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Phytoconstituents and Antioxidant Profiling of *Avicennia marina* (Forsk.) Vierh. Heartwood Extract Sourced from Bagan Kuala: A Novel Natural Source for Radical Scavenging Applications

Firdha Sekar Rahayu[1], Julia Reveny[2*], Yuandani[3]
[1]*Post Graduate Program of Pharmacy, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, 20155 Indonesia*
[2]*Pharmaceutical Technology Department, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, 20155 Indonesia City*
[3]*Pharmacology Department, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, 20155 Indonesia*
[*]Corresponding Author: julia.reveny58@gmail.com

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ABSTRACT
Avicennia marina (Forssk.) Vierh., commonly known as 'api-api' or white mangroves, are woody shrubs and tropical plants that inhabit mangrove ecosystems, which occur at the interface between terrestrial and marine environments. Salinity is a critical environmental factor in mangrove habitats. Plants growing in such extreme conditions exhibit elevated concentrations of specific antioxidant compounds. Various parts of plants, including heartwood, are known to contain an abundance of extractive components compared to sapwood. Objective: This study was the first to evaluate the antioxidant activity of *A. marina* heartwood extract. Method: The applied method of sample extraction comprises ethanol-based maceration, qualitative phytochemical analysis based on observable visual changes, and the assessment of antioxidant activity employing the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay using UV-Vis Spectrophotometry. Result: heartwood extract of *Avicennia marina* exhibits phytochemical constituents that comprise alkaloids, tannins, saponins, triterpenoids, and flavonoids. The antioxidant capacity of the extract showed strong antioxidant activity with an IC_{50} value of 61.50 µg/mL (R^2 = 0,9962). Conclusion: The current study confirms the promising antioxidant activities of *Avicennia marina* heartwood sourced from Bagan Kuala. These marine biological resources can be explored further as an ailment for degenerative disease induced by Reactive Oxidative Stress (ROS), which can be made into an oral supplement or topical preparations, thus generating a greater economic value.

Keyword: *Avicennia marina*, DPPH, Heartwood, IC_{50}, Phytoconstituents

1. Introduction

Bagan Kuala Village is part of Tanjung Beringin, the existence of a forest area and mangrove ecosystem covering an area of 7.417 Ha in Tanjung Beringin sub-district consisting of dominant species such as *Avicennia* sp., *Rhizophora* sp. *Bruguiera cylindrica*, *Sonneratia alba* [1], [2] makes the community utilize the mangrove area as a source of family income.

Mangroves refer to a collection of various species of woody shrub plants that grow in salty tidal wetlands located along tropical and subtropical coasts [3], with the presence of repeated sea air reservoirs, mangrove plants must survive in extreme conditions such as high temperatures, relatively low humidity, and high salinity levels [4]. Plants thriving in harsh conditions generally have high levels of antioxidant chemicals [5]. The antioxidants found in these plants can be used as a source of antioxidants that can be used to treat chronic diseases caused by tissue damage due to oxidative stress.

Avicennia marina (Forssk.) Vierh. or also known as 'api-api' or white mangrove has been widely used empirically as an antiulcer, antirheumatic, treatment for abscesses, burns, and contraceptive [6], and can provide protection against oxidative stress, inflammation, apoptosis and fat peroxidation in diabetic rat models [7-8]. Many previous studies have reported the results of research on the activity of *A. marina* on several parts of the plant such as leaves, aerial parts, fruits, twigs, and tree bark. However, there is a lack of research specifically investigating heartwood of *A. marina*. Recent findings have shown that *A. marina* heartwood possesses inhibitory activity against the 5α-R1 receptor in dermal papilla cells, indicating its potential role in managing androgenetic alopecia [9-10]. Mangrove plants have a promising strong antioxidant activity, for instance *Sonneratia alba* (IC_{50} = 62.38 µg/ml) and *Avicennia officinalis* (IC_{50} = 257.04 µg/ml). Furthermore, heartwood extracts from other plant species, such as *Caesalpinia sappan* and *Acacia* sp., have demonstrated strong antioxidant properties. Despite these promising preliminary results, the specific studies are scarce, this study was necessary to conduct and can be the first to evaluate the antioxidant activity of *A. marina* heartwood extract. Furthermore, a systematic comparison of *A. marina* heartwood remain largely unexplored. [11-13].

