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Phytochemical Screening and Antioxidant Activity Analysis of N-Hexane Extract of *Sonneratia alba* Mangrove Leaves

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Abstract

Mangrove plants have been widely used by people in various regions of Indonesia as traditional medicine for the treatment of wounds, fever, diarrhea, and others. Extreme habitat in coastal areas with high salinity and low oxygen level makes mangrove plants produce a variety of unique secondary metabolites and are rich in antioxidants. Although there have been studies on phytochemicals and antioxidant activity conducted, the study of mangroves in Bali, Indonesia, has not been widely explored. This study aims to determine the compounds contained in mangrove leaf extract and their antioxidant activity. The species to be studied is *Sonneratia alba*, which is commonly found in the Ngurah Rai Mangrove Forest, Bali, Indonesia. In this experimental study, compound extraction was carried out by maceration method using n-hexane solvent. Phytochemical screening using qualitative methods and GC-MS was carried out to determine the phytochemical compounds in the extract, while 1,1-diphenyl-2-picrylhydrazyl (DPPH) method was used to determine antioxidant activity. The result showed that the n-hexane *S. alba* mangrove leaves extract display strong antioxidant activity with IC_{50} value of 64.432 ± 7.675 ppm. The qualitative phytochemical tests showed that the extract contained phenol and steroid, which are known to have antioxidant properties. Moreover, GC-MS analysis showed that there are two compounds with the largest %area namely gamma-sitosterol and dl-alpha-tocopherol (52.88% and 7.77%, respectively), which have been reported to have antioxidant activities. To conclude, these findings demonstrate that n-hexane extract of *S. alba* mangrove leaves from Ngurah Rai Mangrove Forest, Bali, to have potential antioxidant activity.

Keywords: Antioxidant; DPPH; Mangrove; Phytochemicals; *Sonneratia alba*.

INTRODUCTION

Indonesia is a mega biodiversity tropical country. Indonesia has the largest mangrove forest in the world, with an area of about 42,278 km^2 (Bibi et al. 2019). Mangroves are halophytic plants that are well adapted to salt water and flourish in the intertidal zone of tropical and subtropical regions (Bibi et al. 2019). Furthermore, these plants are adaptable to living in low-oxygen and high temperature environments (Dahibhate et al. 2019). These extreme environmental circumstances make these plants to create distinctive substances with interesting pharmacological effects and make it as a source of potential medicinal plants (Abdel-Aziz et al. 2016; Sadeer et al. 2023).

In Indonesia, mangrove plants have been traditionally used for treatment of skin diseases, rheumatic, epilepsy, and diarrhea (Kusmana & Sukristijiono, 2016). In Gedangan Village, Central Java, these plants have been widely used as traditional medicine for fever, diarrhea, treatment of wounds and ulcers, stomach ache, and others (Rahayu & Sunarto, 2020). Furthermore, mangrove plants are utilized as medicine for hematuria, treating bruises and wound, as well as contraception and appetite enhancer by people in Mempawah Distric, West Kalimantan (Arbiastutie et al. 2021). Thus, mangrove plants have great potential to be explored as medicinal sources because they have been utilized empirically and are known to contain a wide range of unique and useful secondary metabolites.

Mangrove plants are notable for their diverse secondary metabolites such as flavonoids, alkaloids, saponins, tannins, steroids and triterpenes (Abdel-Aziz et al. 2016). These metabolites are not only useful for plants but also have useful therapeutic activities for humans such as antibacterial, anticholesterol, antiinflammatory, antifungal, antiviral, antidiabetic, anticancer, and antioxidant (Dahibhate et al. 2019; Genilar et al. 2021; Vinoth et al. 2019). Compounds with high antioxidant activity are quite widely researched in the recent years due to their great benefits in the prevention and treatment of oxidative stress-related pathology.

Antioxidants are compounds that able to neutralize free radicals and reactive oxygen species (ROS), as well

as inhibit oxidation (Neha et al. 2019). Exaggerated amount of ROS in the body could cause oxidative stress, a condition that is closely related to diseases such as diabetes, cardiovascular diseases, cancer, neurodegeneration, and rheumatoid arthritis (Pisoschi et al. 2021). Mangrove plants are rich in natural phenolic substances that contribute to their antioxidant capacity, including phenolic acids, flavonoids, lignan, and tannins (Abdel-Aziz et al. 2016; Amarowicz & Pegg, 2019).

