

# Antioxidant Activity, Total Phenolic, Phytochemical Content, and HPLC Profile of Selected Mangrove Species from Tanjung Api-Api Port Area, South Sumatra, Indonesia

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**Submission date:** 31-Jul-2023 08:34PM (UTC+0700)

**Submission ID:** 2139522517

**File name:** For\_Turnitin\_29-TJNPR-2023-M305\_Galley\_Proof-CI.pdf (481.49K)

**Word count:** 5126

**Character count:** 27348

**Antioxidant Activity, Total Phenolic, Phytochemical Content, and HPLC Profile of Selected Mangrove Species from Tanjung Api-Api Port Area, South Sumatra, Indonesia**Rozirwan Rozirwan<sup>1\*</sup>, Hamid Hananda<sup>1</sup>, Redho Y. Nugroho<sup>2</sup>, Rezi Apri<sup>1</sup>, Nadila N. Khotimah<sup>2</sup>, Fauziyah Fauziyah<sup>1</sup>, Wike A.E. Putri<sup>1</sup>, Riris Aryawati<sup>1</sup><sup>1</sup>Department of Marine Science, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Indralaya 30862, South Sumatra, Indonesia<sup>2</sup>Environmental Management Study Program, Graduate Program, Sriwijaya University, Palembang 30139, Indonesia

## ARTICLE INFO

## ABSTRACT

## Article history:

Received 30 June 2023

Revised 15 July 2023

Accepted 21 July 2023

Published online 01 August 2023

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The development of bioactive chemicals in response to mangroves self-defense mechanisms and environmental adaptation has led to an improvement in antioxidant capacity. This study aims to determine the antioxidant activity (DPPH method), total phenol, phytochemical content, and HPLC profile of some mangrove species. Samples were taken in the mangrove area around the port of Tanjung Api-Api in South Sumatra, Indonesia. Maceration and extraction of all samples was done using methanol as a solvent. Samples were tested for antioxidant against DPPH free radicals, total phenol with Folin-Ciocalteu method, preliminary phytochemical qualitatives, and measuring antioxidant compounds with HPLC. Based on the results, the IC<sub>50</sub> of antioxidants of all samples revealed that *A. marina* (171.16 g/mL) (low), *B. gymnorhiza* (105.09 g/mL) (moderate), and *S. alba* (28.064 g/mL) (very strong) had antioxidant activity. Furthermore, the phenolic content of *A. marina* is 9.0258 mg GAE/g, that of *B. gymnorhiza* is 13.8222 mg GAE/g, and that of *S. alba* is 9.4969 mg GAE/g. A phytochemical test of *A. marina* revealed flavonoids, steroids, and saponins. *B. gymnorhiza* revealed alkaloids, terpenoids, steroids, and saponins. *S. alba* revealed flavonoids, terpenoids, steroids, saponins, and tannins. The HPLC profile of antioxidant activity using ascorbic acid showed that *A. marina* and *S. alba* were lower than the standards of 64.224 ppm and 67.640 ppm, while *B. gymnorhiza* was higher than the standard of 109.510 ppm. All three species of mangroves had potential to inhibit free radical reactions in the low, moderate, and very strong categories.

**Keywords:** antioxidant, HPLC profile, mangrove, phytochemical, total phenol.**Introduction**

Ecologically, mangrove vegetation provides many benefits for aquatic ecosystems.<sup>1,2</sup> Plants spread in the intertidal zone in the tropics and subtropics can act as a barrier for tsunamis and strong currents from the waters towards land.<sup>3</sup> Mangroves are also spawning grounds and foraging grounds for aquatic biota and migratory birds.<sup>4</sup> As a plant that grows in estuary areas, mangroves have unique adaptations to deal with environmental pressures in the form of salinity, temperature, nutrients, and solar radiation.<sup>5</sup> The ability to adapt is not only due to intrinsic factors but also to extrinsic factors such as port activity.<sup>6</sup> The port is a hub of sea transport traffic and fishermen's activities in catching fish, so the waters in the area have the potential to accumulate pollutants in the water column.<sup>7</sup> As a result of environmental pressure factors, mangroves can naturally produce secondary metabolites as a form of self-defense.