Based on the results of the literature review that has been conducted, this study was conducted to see the phytochemical constituents and evaluate the antioxidant activity of *A. marina* extract in the heartwood sourced from Bagan Kuala as a prospective free radical scavenging agent and can be used as a source of economic improvement for local residents.

2. Methodology

2.1. Sample Preparation

The heartwood samples of *A. marina* were taken from the mangrove forest of Bagan Kuala Village, Tanjung Beringin District, Serdang Bedagai Regency, Indonesia with the stipulation that the plants were >7 years old according to the statement of local villager and the record of planting year. The part taken was the inner part of the core stem which was grayish black as (Figure 1). The samples were planted to separate them from the lighter colored sapwood. The sample that has been planted, then chopped into small pieces which are then dried in a drying cabinet at a temperature of 50°C until the sample dries. The dried sample is then powdered to obtain dry simplicia powder of *A. marina* heartwood.

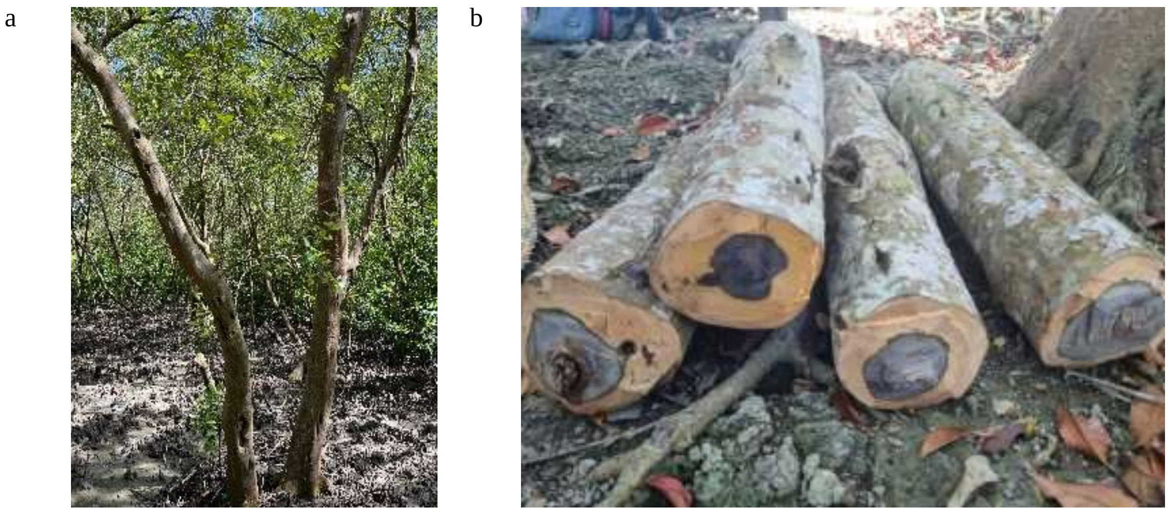

Figure 1. (a) Tree of *A. marina*; (b) Heartwood of *A. marina*

2.2. Sample Extraction

The sample extraction was done accordingly to Indonesian Herbal Pharmacopeia II, the dry powder of *A. marina* was extracted using a maceration method based on 96% ethanol. The sample was soaked in a ratio of 1:10 for the first 6 hours while stirring occasionally, then left for 18 hours. The sample was filtered and the extraction process was repeated once again in half the amount of solvent before. The collected macerate was evaporated using a rotary evaporator at a temperature of 60 ± 2°C until a thick extract was obtained [14].

2.3. Sample Characterization Determination

Sample characterization was carried out with the aim of determining the non-specific parameter values of the simplicia and the bright stem extract of *A. marina*. The parameters tested were the determination of water content, total ash content and acid-insoluble ash as well as water-soluble and ethanol-soluble extract levels.

2.4. Profiling of Phytoconstituents

Phytoconstituent profiling was conducted to analyze the secondary metabolite contained in the dry powder samples and extracts from the *A. marina* heartwood. The test was conducted qualitatively by observing changes and formation of reaction products visually. This test includes alkaloid, flavonoid, tannin, saponin, and triterpenoid tests.

