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PHYTOCHEMICALS AND IDENTIFICATION OF ANTIOXIDANT COMPOUNDS FROM ETHANOL EXTRACT OF SONNERATIA ALBA LEAVES AND BARK

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ABSTRACT
The purpose of this study was the bioactive identification of ethanol extracts (EtOH) from the bark and leaves of *Sonneratia alba* that have antioxidant activity. Mangrove bark and leaf powder each added with ethanol (1: 3, w/v) sonicated for 10 minutes. The obtained extract was tested for phytochemicals, antioxidant activity using DPPH and identification of the compounds using UPLC-ESI-MS. The ethanol extract from the bark and leaves of *S. alba* contains bioactive compounds that have antioxidant activity from the group of phenol compounds and the both extracts contain 7-hydroxycoumarin or scopoletin. The antioxidant activity (IC_{50} value) of each extract was 67,211±2,482 μg/ml and 88,706±4,782 μg/ml, respectively. Ethanol extract from bark and leaves of *S. alba* has the potential to be an antioxidant agent.

KEY WORDS
Antioxidant, bark, ethanol extract, leaves, *Sonneratia alba*.

The superiority of natural antioxidant has been proven and there is no doubt that besides its ability to break free chain reactions, it is also relatively safe to use. The natural antioxidants are increasingly needed in the health, pharmaceutical and other industries, including the food and beverage industry. At least the last two decades, the demand for bioactive compounds has increased rapidly, especially antioxidant compounds must be balanced by exploring resources.

Mangroves are a potential source of natural antioxidants, where this plant is one of the abundant biological resources in Indonesia. Research into the use of this plant shows rapid progress, especially in the last two decades. Some families such as Rhizopora, Avicennia, Bruguiera and Sonneratia are reported as additional bioactive sources. *Rhizopora* spp mangroves have been tested and used for functional nutrition, namely antidiabetics and strong antioxidants (Hardoko, Puspitasari and Suprayitno, 2015). The fruit of *Avicennia marina* is known to contain a lot of triterpenoid saponins which have anti-tumor activity (Yang *et al*., 2019). In addition to the fruit pericarp, *Sonneratia apetala* seeds have the potential as a source of antioxidants, antidiabetics and antibacterial compounds (Hossain *et al.*, 2013). Similarly, hypocotyl of *Bruguiera gymnorrhiza* was found to contain many compounds of terpene, triterpenes, flavonoids and aromatics as secondary metabolites with relatively high antioxidant activity (Nebula, Harisankar and Chandramohanakumar, 2013; Yao *et al.*, 2017). The leaves, fruit and bark of some mangrove plants are reported to contain bioactives as antimicrobial, antidiabetic, anti-inflammatory, antitumor and antioxidant. Most bioactive substances, including antioxidants from mangroves, are phenolic compounds, e.i. coumarine. As one of the phenolic compounds that had an antioxidant, antibacterial, analgesic and anti-inflammatory effect.

Sonneratia alba is known to contain antioxidants and can be used as a functional food for diabetics. While the leaves and bark have the potential to be used as tea and functional drinks. However, there is no information available on antioxidant bioactive compounds in the leaf and bark extract of the plant. In this study, antioxidant compounds in EtOH extracts of *S. alba* leaves and bark were identified using UPLC-ESI-MS.

MATERIALS AND METHODS OF RESEARCH

Fresh *Sonneratia alba* bark and leaves were collected from Probolinggo East Java and used to retrieve their extracts. The bark and leaves were rinsed thoroughly with tap water, followed by distilled water to remove the dust and other particles, air dried and sliced into small pieces. 20 g of finely cut bark and leaves were weighed and put into 250-mL Erlenmeyer flask containing 160 mL ethanol and sonicated for 10 min. The crude extract obtained was filtered and removed the solvent by rotary evaporator for 30 min. For all work was replicated for 3 times. Ethanol extract of bark (EEB) and leaf (EEL) were used to the sample for determine of phytochemical, total poliphenol content (TPC) and antioxidant activity (DPPH, radical scavenging abillity). The antioxidant compounds were determined by LC-MS. EtOH of sample were screened for the presence of alkaloids, flavonoids, terpenoids, steroids, phenolics, saponins and tannins (Harborne, 1984). It was done to assess the qualitative chemical composition of crude extracts using commonly employed precipitation and discoloration reaction. General reactions in these analyses revealed the presence or absence of these compounds in the crude extracts.