Ngurah Rai Mangrove Forest, that is located in the south area of Bali Island, is the greatest mangrove forest in Bali. Currently, there are 24 mangrove species from about 17 families spread throughout this mangrove forest, with *Sonnerratia alba* being one of the prominent species (Setiastri et al. 2019). Although the pharmacological effects of mangroves have been widely studied, no research has yet looked at the antioxidant activity of the *S. alba* mangrove leaves that inhabit Ngurah Rai Mangrove Forest, Bali. In addition, differences in habitat and environment, including differences in rainfall, soil quality, and sunlight between one area and another might create different results compared to similar studies in different areas. Therefore, in this study, we aim to figure out the phytochemicals and antioxidant activity of *S. alba* mangrove leaves extract from Ngurah Rai Mangrove Forest, Bali. This study is expected to provide new information about the antioxidant potential of mangroves that can be developed into new therapeutic agents.

MATERIALS AND METHODS

Materials

Sonneratia alba leaves were obtained from Ngurah Rai Mangrove Forest, to be precise in the KUB Simbar Segara area, Pemogan Village, South Denpasar, Bali, in September 2022. N-hexane solvent was purchased from Merk-Supelco, Germany, while 1,1-diphenyl-2-picryhydrazil (DPPH) kit was acquired from Sigma Aldrich, USA. The equipment used in this study were oven, grinder, mesh, analytical balances, maceration containers, glassware, filter papers, vacuum rotary evaporator, cuvettes, and UV-visible spectrophotometer.

Methods

Sample preparation

The mature leaves of *S. alba* mangrove were collected from five different plants. The leaves were washed with running water and then dried using an oven for 24 hours at 60°C. The dried leaves were then grinded into powder and stored in a tight closed containers until further research. Voucher specimen was made for sample identification (Figure 1), that was performed at Research Centre for Plant Conservation, Botanical Gardens, and Forestry, located in Bedugul, Bali.

Figure 1. Voucher specimen of *Sonneratia alba* sample.

Extraction

An amount of 100 grams leaf powder was added with 500 ml n-hexane, and then extracted using maceration method at room temperature for 2x24 hours with continuous stirring. The extract was filtered using filter paper and the solvent was evaporated using vacuum rotary evaporator at 50°C and 90 rpm. The obtained semisolid extract was stored at 4°C until further experiment (Wijaya & Indraningrat, 2021).

DPPH Assay

The antioxidant activity analysis was performed using the DPPH method. A total of 10 mg of mangrove leaf extract was dissolved in 100 ml of methanol pro analysis (PA). The dilution was carried out using methanol PA by series concentration of 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm. The mixtures were then left for 30 minutes in a dark room at room temperature. The absorbance value was measured using UV-visible spectrophotometer at a wavelength of 517 nm. The test was repeated three times. The IC_{50} value was calculated using the linear regression formula from the concentration series in Microsoft Excel. The level of antioxidant activity was divided into 4 categories: very strong (<50 ppm), strong (50-100 ppm), moderate (101-150 ppm), and weak (151-200 ppm) (Surjanto et al. 2019).

Phytochemical Analysis

The qualitative phytochemical tests were carried out to identify several compounds in the extract, namely flavonoids, alkaloids, tannins, saponins, phenolics, steroids, and terpenoids. Meanwhile, the GC-MS analysis was conducted at Forensic Laboratory of Bali Regional Police. The compounds were determined using a comparison of the mass spectrum and retention times between each sample and the standard.

RESULTS AND DISCUSSION

Sample identification

Sample determination was conducted to ensure that the sample used was the correct species, *Sonneratia alba*, and thus, the sampling error could be avoided. The result showed that the leaves sample harvested from the mangrove forest was certainly *Sonneratia alba* (Table 1).

Table 1. Taxonomy of *Sonneratia alba.*

Rank	Name
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Order	Myrtales
Family	Lythraceae
Genus	Sonneratia L
Species	*Sonneratia alba*

Antioxidant activity

Antioxidant activity analysis of n-hexane extract of *S. alba* mangrove leaves was carried out using the DPPH method. The result showed that the extract possessed strong antioxidant activity, with IC_{50} value of 64.432 ± 7.675 ppm (Table 2). Similar studies showed that ethyl acetate extract of *S. alba* mangrove leaves from Tanjung Jabung, Jambi displayed weak antioxidant activity with IC_{50} value of 223.67 ppm (Latief et al. 2019). Meanwhile, a study in Dumai, Riau, showed strong to very strong antioxidant activity of young and old *S. alba* leaves, with IC_{50} value of 50.12 ppm and 49.87 ppm respectively (Sumartini et al. 2022). The variation in the antioxidant activities of these extracts from the same species could be affected by differences in the solvents, habitats, or extraction methods (Elsharkawy et al. 2021; Onyebuchi & Kavaz, 2020).

Table 2. IC_{50} value of n-hexane extract of *S. alba* leaves.