2.4.1 Alkaloid screening

The sample was weighed and then added to a mixture of 2N HCl solution: distilled water (1:9), extraction was carried out for 2 minutes by heating, then filtered. The filtrate obtained was taken, each 0.5 ml of filtrate was taken into a test tube, where each test tube was added with 2 drops of Mayer's, Dragendorff's, and Bouchardat's reagents. The sample is said to be positive for alkaloids if the addition of the sample with Mayer's reagent forms a white or yellow precipitate, Dragendorff's reagent forms a brown or orange-brown precipitate, and Bouchardat forms a brown to blackish precipitate [15].

2.4.2 Flavonoid screening

The sample was extracted by heating, filtered until a filtrate was obtained which was added with 0.1 grams of magnesium powder and 1 ml of HCl, the sample was positive for containing flavonoids if a color change from red to orange was observed [16].

2.4.3 Tannin screening

The sample that has been extracted by heating is filtered until a filtrate is obtained, the filtrate is then dripped with 1-2 drops of 1% FeCl3 reagent, it is said to be positive if there is a color change from blackish blue or brownish green [17].

2.4.4 Saponin Screening

The weighed sample was added with 10 ml of distilled water, then shaken vigorously for 10 seconds. A positive result is indicated by the formation of 1 cm high foam [17].

2.4.5 Terpenoid screening

The weighed sample was extracted using 20 ml of n-hexane for 2 hours, then filtered. The filtrate obtained was then evaporated and the sample was dripped with Liebermann-Burchard reagent, positive triterpenoid samples with the formation of red to purple colors, while positive steroids with the formation of green to blue colors [15].

2.5 Profiling of Antioxidant Activity

Antioxidant activity profiling of the extract was carried out using the DPPH free radical scavenging method, the absorbance of which was measured using a UV-Vis spectrophotometer at λ = 517 nm. The reference used was ascorbic acid as an antioxidant with very strong activity.

2.5.1 Preparation of dpph solution

An amount of 0.5 mM DPPH stock solution (2×10^{-1} mg/ml) was prepared by dissolving 5 mg of DPPH powder with methanol p.a into a 25 ml volumetric flask, homogenized. A total of 0.1 mM DPPH was taken from the stock solution and added to the extract test solution and ascorbic acid standard.

2.5.2 Preparation of sample solution

A sample stock solution of *A. marina* heartwood extract sample was prepared with a concentration of 2 x 10-1 mg/ml, then homogenized. From the stock solution, a working solution was made with a concentration of 2×10^{-2}; 3×10^{-2}; 4×10^{-2}; 5×10^{-2}; 6×10^{-2} mg/ml.

2.5.3 Preparation of standard solution

A stock solution of the standard ascorbic acid with a concentration of 1×10^{-1} mg/ml was prepared and then homogenized. From the stock solution, working stock solutions with concentrations of 5×10^{-5}; 10×10^{-4}; 15×10^{-4}; 20×10^{-4}; 25×10^{-4} mg/ml were prepared.

2.5.4 Antioxidant activity assay

The test solution of *A. marina* heartwood extract and the ascorbic acid standard were taken in their concentrations and transferred into a 5 ml volumetric flask. Each working flask was then added with 1 ml of 0.1 mM DPPH solution and topped up with methanol p.a., then homogenized using a sonicator and incubated in a light-proof place for about 30 minutes. The absorbance value of the DPPH inhibition obtained is then calculated as a percentage using the following equation (1)

$$\% \text{ Scavenging} = \frac{\text{Absorbance of Blank-Absorbance of Sample / Standard}}{\text{Absorbance of Blank}} \text{ x } 100\% \quad (1)$$

Where :
Absorbance of Blank = Absorbance value of 0.1 mM DPPH solution
Absosrbance of Sample / Standard = Absorbance value of sample / standard with 0.1 mM DPPH solution