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**Citation:** Rozirwan R, Hananda H, Nugroho RY, Apri R, Khotimah NN, Fauziyah F, Putri WAE, Aryawati R. Antioxidant Activity, Total Phenolic, Phytochemical Content, and HPLC Profile of Selected Mangrove Species from Tanjung Api-Api Port Area, South Sumatra, Indonesia. Trop J Nat Prod Res. 2023; 7(7):3482-3489 <http://www.doi.org/10.26538/tjnpr/v7i7.29>

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Due to environmental changes, the self-defense mechanism can be found in bioactive compounds production. As a potential resource, the activity of bioactive compounds contained in mangroves can produce a variety of natural products that are widely used in pharmaceuticals and food supplements.<sup>3</sup> Secondary metabolites in mangrove plants include alkaloids, phenolics, steroids, terpenoids, and other compounds.<sup>8</sup> These compounds have important toxic, pharmacological, and ecological effects.<sup>5</sup> Mangrove plant extracts show biological activities such as antioxidants, antibacterials, and antimalarials.<sup>9</sup> The activity of antioxidant compounds can play a role in inhibiting free radicals that cause cell damage or slow oxidation reactions, even at small concentrations. Because of its association with beneficial health effects against a variety of diseases, there has been an increase in interest in the application of this antioxidant in the field of medicine in recent years, such as cardiovascular, cancer, cataract, atherosclerosis, retinopathy, arthritis, emphysema, and neuro-degenerative.<sup>10</sup> In addition, natural antioxidant compounds are significant in the health sector and have a direct effect on the food industry as natural preservatives for food products.

Research on the activity of bioactive compounds in mangrove leaves has been carried out in recent years, phytochemical content and toxicity effects to *Avicennia marina*,<sup>7</sup> and utilization of mangrove leaf extract of *Bruguiera gymnorhiza* with various solvents has been studied as an antibacterial, antifungal, and antioxidant.<sup>11</sup> antioxidant activity of from *Sonneratia caseolaris* extract has been studied as super antioxidant.<sup>12</sup> Moreover, the phenolic properties of mangrove plants and the activity of their strong bioactive compounds have been discussed in many studies.

However, polycyclic aromatic hydrocarbons (PAHs) have caused organic contamination in the Tanjung Api-Api Port area. In water,

naphthalene 15.848 ppb and benzo[a]pyrene 76.493 ppb were detected, whereas benzo[a]anthracene 3.16 ppb was found in sediments.<sup>13</sup> Some forms of PAHs are detected above the threshold. Additionally, sediments have a Cd 0.03 and Hg 0.01 mg/L concentration of heavy metals, compared to waters that have 0.02 mg/L and 0.012 mg/L, respectively.<sup>14</sup> There is no doubt that port activities has a significant and diversified impact on the environment. Port operations have a substantial negative influence on the ecology in the area. For example, siltation is accelerated, which lowers the water levels that reach mangrove forests,<sup>15</sup> and polluting elements such as heavy metal debris, garbage, and smoke pollution are increased.<sup>16</sup> Exposure to pollutants encourages an increase in the body's antioxidant defense system in mangrove exposure by producing bioactive compounds.<sup>7</sup> As a result, this study aimed to characterized the antioxidants of several mangrove species, such as *A. marina*, *B. gymnorhiza*, and *S. alba*, from the port area.

## Materials and Methods

### Collection of mangrove leaves

Mangrove leaves were collected around the Tanjung Api-Api port area in August 2020 at °S 2.3719750 and °E 104.8046833 Banyuasin Regency, South Sumatra (Figure 1). Mangrove leaves were identified in Marine Bioecology Laboratorium, Sriwijaya University, Indonesia. This location has a lot of mangrove vegetation that grows along the coast.<sup>7,17</sup> Many anthropogenic activities produce pollutants at the sampling location, such as domestic port activities and heavy shipping activities. Pollutant exposure to mangrove induces the production of bioactive compounds, which promotes an increase in the body's antioxidant defense system. The mangroves selected were three species, which included the major components in this region, with each of the mangrove species being *A. marina*, *B. gymnorhiza*, and *S. alba*. Identification of mangrove species using a guidebook.<sup>18</sup> The leaves were collected by random sampling to represent the overall characteristics of mangrove. Old and fresh leaves were chosen because they contain more secondary metabolites than young leaves.<sup>19,20</sup>

### Environmental quality measurement

The environment influences the existence of antioxidant bioactive compounds in mangrove vegetation.<sup>21</sup> This ability originates due to increased secondary metabolism of mangroves to defend themselves by producing active antioxidant compounds as an effort to balance physiological changes.<sup>22</sup> Environmental parameters were measured around the sampling location. These parameters include dissolved oxygen concentration, salinity, acidity level, and temperature.<sup>23</sup> All environmental parameters were measured using a multiparameter (Hanna HI 9829-01042, USA) and salinity was measured using a Handrefraktometer (ATC Portable, China).

