota. 2004;IX(2):124-126. [In Indonesian]
4. Bandaranayake WM. Bioactivities, bioactive compounds and chemalconstituents of *mangrove* plants. Wetlands Ecol. Manage. 2002;10:421-452.
5. Rout P,Basak UC. Antioxidant properties in leaf and root extracts of some medicinally important mangrove species of *Odisha Coast*. Am. J. PharmTech Res. 2014;4(4):606-617.
6. Jahan IA, Hossain H, Akbar PN, Rahman MM, Khan TA, Rahman SE, Siraj MA. Antioxidant properties and HPLC assay of bioactive polyphenols of the ethanol extract of *Excoecaria agallocha* stem bark growing in Bangladesh. British Journal of Pharmaceutical Research. 2014;4(17): 2116-2125.
7. Asha KK, Suseela M, Laksmanan PT. Flavonoid and phenolic compounds in two mangrove species and their antioxydant property. Journal Og Geo-Marine Science. 2012;41(3):259-264.
8. Herawati N, Jalaludin N, Daha L, Zenta F. Antioxidant potential of methanol extract from stems of mangrove plants *S. alba* . Farmasidan Farmakologi. 2011;15(1):23-25. [In Indonesian]
9. Wonggo D, Berhimpon S, Kurnia D, Dotulong V. Antioxidant activities of mangrove fruit (*Sonneratia alba*) taken

from Wori Village, North Sulawesi, Indonesia. International Journal of ChemTech Research. 2017;10(12):284-290

10. Gawali P, Jadhav BL. Antioxidant activity and antioxidant phytochemical analysis of mangrove species *Sonneratia alba* and *Bruguiera cylindrica.* Asian Jr. of Microbiol. Biotech. Env. Sc. 2011;13(2):257-261.
11. DotulongV, Wonggo D,Montolalu LADY. Phytochemical content, total phenols,and antioxidant activity of mangrove*Sonneratia alba* young leaf throughdifferent extraction methods and solvents. International Journal of Chem Tech Resaearch. 2018a; 11(11):356-363.
12. Darwis D. A basic laboratories in the study compound natural ingridients biological, Workshop The Devolopment of Human Resources in the Field of Organic Chemistry of Biological, FMIPA University of Andalas Padang; 2000.
13. Lestari SB, Pari G. Chemical analysis of several Indonesian wood types. Journal of Forest Products Research. Center for Forest Product Research and Development. 1990;11(3):96-100. [In Indonesian]
14. Harbone JB. Phytochemical methods. Guidelines for modern ways to analyze plants. 2nd Ed. Translated by Kosasih Padmawinata and Iwang Soediro. Editor Sofia Mansoor. ITB. Bandung. 2006;354. [In Indonesian]
15. Ganesan M, Mairh OP, Rao PVS. Effect of Shelf life of brown algae *Sargassum wightii* (Fucales/ Phaeophyta) and *Turbinaria conoides* (Fuales/Phaeophyta) on alginic acid yield. Indian Journal of Marine Science. 2001; 30:108-110.
16. Devi KP, Suganthy P, Kesika SK. Pandian. Bioprotective Properties of seaweed: In vitro evaluation of antioxidant activity and antimicrobial actvityagaints food borne bacteria in relation to polyphenolic content. BMC Complementory and Alternative Medicine. 2008;8(38).
17. Podungge F, Purwaningsih S, Nurhayati T. Characteristics of black mangrove fruit as antioxidant source extract preparations. J PHPI. 2015;18(2):140-149. [In Indonesian]
18. Nurjanah N, Jacoeb AM, Hidayat T, Hazar, S, danNugraha R. antioxidant activity, total phenol content, and bioactive components of lindur leave (*Bruguiera gymnorrhiza*). American Journal of Food Science and Health. 2016;2(4):65-70.
19. Wulandari A. Activity of red mangrove extract (*Rhizophora stylosa* Griff.) as an antidiarrheal by *in vitro* analysis. Thesis. ITB. Bandung; 2014. [in Indonesian]
20. Wonggo. Antioxidant of mangrove fruit (*Sonneratiaalba*) taken from Wori Village, Regency North Minahasa, North Sulawesi. Disertration. Sam Ratulangi University. 2018;181. [In Indonesian]
21. Dotulong V, Wonggo D, Montolalu LADY. Antioxidant potential of young leaves mangrove*Sonneratiaalba* as a functional drink. Final report of applied research. funded by Sam Ratulangi University Manado. 2018b;29. [in Indonesian]
22. Sirait M. Phytochemical guides in pharmacy. ITB. Bandung. 2007;246. [In Indonesian]
23. Agati G, Matteinni P, Goti A, danTattini M. Chloroplast located Ca Scavenge Singlet Oxygen. New Phytologist. 2007;174:77-82.
24. Banerjee D, Chakrabarti S, Hazra AK, Banerjee S, Ray J, Mukherjee B. Antioxidant activity and total phenolics of some *Mangrove*s in Sundarbans. African Journal of Biotechnology. 2008;7(6):805.
25. Es-Sefi, Ghidouche NES, Ducrot PH. Flavonboids, hemisynthesis, reactivirty, characerition, and free radical scavenging activity. Molecule. 2007;12(9):2228–2258.
26. Prakash A. Antioxidant activity. Medallion Laboratories Analitical Progress. 2001; 19(2) Minesota.
27. Tang Ho C, Lee CY, Huang MT. Phenolic compound in food. Departement of Food Science. 1992;1(14):338.
28. Sudirman S, Nurjanah, danJacoeb AM. Proximate compositions, bioactive compounds and antioxidant activity from large-leafed mangrove (*Bruguiera gymnorrhiza*) fruit. International Food Research Journal. 2014;21(6):2387-2391.
29. Wei SD, Zhou HC, Lin YM. Antioxidant activities of extract and fractions from the hypocotyls of the mangrove plant *Kandelia candel.* International Journal of Molecular Sciences. 2010;11:4080-4083.
30. Jacoeb AM, Purwaningsih S, Rinto. Anatomy, bioactive compound and antioxidant activity of mangrove Api-Api (*Avicenia marina*) leaf. J. PHPI. 2011; 14(2):143-152.

31. Putri IJ, FauziyahdanElfita. Antioxidant Activity with DPPH of Nipah (*Nypa fruticans*) leaves and seeds from of Banyuasin Sand, South Sumatra. Journal Maspari. 2013;5(1):16-21. [In Indonesian]

32. Moteriya P, Dalsaniya A, Chanda S. Antioxidant and antimicrobial activity of a mangrove plant *Avicennia marina* (Forsk.) Journal of Coastal Life Medicine. 2015; 3(9):713-717.

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