The linear regression equation (y = a + bx) was determined to calculate the antioxidant activity, where x-axis was valued as sample / standard concetration and y-axis as % Scavenging. The value of coefficient a and b were obtained by the equation (2) and (3):

$$a = \frac{(\sum xy)-(\sum x)(\sum y)/n}{(\sum x^2)-(\sum x)^2/n} \quad (2)$$

$$b = \frac{y-a}{x} \quad (3)$$

Where :
x = sample / standard concetration
y = % scavenging
n = number of sample

Antioxidant activity was obtained by calculating the loss of 50% of DPPH activity (IC_{50}) by substituting the "y" value into the linear regression equation formula (y = a + bx) (equation 4). Antioxidant testing was carried out in triplicate.

$$50 = a + bx$$

$$x = \frac{50-a}{b}$$

$$x = IC_{50}\ (\mu g/ml) \quad (4)$$

Coefficient of determination or regression linearity (R^2) was calculated to show how proportionate the data fit the regression model, it was obtained by the equation (5):

$$R^2 = \left(\frac{\sum XY-(\sum X)(\sum Y)/n}{\sqrt{\left(\sum x^2 - \frac{(\sum x)^2}{n}\right)(\sum y^2 - \frac{(\sum y)^2}{n})}}\right)^2 \quad (5)$$

3. Result and Discussion

3.1 Yield of Extraction

The extract of *A. marina* obtained is a thick, reddish-brown extract with a characteristic mangrove wood aroma and a slightly bitter taste, yielding 7.35% of the total extract.

3.2 Sample Characterization Result

The results of the characterization of the dry powder sample and the heartwood extract of *A. marina* can be seen in Table 1. The determination of water content is carried out to ascertain the water content present in the sample after the drying process. Elevated water content promotes enzymatic and oxidative degradation and facilitates microbial proliferation, thereby diminishing the material's quality and stability. Determination of total ash content to identify the inorganic content contained in the sample, while the determination of acid-insoluble ash to identify the contamination of external inorganic compounds that are insoluble in acid within the sample. high values may indicate soil contamination, sand, or the addition of inorganic materials which can reduce quality of the sample [18]-[20]. The determination of the water and ethanol soluble extractive content is carried out to understand the ability of the compounds present in the simplicia to be extracted with either water or ethanol as solvents, differences in values indicate differences in chemical composition depending on the source and processing method; related to pharmacological potency. Low/high values should be interpreted in conjunction with the chromatographic profile [21]-[22].

Table 1. Characterization profile of dry powder sample and heartwood extract of *A. marina*

Evaluation	Result	
	Dry Powder	Extract
Moisture Content	7,32 ± 1,15%	7,99 ± 1,99%
Total Ash Content	2,59 ± 0,18%	2,56 ± 0,32%
Acid-Insoluble Ash Content	0,06 ± 0,01%	0,11 ± 0,01%
Water-Soluble Extract Content	28,04 ± 3,74%	Not Tested
Ethanol Soluble Extract Content	23,58 ± 3,25%	Not Tested

3.3 Phytoconstituent Profile

Phytoconstituents are secondary compounds that play additional roles such as aiding plant development, protecting plants, and providing color, flavor, and aroma to plants. Phytoconstituents are widely used commercially as preservatives, colorants, and others. Active components of phytoconstituents such as flavonoids, terpenoids, alkaloids, quinine, and so on belong to the class of molecular structures that are biologically active, thus can be utilized for therapeutic purposes [23]-[24]. Based on the test results in Table 2, both the simplicia sample and the extract show that *A. marina* contains alkaloid, flavonoid, saponin, tannin, and terpenoid compounds. Samples containing alkaloid compounds have cyclic nitrogen groups that will then interact with K^+ ions contained in Mayer, Bouchardat, and Dragendroff reagents. Each will form a precipitate in the form of a complex compound. Flavonoid compounds that give a positive reaction are usually reduced by the addition of magnesium in hydrochloric acid solution, resulting in a characteristic color ranging from red to orange. Whereas tannins are polyphenolic compounds in which the phenolic hydroxyl groups complex with Fe^{3+} ions, resulting in a brownish-green color [25]

Table 2. Phytoconstituents profile of dry powder and heartwood extract of *A. marina*

Compound Group	**Reagent**	**Result**	
		Dry Powder	**Extract**
Alkaloida	Dragendorf	+	+
	Bouchardat	+	+
	Mayer	+	+
Flavonoida	Mg(s) + HCl (p)	+	+
Saponin	S + Aquadest	+	+
Tanin	$FeCl_3$ 1%	+	+
Terpenoid	Liebermann-Burchard	+	+