### Plant maceration and extraction

Three species of mangrove leaves were taken as much as 500 g (dry weight) using an analytical scale (NIMBUS NBL254), which were mashed and macerated with 2 L of methanol (1:4 b/v) for 2 x 24 h. According to,<sup>24</sup> the methanol solvent was reported to be able to extract polyphenols and flavonoids at higher levels than other solvents. In line with research,<sup>25</sup> polar solvents tend to be able to extract more bioactive compounds. The success and efficiency of the method were determined by the type of solvent chosen.<sup>26</sup> The extract solution was filtered using Whatman No. 40 (Whatman International Ltd, UK). It was evaporated using a rotary evaporator (DLAB RE100-Pro, China) at temperature 40°C. Evaporation was carried out until the solvent completely evaporated, producing a paste texture (crude extract) of mangrove leaves. The crude extract was stored at 4 °C. This method is suitable for thermolabile plant extracts.<sup>27</sup>

### Determination of antioxidant activity by DPPH assay

The 0.02 g of leaves crude extract was dissolved in methanol to a final volume of 10 mL in a beaker glass to create a 2000 ppm concentrated extract mother liquor. Weighing 0.02 g of pure vitamin C, it is then dissolved in methanol until the final volume reaches 10 mL in a beaker glass, resulting in a mother liquor of ascorbic acid or pure vitamin C

with a concentration of 2000 ppm. 0.002 g of DPPH crystals (Merck, Germany) were dissolved in 50 mL of methanol to create a blank solution with a concentration of 0.1 mM. The solution is then homogenized and incubated for 30 minutes in a dark place. Furthermore, absorption was measured with a UV-Vis spectrophotometer (Shimadzu UV-1900, Japan) at a wavelength of 517 nm.<sup>25</sup> The characteristics of antioxidant activity were expressed as IC<sub>50</sub> to determine the strength of the antioxidant content (Table 1). The IC<sub>50</sub> is defined as the concentration in mg of dry matter per mL that inhibits DPPH radical formation by 50%, with the following formula.

$$\% \text{ inhibisi} = \frac{\text{blank abs} - \text{sample abs.}}{\text{blank abs}} \times 100\% \quad (1)$$

The IC<sub>50</sub> results were entered in the linear regression equation  $y = ax + B$ . The concentration of the sample is the abscissa (X axis), and the percentage of antioxidant inhibition is the ordinate (Y axis).<sup>28</sup>

### Determination of phenol content

The analysis of total phenol was carried out using the Folin-Ciocalteu method.<sup>29,30,31</sup> A standard solution of 1000 ppm gallic acid (Merck, Germany) was diluted into each series concentrations of 0 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm. The 50 mg leaves crude extract was dissolved in 2 mL of methanol and 5 mL of distilled water.

A half milliliter of 50% Folin-Ciocalteu (Merck, Germany) was added to each series concentration, then distilled water up to the mark for 5 minutes, and 1 mL of 5% Na<sub>2</sub>CO<sub>3</sub> (Merck, Germany) was added. Then the solution was incubated in a dark place without light for one hour. After incubation, the samples were measured using a UV-Vis spectrophotometer (Shimadzu UV-1900, Japan) with a 750 nm wavelength.