IC50 value (ppm)				Category
I	II	III	Average	
72.611	63.298	57.387	64.432 ± 7.675	**Strong**

Phytochemical analysis

The qualitative phytochemical analysis showed that the n-hexane extract contained two compounds, namely phenol and steroids (Table 3). Both of these compounds are known to exhibit antioxidant bioactivity (Llauradó Maury et al. 2020). However, a study conducted in Dumai, Riau, showed that compounds detected in n-hexane extract of *S. alba* leaves were alkaloids, flavonoids, saponins, and tannins (Sumartini et al. 2022). Different extraction methods used and different habitat of the *S. alba* mangroves can both contribute to these variations (Elsharkawy et al. 2021; Onyebuchi & Kavaz, 2020).

Table 3. Phytochemical analysis results of n-hexane extract of *S. alba* leaves.

Phytochemicals	Reagent used	Results
Terpenoids	Liebermann Burchard	(-)
Phenols	FeCl3 1%	**(+)**
Alkaloids	Dragendorff	(-)
Steroids	Liebermann Burchard	**(+)**
Saponins	Distilled water	(-)
Tannins	FeCl3 1%	(-)
Flavonoids	Mg+HC	(-)

The GC-MS analysis results showed that there were five prominent compounds found in the n-hexane extract, namely gamma-sitosterol, dl-alpha-tocopherol, Benzene-1 ,2,4-trimethyl, 3-Methyl-4- (phenylthio)-2-prop-2-enyl-2,5-dihydrothiophene 1,1-dioxide, and Benzo [h] quinoline, 2,4-dimethyl- (Table 4). Gamma-sitosterol was dominantly found in the n-hexane leaves extract of *S. alba* in this study, while stigmasterol was prominent in the n-hexane root extract of this species (Wijaya et al. 2023). Gamma-sitosterol has been shown to has a strong anticancer activity, as well as antioxidant and anticholesterol properties (Dede et al. 2019). The compound dl-alpha-tocopherol or also known as vitamin E is a lipophilic antioxidant (Zeece, 2020). Meanwhile, benzene-1,2,4-trimethyl is known to have potential antioxidants effects, and also have efficacy against pancreatic cancer (Marikkannu & Ganesan, 2021; Ogunmefun et al. 2023). Benzo[h]quinolone has the potential as an antitumor because its cytotoxic effect and is able to inhibit the growth of tumor cells (Jafari et al. 2019). However, no studies have demonstrated the antioxidant activity of this substance. In addition, no evidence has been found that clarify the pharmacological activity of the 3-Methyl-4- (phenylthio)-2-prop-2-enyl-2,5-dihydrothiophene 1,1-dioxide compound.

Table 4. GC-MS results of n-hexane extract of *S. alba* leaves.

Peak	Retention time	Compounds	% area
1	49.168	Gamma-sitosterol	52.88
2	47.053	dl-alpha-Tocopherol	7.77
3	4.413	Benzene- 1,2,4-trimethyl	4.07
4	31.948	3-Methyl-4- (phenylthio)-2-prop-2-enyl-2,5-dihydrothiophene 1,1-dioxide	3.85
5	48.458	Benzo [h] quinoline, 2,4-dimethyl-	3.82
6	50.094	Thymol, TMS derivative	3.27
7	49.484	2-Ethylacridine	3.05
8	43.111	1H-Indole, 5-methyl-2-phenyl-	3.03
9	5.474	3,4-Dichloro-5-phenylimino-2(5H)-furanone	2.17
10	4.554	dl-Allo-cystathionine	2.04
11	3.650	Benzene, 1-ethyl-3-methyl-	1.94
12	7.820	Aluminum, tripropyl-	1.83
13	8.407	2-Acetyl-5-bromo-1-methyl-1H-indol-3-yl difluoroborinate	1.78
14	49.451	(E)-2-bromobutyloxychalcone	1.38
15	3.793	1,3-Cyclopentadiene, 5-(1-methylpropylidene)-	1.01
16	41.319	Histidine, 1, N-dimethyl-4-nitro-	0.74

CONCLUSIONS

Based on the findings of this study, it can be concluded that the n-hexane extract of *S. alba* leaves displayed strong antioxidant activity with an IC50 value of 64.432 ± 7.675 ppm. The phytochemicals found in the extract were phenols and steroids, while GC-MS analysis showed that gamma-sitosterol was the most prominent compound with % area of 52.88.

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Authors' Contributions: Putu Rissa Almadea Surya, Made Dharmesti Wijaya, & Desak Putu Citra Udiyani designed the study. Putu Rissa Almadea Surya carried out the laboratory work and analyzed the data. Putu Rissa Almadea Surya & Made Dharmesti Wijaya wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests.

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