Where:
(+): Contain Compound Group
(–): Not Contain Compound Group

The formation of foam as a positive result of saponin content in the sample indicates the presence of polar and non-polar groups that, when shaken, will create interfacial tension [26]. The terpenoid results showed a positive outcome with the sulfonation reaction on the sterol group, resulting in a color change to reddish-purple in the result [25]. Based on previous research, *A. marina* in the leaf part contains phytoconstituents such as alkaloids, saponins, tannins, flavonoids, glycosides, and triterpenoids/steroids [26]. Phytoconstituent Profile Result can be seen in Figure 2.

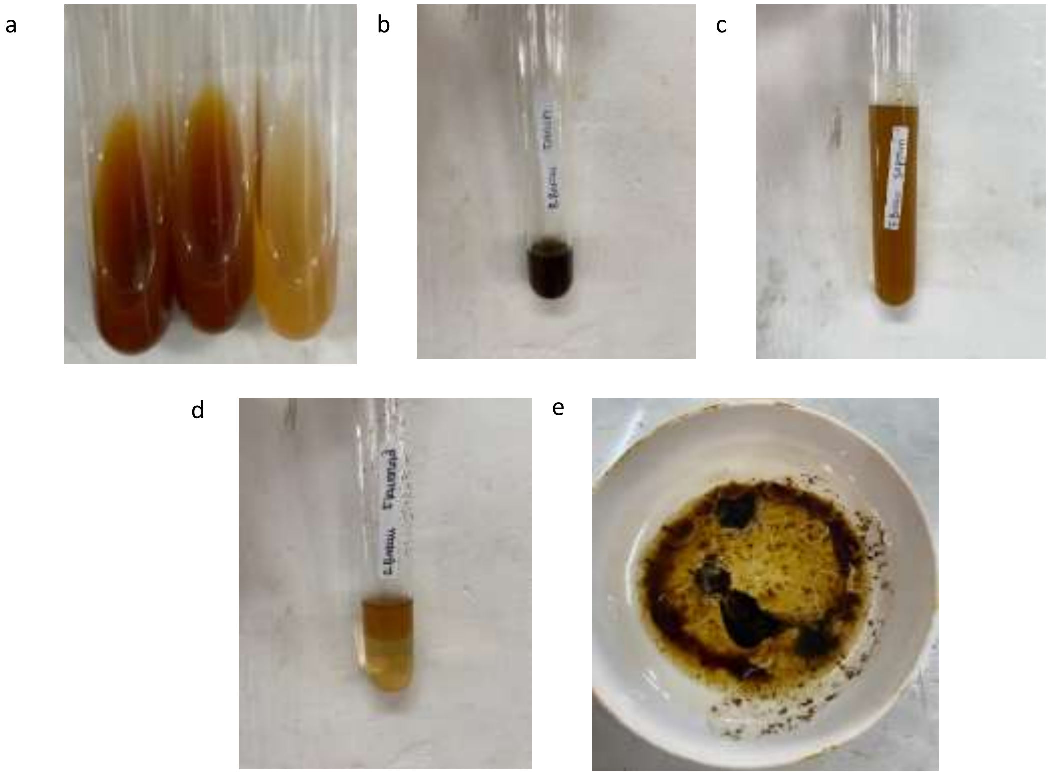

Figure 2. Results of phytoconstituents profile of *A. marina* (a) Alkaloid; (b) Tannin; (c) Saponin; (d) Flavonoid; (e) Terpenoid

3.4 Antioxidant Activity Profile

1,1-diphenyl-2-picrylhydrazyl, commonly known as DPPH, is a stable free radical compound with a deep purple color. Mixing a DPPH solution with a compound that can donate a hydrogen atom will cause a quenching reaction between the divalent nitrogen atom in the DPPH* structure, resulting in the formation of hydrazine (DPPH-H). Antioxidants that act as H atom donors can trigger this reaction, which is marked by the color change of the DPPH reagent from purple to pale yellow [27]. The addition of the test sample solution concentration will increase the DPPH scavenging activity, as indicated by the decrease in its absorbance value. The results of DPPH scavenging by the stem core extract of *A. marina* and the IC_{50} value can be seen in Table 3 and Figure 2.