### Phytochemical analysis

The phytochemical test of the leaf extracts of *A. marina*, *B. gymnorhiza*, and *S. alba* was carried out using a qualitative method, such as alkaloids, flavonoids, terpenoids/steroids, saponins, and tannins as described in.<sup>32-34</sup>



Figure 1: Map of sample collection<sup>57</sup>

Table 1: The characteristic value of IC<sub>50</sub>

Concentration (µg/mL)	Characteristic
<50	Very strong
50-100	Strong
100-150	Moderate
150-200	Low

**High-performance liquid chromatography analysis of L-Ascorbic Acid**  
HPLC (Shimadzu LC-2030, Japan) was used to analyze leaves extract of *A. marina*, *B. gymnorrhiza*, and *S. alba* in the column C18 Shim-pack VP-ODS 150 x 4.6 mm.<sup>34-37</sup> The mobile phase of phosphate buffer solution at pH 2.8 was prepared using a homogenized dilution of aquabidest and NaOH (80 v: 20 v) and filtered through 0.45 µm Whatman paper. Mother liquor with a concentration of 500 ppm was prepared with methanol solution. The solution was diluted to concentrations of 0 ppm, 20 ppm, 50 ppm, 70 ppm, and 100 ppm and stored at 4 °C. As much as 0.5 g of the extract was dissolved in 20 mL of methanol, then ultrasonicated for 15 minutes. The solution was filtered using filter paper with a pore size of 0.45 µm. Samples were injected into HPLC to determine and measure the ascorbic acid content, with a characteristic wavelength of 210 nm and a retention time of 3.08 min.

## Results and Discussion

### Description of mangrove leaves

Mangroves had different generative characteristics for each species. The abundance of characters lies in the color of leaves, type and arrangement of flowers, size and surface of the fruit, roots, and bark. The leaves of *A. marina* were green, with veins that ran from the base to the tip of the leaf. The elongated elliptical leaf is rounded, and the ends were tapered to be slightly rounded. The surface of the leaf was green, and the underside was grayish white. The leaves of *B. gymnorrhiza* had an elliptical shape with a tapered tip. The leaves were green on the surface and yellowish on the bottom, with black spots. The leaves of *S. alba* had an inverted oval shape with rounded ends and were located opposite (Figure 2).

The Tanjung Api-api port area is on the east coast of South Sumatra, surrounded by mangrove vegetation. Several mangrove species can be found in this area, such as *Rhizophora apiculata*, *Avicennia marina*, *Xylocarpus granatum*, *Bruguiera gymnorrhiza*, *Sonneratia alba*, *Lumnitzera racemosa*, *Nypa fruticans*, and *Xylocarpus granatum*.<sup>38</sup> The three species selected in this study, apart from having a high degree of dominance in the area, also have different morphology and physiology, starting from their roots, stems, leaves, and content of bioactive compounds. Moreover, leaf types play an important role in adapting to environmental changes.<sup>39</sup> The mangrove leaves were old leaves marked with a dark green color.

### Environmental characteristics

The ecological growth and development of mangroves in the Tanjung Api-api port area were influenced by the surrounding conditions, especially ship transportation. In addition, aquatic environmental parameters include DO, pH, temperature, and salinity (Table 2). The mangroves *A. marina*, *B. gymnorrhiza*, and *S. alba* found at live sampling sites included major and adjacent mangrove species, all of which were associated with mangrove species.

Water quality sampling and measurement was carried out at low tide. Sampling at low tide shows that the water tends to be calmer and does not experience much turbulence. The results of the measured environmental characteristics were temperature 25.2 - 27.4 °C, dissolved oxygen 1.98 - 2.64 mg/L, pH 6.5-7, and salinity 15 - 19 ‰. This condition tends to lower dissolved oxygen levels and water salinity in

conditions that were not too high because, when the tide is low, the salt level approaches the salinity of seawater.<sup>40</sup>

Environmental factors can affect the growth and development of mangroves. Environmental factors were involved, including DO, pH, temperature, and salinity. During the sampling process, the condition of the water improved. Low dissolved oxygen indicates that common tide conditions were calmer than high tide, and there is no turbulence due to the semi-open estuary area.<sup>41</sup> In addition, the salinity is not high because the influence of river water is more significant than seawater's.<sup>7</sup> Changes in environmental conditions that always occur in mangrove habitats make this plant a unique adaptation model to combat ever-changing ecological conditions such as high salinity, high temperature, low nutrients, and excessive radiation.<sup>42</sup>

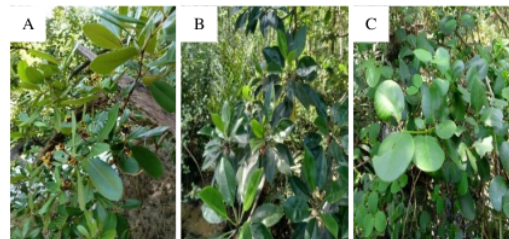
### Extract characteristics of mangrove leaves

The percentage of wet and dry leaves weight loss for each sample was obtained from the results of the indirect sunlight method for five consecutive days.<sup>7</sup> The value of the proportion of depreciation could be referred to as the proportion of water content in the leaves (Table 3).