The phenolic content (TPC) in the EtOH extract were determined by the Folin-ceocalteu reagent (Sasmito *et al.*, 2013). 250 µl extract was added with 1250 µl aquades, 250 µl ethanol and 125 µl Folin-ceocalteu reagents. The mixture was incubated at room temperature for 5 min, added 250 µl 5% Na_2CO_3, and stored in a dark room for a hour. Absorbance was measured by using a spectrophotometer at λ 725 nm. TPC was calculated based on the standard formula (gallic acid), $y = 0.0085x - 0.018$ and expressed in mg gallic acid equivalent per g sample.

The antioxidant activity of the EtOH extract from the bark and leaf of *S. alba* were determined using DPPH radical scavenging assay according to the method used (Sasmito *et al.*, 2013). Each aliquot (0.1 ml) of the sample with varying concentrations (25, 50, 100 and 200 µg/ml) was added to 3.0 ml of DPPH ethanol solution, shaken and stored for 30 minutes in a dark room. The scavenging effect on DPPH radicals was determined by spectrophotometer at λ 517 nm. DPPH is calculated by the following equation:

$$\text{Percentage of the RSA} = \frac{Ac - As}{Ac}\ x\ 100\%$$

Where: A_C =absorbance of control and A_S = absorbance of the sample. The DPPH solution without sample solution was used as control. IC_{50} value is the concentration of sample required to scavenge 50% of DPPH free radical and was calculated by using the plotted graph of radical scavenging activity (RSA) against the concentration of samples.

Bioactive compounds were investigated using liquid chromatography instruments (ACQUITY UPLCH-Class System, Water, USA) with a C18 column (1.8 µm 2.1 x 100 mm). Mass spectrometry: Electropray ionization (ESI), positive ion. Mass analysis range: 50 - 1200 m/z. A total of 5 µl of the sample were injected and through the first 0.2 µl syringe filter. Mobile phase: water + 5 mM format ammonium and acetonitrile + 0.05% formic acid; flow rate: 0.2 ml/min gradually for 18 minutes. The mass spectrum of compounds was analyzed using Masslynx software to obtain molecular formulas (compounds and their relative masses), and then the determination of compounds was confirmed with data bases at Chemspider, Massbank, and others. Except for the identification of antioxidant compounds by LCMS, all analyzes were carried out and repeated three times. The results of data analysis are displayed as the mean values ± standard deviation (S.D); and linear regression analysis and calculation were done by using MS-Excel software.

RESULTS AND DISCUSSION

The ethanolic extract from *S. alba* bark and leaves was 9.37 ± 0.19% and 10.26 ± 0.29%, with a moisture content of 17.50 ± 0.07% and 19.30 ± 0.03%, respectively. In

addition, the results of the phytochemical analysis of ethanolate extracts are both shown in Table 1.

Table 1 – Phytochemical, antioxidant activity (IC_{50}) and total phenol of the EtOH extract

Compounds		Bark	Leaves
Alcaloids		-	-
Flavonoids		+++	++
Terpenoids		++	+
Steroids		+	-
Phenolics		++	++
Saponins		++	++
Tannins		++	+
Antioxidant activity	IC_{50} (µg/ml)	67.211±2.482	88.706±4.782
Total phenolic content	mgGAE/g	10.248±0.174	5.847±0.315

The EtOH extract from *S. alba* bark was not contain alkaloids but positively contains flavonoids, terpenoids, steroids, phenolics and tannins. Whereas the extract from *S. alba* leaves, besides showing no alkaloid content, also the presence of steroid compounds was not detected (Table 1). Furthermore, the antioxidant activity of ethanolate extract from the bark and leaves of *S. alba* to scavenge a free radical (DPPH) is very strong. The IC_{50} values of each of the above extracts were 67,211 ± 2,482 µg/ml and 88,706 ± 4,782 µg/ml, respectively. The total content of polyphenols in the bark extract was 10.248 ± 0.174 mgGAE/g sample, while the EtOH extract of mangrove leaves contained a total polyphenol of 5.847 ± 0.315 mgGAE/g sample.

The types of antioxidant compounds detected in the leaves of EtOH extract were fewer than those found in the bark extract. Bioactive compounds suspected of having antioxidant activity of mangrove bark are approximately 8 compounds; whereas only about 5 antioxidant compounds were detected in mangrove leaves (Table 2).

