Antioxidant Property of Methanol Extract of *Sonneratia alba* Mangrove Leaves from Bali-Indonesia

Made Dharmesti Wijaya[1], Putu Rissa Almadea Surya, Anak Agung Gede Indraningrat, Desak Putu Citra Udiyani

{dharmestiwijaya@gmail.com[1]}

Faculty of Medicine and Health Sciences, Warmadewa University, Denpasar-Bali, 80235, Indonesia

Abstract. The rising incidence of oxidative stress-associated diseases e.g. cancer, diabetes, as well as neurodegenerative and cardiovascular diseases in the last decades makes exploration of new natural antioxidant compounds very crucial. Mangrove plants stand out among other natural sources due to the distinctive secondary metabolites they produce to adapt to their extreme habitat. Numerous studies have been done to explore the pharmaceutical activities of mangrove plants, yet little is known about the antioxidant properties of mangroves in Bali, Indonesia. This research aims to discover the antioxidant activity and phytochemical content of the *Sonneratia alba* mangrove leaves methanolic extract collected from Ngurah Rai Mangrove Forest, Bali, Indonesia. The extract was obtained by maceration of the leaves powder using methanol (1:5 ratio) for 2x24 hours. The antioxidant activity was analyzed based on DPPH assay, while chemical profiles of the extract was detected using both the qualitative and GC-MS methods. Results showed that the methanol extract from *S. alba* leaves demonstrated potent antioxidant activity, indicated by an IC_{50} value of 0.60 ± 0.08 ppm. This great activity is supported by its phytochemical content, including phenols, flavonoids, tannins, and steroids, with their notable antioxidant activities. The GC-MS analysis identified 1,2,3-benzenetriol, a compound renowned for its antioxidant properties. In conclusion, these results display promising potential of the methanol extract of *S. alba* mangrove leaves to be further explored and developed as a new natural antioxidant source.

Keywords: *Sonneratia alba,* Antioxidant, Phytochemicals

1. Introduction

Oxidative stress has been proposed as a major contributor to the rate of ageing and contributes to a number of age-related and chronic diseases, such as cancer, neurodegenerative diseases, diabetes, and cardiovascular diseases [1, 2]. Oxidative stress occurs when oxidants outweigh antioxidants in the body, causing disturbances in redox signaling and regulation as well as instigating molecular damage [3]. Free radical and oxygen radicals that are often by products from a variety of metabolic activities are normally counteracted by endogenous and exogenous antioxidants to maintain the oxidant-antioxidant balance and protect cells from oxidative damage [4]. However, conditions such as long-term stress, unhealthy diet, excessive physical workouts, and UV radiation exposure can elevate the free radicals production and cause harm to the body [5].

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The essential function of antioxidants in the defense mechanism against free radicals has led to widespread research on new sources of natural antioxidants in the last few decades. Besides, more focus is being placed on natural antioxidants, as the majority of commonly used artificial antioxidants are accused to give adverse effects on human health [6]. Among natural sources, exogenous non-enzymatic antioxidants from plants are extensively studied. A number of phytochemical molecules have been renowned for their antioxidant effects including vitamin C and E, carotenoids, polyphenols, catechins, flavonoids, amino acids, organosulfur, and several minerals [2, 4]. These plant-derived compounds are thought to have beneficial effects on antioxidant defenses and are expected to prevent oxidative stress-related diseases [7].

Mangroves are one of the potential sources of natural antioxidants. Mangrove plants have been used for ethnomedical treatments all around the world [8]. These plants live in stressful habitats with high water salinity and temperature, tidal upsurges, and poor oxygen levels, and have adapted well by producing several unique bioactive compounds to help them flourish in this harsh environment [8, 9]. These compounds include phenols, flavonoids, tannins, alkaloids, terpenoids, saponins, and steroids [9]. Among them, polyphenolic compounds, including phenolic acids, flavonoids, and tannins, are well-known for their antioxidant properties [6, 10]. The abundance of phytochemicals found in mangroves and their potential to be developed as pharmaceutical products make thorough research supported by reliable scientific evidence noticeably needed [11].

Indonesia boasts the world's largest mangrove forest, covering an impressive over forty thousand or approximately 26% of the global mangrove forest area [11]. Bali is one of the provinces with a large and well-maintained mangrove area, with Ngurah Rai Mangrove Forest standing as the largest mangrove habitat within the province. *Sonneratia alba* is the most dominant species found in this forest [12]. There have been many studies on the Sonneratia genus, but they are still mostly focused on *S. caseolaris* [13, 14]. Meanwhile, research related to *S. alba* is still limited. Furthermore, no research has been conducted to investigate the antioxidant property of the methanol extract from *S. alba* leaves found specifically in the Ngurah Rai Mangrove Forest. Therefore, exploratory research is needed regarding the antioxidant activity of the methanol extract of *S. alba* leaves from Ngurah Rai Mangrove Forest, Bali, Indonesia. This research aims to provide novel insights into the promising antioxidant properties of the extract and serve as a foundational step in the development of new natural antioxidant compounds.

2. Method

Sonneratia alba mangrove leaves were gathered from the Simbar Segara area of Ngurah Rai Mangrove Forest, Denpasar, Bali, Indonesia, around September 2022. A voucher specimen was prepared and sent to the Research Centre for Plant Conservation, Bedugul, Bali, for plant determination. The obtained leaf samples were carefully cleaned under running water and then dehydrated in an oven at 60°C for 24 hours. Following, the dried samples were powdered using a blender and saved in airtight storage until further use.

The powdered plant material was subjected to a maceration process using methanol solvent (Smart Lab, Indonesia). A 1:5 ratio of plant material to solvent was employed, and the mixture was allowed to macerate at room temperature for a 24-hour period. This procedure was repeated once more with fresh solvent. A vacuum rotary evaporator at 90 rpm, 50°C was used to dry the extract and subsequently the obtained extract was stored at 4^{o}C until further used.