The depreciation of *A. marina*, *B. gymnorrhiza*, and *S. alba* leaves from wet to dry leaves was 67.5%, 75%, and 65%, respectively. The process was carried out to reduce the possibility of chemical changes occurring in the roots, leaves, fruit, and bark so that they do not rot quickly and experience changes in the content of chemical compounds in them.

Evaporation results obtained in the form of thick and crude extracts. Based on Table 4, it is known that the proportion of the extract obtained from the extraction of sample powder using methanol solution.

The weight percentage of the extract produced from each mangrove in the methanol extract was 3.092% for *A. marina*, 9.17% for *B. gymnorrhiza*, and 8.63% for *S. alba*. Each solvent depreciated by 96.9%, 90.8%, and 91.3%, respectively.



**Figure 2:** Morphology of leaves from several species, A) *A. marina*, B) *B. gymnorrhiza*, and C) *S. alba*

**Table 2:** Environmental quality

Environment quality	Score		
	<i>A. marina</i>	<i>B. gymnorrhiza</i>	<i>S. alba</i>
Dissolved oxygen (mg/L)	1.98	1.98	2.64
Acidity (pH)	7	7	6.5
Temperature (°C)	25.2	25.2	27.4
Salinity (‰)	15	15	19

**Table 3:** Depreciation percentage of weight

Sample leaves	Sample weight (g)		Depreciation percentage (%)	Weight percentage (%)
	Wet	Dry		
<i>A. marina</i>	2.000	650	67.5	32.5
<i>B. gymnorrhiza</i>	1.000	250	75	25
<i>S. alba</i>	1.000	350	65	35

**Table 4:** Percentage of methanol extract

Sample leaves	Extract weight (g)		Depreciation percentage (%)	Extract percentage (%)
	Dry powder	Crude extract		
<i>A. marina</i>	500	15.46	96.9	3.09
<i>B. gymnorhiza</i>	234	21.47	90.8	9.17
<i>S. alba</i>	250	21.58	91.3	8.63

**Table 5:** Classification of IC<sub>50</sub>

Sample leaves	Linear regression			IC <sub>50</sub> (µg/mL)	Category
	a	b	R <sup>2</sup>		
<i>A. marina</i>	1.4165	1.6045	0.9892	171.160	Low
<i>B. gymnorhiza</i>	2.3137	1.3288	0.8916	105.090	Moderate
<i>S. alba</i>	3.8839	0.7707	0.9778	28.064	Very strong

**Table 6:** Determination of total phenol of methanol extract

Sample	Phenol (mg GAE/g)
<i>A. marina</i>	9.0258
<i>B. gymnorhiza</i>	13.8222
<i>S. alba</i>	9.4969

Maceration and extraction were processes for analyzing bioactive compounds in plants. Solvent extraction can be used to separate complex bioactive compounds from mangrove leaves.<sup>9</sup> The choice of solvent depends on the samples, the part of the plant to be extracted, the nature of the bioactive compound, and the solvent's availability. Based on the weight percentage of the extract, (Table 3) shows the depreciation obtained through the most significant methanol extraction in *B. gymnorhiza*. Another study on *Bruguiera gymnorhiza* found 67.2% aqueous extract followed by 67% methanol extract, whereas in the case of *Excoecaria agallocha*, 64.9% were in ethanol and 46.2% were in chloroform extract.<sup>43</sup> The crude methanol extract of selected mangroves has free radical scavenging.<sup>44</sup>

#### DPPH radical scavenging activity of mangrove leaves

Quantitative analysis of antioxidant activity using the percentage of free radical inhibition and the IC<sub>50</sub>. The DPPH radical scavenging method with methanol was used to conduct antioxidant tests on three mangroves (Table 5).