Table 2 shows that 7-hydroxycoumarin ($C_9H_6O_3$) was detected at the earliest retention time (Rt 4.22 min). As an organic compound, hydroxycoumarin is found in many types of plants and are widely distributed in parts such as leaves, stems and roots of plants. These compound is also called umbelliferone, as derivative compound of coumarin which has antioxidant, antibacterial and antifungal activities (Repčák, Imrich and Franeková, 2001; Abdel-Farid, Sheded and Mohamed, 2014; Witaicenis *et al.*, 2014; Mazimba, 2017). Coumarin is a phenolic compound found in many plants that has been known to have good antioxidant activity, and is soluble in polar solvents such as methanol and ethanol (Yang and Lee, 2012). This is supported by the results of phytochemical analysis (Table 1) which shows the presence of phenolic compounds in the EtOH extracts of bark and *S. alba* leaves. Coumarin has antioxidant activity as reported by Wang (Wang *et al.*, 2016) and also has activity as an antitumor and antihyperlipidemic (Iyer and Patil, 2014).

Table 2 – Results of identification of antioxidant compounds in EtOH extracts

Source	Rt*	m/z (Da)	Mol formula	Compounds
Bark	4.22	162.032	$C_9H_6O_3$	7-hydroxycoumarin
	4.76	138.121	$C_7H_6O_3$	3-hydroxybenzoic acid, or Salicylic acid
	4.79	306.074	$C_{15}H_{14}O_7$	Epigallocatechin
	5.096	578.520	$C_{30}H_{26}O_{12}$	Procyanidin B1 or B2
	6.689	170.120	$C_7H_6O_5$	Gallic acid
	7.406	236.265	$C_{16}H_{12}O_2$	3-benzyl-2H-chromen-2-one, or Coumarin
		328.359	$C_{19}H_{20}O_5$	Decursin or acutilobin
	7.509	278.302	$C_{18}H_{14}O_3$	Tanshinone
	8.057	344.272	$C_{17}H_{12}O_8$	2,3,8-tri-O-methylellagic acid
Leaves	4.22	162.032	$C_9H_6O_3$	7-hydroxycoumarin,
	4.84	256.253	$C_{15}H_{12}O_4$	Isoliquiritigenin,
	5.06	300.263	$C_{16}H_{12}O_6$	Diosmetin
	5.46	318.235	$C_{15}H_{10}O_8$	Merycetin, Gossypetin, Quercetagetin
	6.64	286.236	$C_{15}H_{10}O_6$	Luteolin

*Note: *Retention times (min).*

Salicylic acid ($C_7H_6O_3$) is a secondary metabolite of plants that has anti-inflammatory, anti-fungal and antibacterial activity. Salisilic acid is a hydroxybenzoic acid in which bensoic acid with a hydroxyl group is in the ortho position(National Center for Biotechnology Information., 2019).

Epigallocatechin ($C_{15}H_{14}O_7$), a phenolic compound that has strong antioxidant activity, and is widely found in plant leaves including mangroves. Rios (2005) has reported that methanol extracts from Acacia pennatula leaves were detected containing catechins and epigallocatechin compounds. Epigallocatechin and catechin derivatives are also found in many plant leaf extracts, like as reported by Romani *et al.* (1999), that the leaves of *Myrtus communis* also contain many catechin derivatives.

Isomeric of $C_{30}H_{26}O_{12}$ (+)-procyanidin B1 from B2 and (+)-catechins including polyphenol compounds found in many parts of plants as reported (Du *et al.*, 2013). This compound has a high ability to reduce free radicals. ESI-MS analysis showed the main molecular weight of procyanidin with a peak value of [M+H]+ was 579.2. This means that the extract contains a dimer from procyanidin, and epicatechin as its basic unit (Ling, Xie and Yang, 2005).

Coumarin, 3-benzyl-2H-chromium-2-one ($C_{16}H_{12}O_2$), coumarin is a heterocyclic molecule that is often associated with its benefits for human health, such as reducing the risk of cancer, diabetes and heart disease. This effect is associated with the effect of capturing free radicals, due to their antioxidant activity. The clean-up effect of the free radical 2,2-diphenyl-1-picrillidrazil (DPPH) shows high activity with a low IC_{50} value (Li *et al.*, 2012).