The ability of the methanol extract from *S. alba* leaves to act as an antioxidant was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) kit (Sigma Aldrich, USA) radical scavenging assay. The linear regression equation was used to calculate the IC_{50} values. The IC_{50} values were categorized into four categories: very strong, strong, moderate, and weak (Table 1).

Table 1. Classification of antioxidant activity [15]

IC_{50} Values	Category
<50 ppm	Very Strong
50-100 ppm	Strong
101-150 ppm	Moderate
151-200 ppm	Weak

Analysis of the phytochemical compounds was carried out using the qualitative method to identify secondary metabolite compounds such as tannins, phenols, saponins, flavonoids, terpenoids, alkaloids, and steroids. Furthermore, gas chromatography coupled to mass spectrometry (GC-MS) analysis was also performed to identify the compounds contained in the extract. The analysis was performed at the Forensic Laboratory of the Bali Regional Police.

3. Result and Discussion

The *S. alba* leaf extract exhibit very strong antioxidant property with an IC_{50} of 0.60 ± 0.08 ppm (Table 2). A similar study found that *S. alba* leaf extract from Malaysia also had very strong antioxidant activity with an IC_{50} of 38 ppm [16]. Another study using different solvents showed slightly different results. The ethanol extract of *S. alba* leaves from East Java, Indonesia, displayed an IC_{50} value of 88.70 ppm and was thus categorized as having strong antioxidant activity [17]. Meanwhile, tea from *S. alba* old and young leaves showed very strong and strong antioxidant properties, with IC_{50} values of 49.87 ppm and 50.12 ppm, respectively [18]. The disparity in IC_{50} values observed between the *S. alba* leaf extracts could be attributed to variations in the types and quantities of phytochemicals present in the samples. These variations may arise from differences in the plant's habitat, the specific extraction methods employed, or the type of solvent used for the extraction [19, 20].

Table 2. IC_{50} value of *S. alba* leaves methanol extract

IC_{50} value (ppm)				Category
I	II	III	Average	
0.512	0.609	0.667	0.60 ± 0.08	Very Strong

The phytochemical analysis revealed the presence of four distinct compounds within the extract, namely phenols, flavonoids, steroids, and tannins (Table 3). Similar studies of *S. alba* leaves extract from different habitats, solvents, and extraction methods showed slightly different phytochemical constituents, but flavonoids, tannins, and phenols were always present [17, 18, 21]. Phenolic compounds are regarded as antioxidants because of their ability to donate an electron and/or a hydrogen atom to free radicals, thus disrupting the oxidation chain reaction [10]. Phenolic compounds consist of hydroxylated aromatic rings, and their antioxidant properties are determined by the quantity of hydroxyl groups in the aromatic rings as well as

their arrangements [6]. Meanwhile, flavonoids are a class of compounds that belong to the polyphenolic group and are notable for their antioxidant activities. The antioxidant mechanisms are through oxidases inhibition, antioxidant enzymes activation, ROS scavenging, and α-tocopheryl radicals' reduction [22, 23]. Tannins also belong to the poliphenolic group. Their antioxidant abilities are based on the distinctive features of any phenol thet neutralize free radicals [24]. In addition, plant steroids, also known as phytosterols, have remarkable antioxidant effect due to their capability to increase antioxidant enzymes, stabilize cell membranes, and have free radical scavenging activity [25].

Table 3. Phytochemical compounds analysis of *S. alba* leaves methanol extract

Compounds	Detection Method	Outcome
Phenols	FeCl3 1%	**(+)**
Flavonoids	Mg+HCl	**(+)**
Tannins	FeCl3 1%	**(+)**
Alkaloids	Dragendorff	(-)
Terpenoids	Liebermann Burchard	(-)
Saponins	Distilled water	(-)
Steroids	Liebermann Burchard	**(+)**

The GC-MS analysis displayed the presence of seven bioactive compounds within the methanol extract of *S. alba* leaves (Table 3). Among them, 1,2,3-benzenetriol or also known as pyrogallic acid was the most prominent, with a peak area of 85.72%. This compound belongs to the polyphenol group and is notable for its antioxidant activity [26]. Another compound detected was 2-ethylacridine, with profound antioxidant and antimicrobial properties [27]. Furthermore, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one or DDMP is also known for its remarkable free radical scavenging capacity, which is derived from the unstable enol structure of this compound [28]. However, the information about the antioxidant activities of 1,2-Propanediol, 3-chloro-; Succindialdehyde; 1,2,4-Thiadiazole, 3,5-bis(carbamoylmethylthio)-; and 2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-butenyl)- is still limited.

Table 4. GC-MS analysis of *S. alba* leaves methanol extract

Peak	Retention Time	Compounds	%Area
1	3.661	1,2-Propanediol, 3-chloro-	1.99
2	9.123	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one	2.58
3	13.582	Succindialdehyde	2.25
4	16.014	1,2,3-Benzenetriol	85.72
5	17.664	1,2,4-Thiadiazole, 3,5-bis(carbamoylmethylthio)-	1.56
6	25.667	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-butenyl)-	1.71
7	49.165	2-Ethylacridine	3.56

4. Conclusion

In conclusion, the methanol extract derived from *S. alba* leaves exhibited exceptionally strong antioxidant activity, as indicated by an IC50 value of 0.60 ± 0.08 ppm. This potent antioxidant property is due to phytochemicals detected in the extract, namely phenols, flavonoids, tannins, and steroids, which are well-known for their antioxidant capacity. In addition, GC-MS analysis showed seven compounds detected in the extract, with 1,2,3-benzenetriol being the most prominent.

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