The IC<sub>50</sub> result for *A. marina* was 171.16 µg/mL, which was classified as weak; *B. gymnorhiza* was 105.09 µg/mL, which was classified as moderate; and *S. alba* was 28.064 µg/mL, which was classified as very strong. The difference in IC<sub>50</sub> in each sample showed the effect of secondary metabolite content in different mangrove plants.

Antioxidant activity in counteracting free radicals uses DPPH, one of the most commercialized organic nitrogen compounds. The difference in the IC<sub>50</sub> values of the three species is influenced by the different contents of secondary metabolites (Table 5). Based on this result, *S. alba* has a powerful ability to inhibit free radicals. Another study on the antioxidant activity of the ethanol extract of the leaves and stem bark of *S. alba* found that both had strong antioxidant potentials of 20.27 ppm and 18.62 ppm.<sup>45</sup> Gazali et al. (2020) using methanol solvent showed that the crude extract of *S. alba* leaves had an IC<sub>50</sub> value of 26.68 ppm. The characteristic IC<sub>50</sub> value is powerful in *S. alba*, which has excellent potential to be developed into various types of materials that can be used as antioxidants.<sup>29</sup> The difference in antioxidant activity in each mangrove species is caused by its characteristics, which are influenced by physical-chemical environmental factors such as habitat, tides, and sediment substrates.<sup>4</sup> *S. alba* has intense antioxidant activity compared to *A. marina* and *B. gymnorhiza* because zonation factors influence this. According to Rozirwan et al. (2023), *S. alba* is included in the open area zone closest to the sea area, so it is suspected that it will be affected by a higher level of environmental stress. Mangrove has adapted to

these changes, the most notable of which is the production of secondary metabolites.<sup>48</sup>

#### Phenol content of mangrove leaves

Quantitative measurements were carried out to determine the phenolic compounds in the methanol extract. Total phenol was measured by adding the Folin-Ciocalteu reagent to the test solution (Table 6). The total phenolic compounds are related to the equivalent value of gallic acid.

The highest total phenol yield was found in *B. gymnorhiza* at 13.8222 mg GAE/g. Furthermore, *S. alba* at 9.4969 mg GAE/g and *A. marina* at 9.0258 mg GAE/g were the smallest in the sample.

The ability of antioxidant activity is inseparable from their total phenol content.<sup>49,50</sup> According to Rozirwan et al. (2023), the total phenol content is directly proportional to the antioxidant activity of a substance; the greater the total phenol, the greater the antioxidant activity in a sample. The total phenol content of the three mangrove species with the highest yield was 13.822 mg GAE/g in *B. gymnorhiza* (Table 6). Many biological activities were known, such as phenols and flavonoids in *B. gymnorhiza* leaves extract.<sup>43</sup> Furthermore, in *S. alba*, it was 9.4969 mg GAE/g. Another study found that *S. alba* contained 8.27 mg GAE/g.<sup>29</sup> *S. alba* leaves contain flavonoids, steroids, triterpenoids, saponins, and tannins.<sup>51</sup> The phenolic and flavonoid content of *A. marina* leaves were determined to be 109 and 23 mg GAE/g, respectively.<sup>52</sup> Core structures are derived from phenolic compounds, including tannins and flavonoids.<sup>12</sup> Some tannins and flavonoids could scavenge radicals due to their higher polymerization ability to form stable structures.<sup>53</sup> This is one of the reasons why so many flavonoids and tannins have potent antioxidative properties.<sup>54</sup>

#### Screening of phytochemical from mangrove leaves

Based on the results, the crude methanol extract of leaves contains a group of bioactive compounds, which include alkaloids, flavonoids, terpenoids, steroids, saponins, and tannins (Table 7).