Decursin or acutilobin is a coumarin derivate compound ($C_{19}H_{20}O_5$). This compound belongs to the class of coumarin derivatives known as linear pyranocoumarin. This is an organic compound containing pyran ring which linearly joins to the coumarin section. Coumarin is a phenolic compound that is naturally synthesized by several plant species. Coumarin and its derivatives have been found to have significant anti-inflammatory and antioxidant activity (Kontogiorgis, Savvoglou and Hadjipavlou-Litina, 2006). Acutilobin is a coumarin derivative that also has biological activity as an antitumor (anticancer), and some coumarin derivatives have also been found to have the ability to scavenge reactive oxygen species (ROS) - free radicals (Kadhum *et al.*, 2011).

Furthermore tanshinone ($C_{18}H_{14}O_3$) is an organic compound from the prenol-lipid class that is terpenoid which also has good biological activity as an antioxidant. The quinone compound that is thought to be present in the ethanol extract of mangrove bark has been used as a natural medicine in China. Dried root *Salvia miltiorrhiza* Bunge is a herbal medicine that is very commonly used in several regions in China. Besides *S. miltiorrhiza* Bunge (purple flowering) also other varieties with white flowers, *S. miltiorrhiza* Bunge *f. Alba* (Zhang *et al.*, 2016). Tansinon, besides having antioxidant activity is also reported to have anti-tumor activity (Yoon *et al.*, 1999; Lee *et al.*, 2010).

Another polyphenol compound detected in the *S. alba* bark extract is $C_{17}H_{12}O_8$ (2,3,8-tri-O-methylellagic acid). These compounds found in plants that are widely distributed. The amount of MeOH solubles in both bark and wood from young trees *Eucalyptus globulus, E. Regnansand E. Deglupta* showed the presence ellagic compound (Yazaki and Hillis, 1976). It is contained in the leaves of *Phyllanthus reticulatus* as part of the metabolites extracted in dichlorometan (Pojchaijongdee *et al.*, 2010). This compound is also contained in the bark of the *Irvingia gabonensis* plant and is thought to have anti-bacterial activity (Ndukwe and Zhao, 2007).

Furthermore, the bioactive compounds suspected of being natural antioxidants in *S. alba* leaves beside the 6-Methoxy-7-hydroxycoumarin, are: Isoliquiritigenin is a flavonoid (isoflavan) which has bioactive potential as an anti-inflammatory, anti-bacterial and antioxidant (Trinh *et al.*, 2015; Xiao *et al.*, 2017). This compound is also widely found in plants such as *Glycyrrhiza uralensis* as reported by Watanabe (Watanabe *et al.*, 2016).

Diosmetin ($C_{16}H_{12}O_6$) or 3',5,7-trihydroxy-4'-methoxyflavone) is the aglycone portion of the diosmin flavonoid glycosides. A phenolic compound like flavonoids, that is also found in many fruit peels, flowers and medicinal plants such as *Chionanthus retusus* (Oleaceae) (Patel *et al.*, 2013; Lee *et al.*, 2019).

$C_{15}H_{10}O_8$ (merycetin, gossypetin, quercetagetin), merisitin, gossypetin are phenolic compounds which are derivatives of flavonoids-glucosides, many of which are found in plant material such as cactus rind, white berries (*Myrtus communis* L. var, *leucocarpa* DC) and others (Fernández-López *et al.*, 2010; Serreli *et al.*, 2017). Quercetagetin is flavonol which is also widely found in plant matter and has antioxidant activity. These compounds besides having antioxidant activity, are also anti-diabetic, antiproliferation and others (Loizzo *et al.*, 2009; Zehl *et al.*, 2011; Wang *et al.*, 2016).

$C_{15}H_{10}O_6$ (Luteolin), is a tetrahydroxyflavone which is basically a planar molecule and contains intramolecular hydrogen bonds (Cox *et al.*, 2003). Luteolin is a flavon that has a hydroxyl group in ring B and is an isomer of epigenin, and inhibits oxidative stress (Huang *et al.*, 2013).

CONCLUSION

The antioxidant compounds in the ethanol extract of Sonneratia alba bark, quantitatively, have more types and contents than ethanol extracts from their leaves. Polyphenols are the constituents in both of the ethanol extracts (bark and leaves) which have antioxidant activities.

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