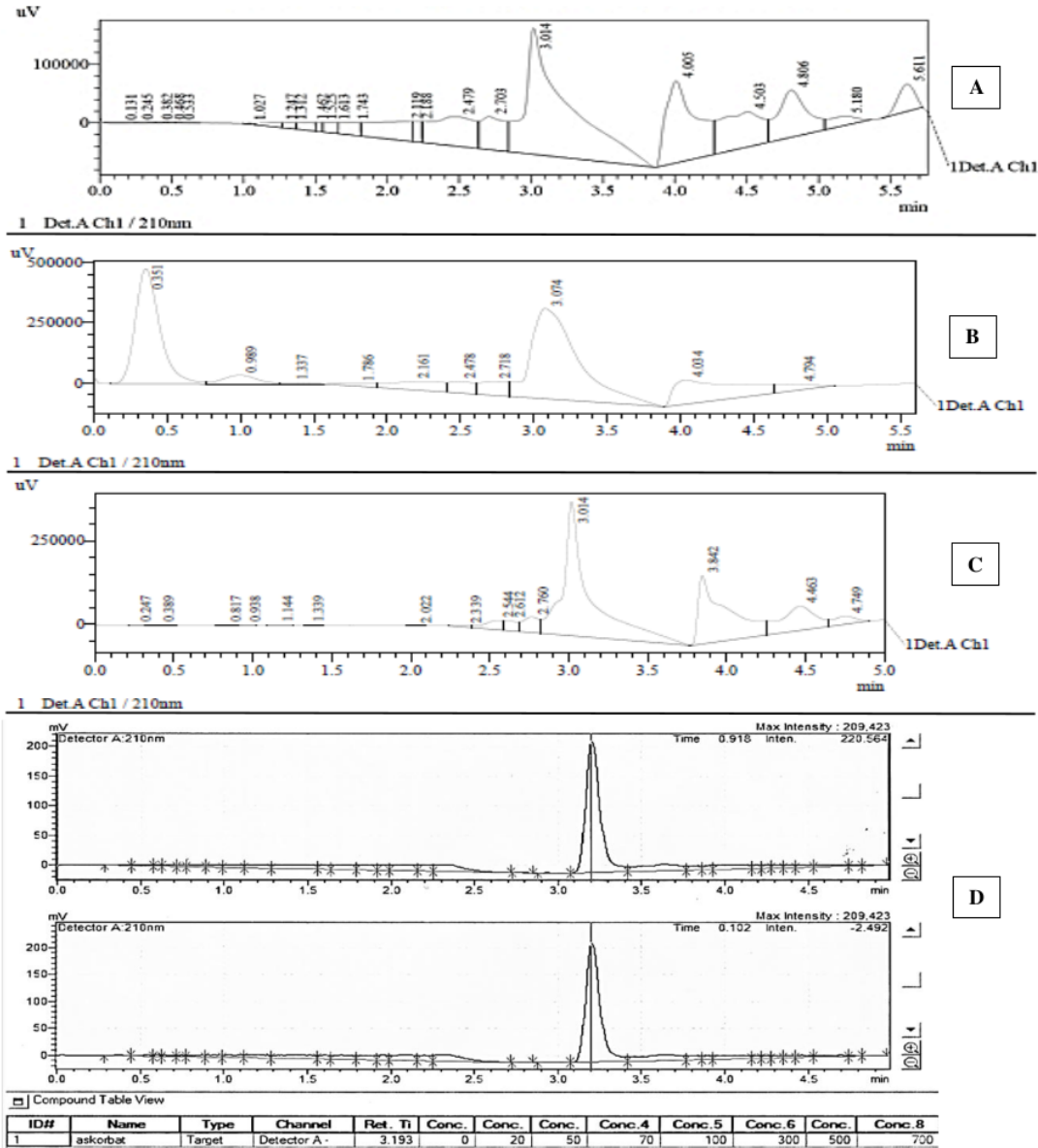
The bioactive compounds in *A. marina* leaf extract consisted of flavonoids, steroids, saponins, and tannins, but the alkaloids and terpenoids were not found in the preliminary phytochemicals test. *B. gymnorhiza* extract contained alkaloids, terpenoids, steroids, and saponins but no flavonoids or tannins. Then, in the extract from *S. alba*, flavonoids, terpenoids, steroids, saponins, and tannins were discovered, while alkaloid was not found in the preliminary phytochemicals test. Phytochemical screening revealed the presence of flavonoids, steroids, saponins, tannins, alkaloids, and terpenoids in the methanol extract of the three mangrove species. Bioactive components in mangrove leaves could affect antioxidant activity.<sup>3,25</sup> Each plant produces different secondary metabolites. Several studies report that flavonoids could function as anti-allergic, anti-inflammatory, antimicrobial, and anticancer agents.<sup>54</sup> Steroid compounds have antibacterial, anti-inflammatory, and anticancer properties.<sup>9</sup> Saponins function as antituberculosis, antibacterial, and antioxidant agents. Saponins are

active on the surface and can form foam when shaken in water.<sup>8</sup> Tannins were efficacious as astringents, antidiarrheals, and inhibitors of free radicals.<sup>8,9</sup> Alkaloids could act as antibacterial and are the most significant secondary substance compounds.<sup>9</sup> Terpenoids have various biological activities, including anticancer, antimicrobial, anti-inflammatory, antioxidant, and anti-allergic.<sup>55</sup>

*High-performance liquid chromatography analysis of L-Ascorbic Acid*  
HPLC chromatograms showed that extracts of *A. marina*, *B. gymnorizha*, and *S. alba* detected ascorbic acid with retention times of

3,014 min, 3,074 min, and 3,014 min, respectively. The results were consistent with the standard at a retention time of 3.194 minutes (Figure 3).

The concentration of ascorbic acid was calculated for all types of mangroves that showed antioxidant activity. The concentration of ascorbic acid in the three extracts was calculated by interpolating the area of the curve on the chromatogram into the standard curve equation (Table 8).



**Figure 3:** HPLC chromatogram of mangrove leaves extract; A) *A. marina*, B) *B. gymnorizha*, C) *S. alba*, dan D) Ascorbate acid as standards (Std)

**Table 7:** Bioactive compound groups in the phytochemical test of methanol extract

Phytochemical	<i>A. marina</i>	<i>B. gymnorhiza</i>	<i>S. alba</i>	Analysis type
Alkaloid	-	+	-	Qualitative
Flavonoid	+	-	+	
Terpenoid	-	+	+	
Steroid	+	+	+	
Saponin	+	+	+	
Tanin	+	-	+	

**Table 8:** The ascorbate acids concentrations of mangrove leaves extract

Sample leaves	Ret.Time (min)	Area	Height (mV)	Ascorbate acids conc. (ppm)
<i>A. marina</i>	3.014	4381059	215668	64.224
<i>B. gymnorhiza</i>	3.074	8476832	375522	109.510
<i>S. alba</i>	3.014	4612381	400059	67.640
Ascorbate acids	3.193	6871180	-	100.000

The ascorbic acid content in *A. marina* extract was 64.224 ppm, *B. gymnorhiza* was 109.510 ppm, and *S. alba* was 67.640 ppm. *A. marina* and *S. alba* had lower ascorbic acid content than pure ascorbic acid standards, while *B. gymnorhiza* had higher concentrations than pure ascorbic acid standards.

Ascorbic acid testing using HPLC is used to analyze the levels of active ingredients in samples and has good sensitivity. Results of HPLC analysis (Table 8) showed that *B. gymnorhiza* leaf extract had a higher concentration of ascorbic acid than the standard used. Meanwhile, *A. marina* and *S. alba* had lower concentrations than the standard specificity of the HPLC method (100 ppm) for the quantity of antioxidant activity, which allows for the interference of the excipients in the preparation. Other interferences, such as antibiotics, do not cause disturbances in the ascorbic acid peak.<sup>56,57</sup> The difference in each species is caused by the decomposition of these compounds that could occur in extraction processes such as heat, light, air, and pH, which are factors that degrade the compound.<sup>36</sup> The HPLC method has provided fast, sensitive, and accurate information and a means that can be reproduced to determine the activity of inhibiting free radicals.

### Conclusion

The IC<sub>50</sub> values of all samples showed antioxidant activity, namely *A. marina* (171.16 g/mL) (low), *B. gymnorhiza* (105.09 g/mL) (medium), and *S. alba* (28.064 g/mL) (very strong). Phytochemical groups of the selected mangroves consisted of flavonoids, steroids, and saponins, alkaloids, terpenoids, and tannins. Based on that, by taking the basic physiological properties into account on a more practical ecological scale, it is believed that our research findings can help advance understanding of antioxidant bioactive chemicals derived from mangrove plants.

### Conflict of Interest

The authors declare no conflict of interest.

### Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

### Acknowledgments

The research of this article was funded by DIPA of Public Service Agency of Universitas Sriwijaya 2023. No. SP DIPA-

023.17.2.677515/2023, On November 30, 2022. In accordance with the Rector's Decree Number: 0188/UN9.3.1/SK/2023, On April 18, 2023.

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