Table 3. Calculation of antioxidant activity of *A. marina* heartwood extract and ascorbic acid standard

Sample	Regression Equation	Regression Linearity (R^2)	IC_{50} Value (µg/ml)
A. marina Heartwood Extract	Y = 0,97x – 9,61	0,9960	61,50 ± 0,0762
Ascorbic Acid	Y = 20,798x – 0,37	0,9931	2,42 ± 0,0063

The IC_{50} value of the *A. marina* stem bark extract is 61.50 ± 0.0762 µg/ml, while ascorbic acid as a standard has a value of 2.42 ± 0.0063 µg/ml, with each IC_{50} value obtained through triplicate repetitions. Referring to the IC_{50} value categorization described by Molyneux (Table 4) the inhibition value of ascorbic acid has very strong inhibition activity (IC_{50} < µg/ml) while the extract of the *A. marina* stem has strong activity (IC_{50} 50-100 µg/ml) [28].

Table 4. IC_{50} Value Based Antioxidant Activity Category (Molyneux, 2004)

IC_{50} Value	Antioxidant Category
< 50 µg/ml	Very Strong
50 – 100 µg/ml	Strong
100 – 150 µg/ml	Moderate
> 150 µg/ml	Weak

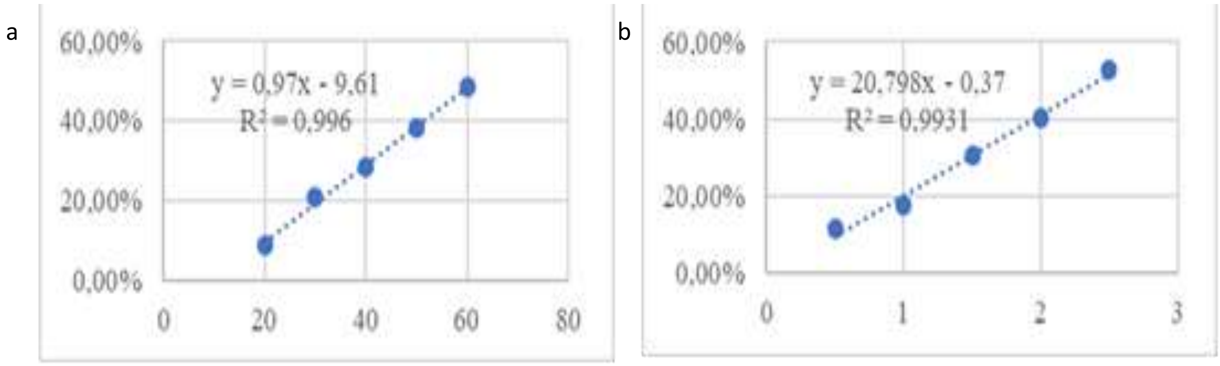

Figure 3. Graph of DPPH Scavanging Ability of Sample (a) *A. marina* Heartwood Extract; (b) Ascorbic Acid Standard

Plants that thrive in harsh conditions generally have a high content of antioxidant chemicals. Antioxidant agents derived from enzymes and metabolites are used to maintain oxidative balance in plants, which is also necessary for environmental adaptation [5]. Antioxidants are substances that can neutralize free radicals, thereby preventing the onset of diseases caused by them. Research conducted on plants shows that plants containing secondary metabolites such as flavonoids and phenols are effective as free radical scavengers with antioxidant activity. Free radicals are one type of reactive chemical compound commonly known as compounds that contain unpaired electrons, making them highly reactive. The presence of highly reactive and unstable free radicals in the body can cause damage to cells, tissues, and genetic material [29].

The extract of *A. marina* heartwood from this study showed a relatively strong IC_{50} value (61.50 µg/ml). Several previous studies stated that the potential of mangrove plants from several species has antioxidant activity that ranges in the category of strong to very strong, such as *Sonneratia alba* fruit (62.38 µg/ml), *Avicennia officinalis* leaves (257.04 µg/ml), *Rhizophora* sp leaves (0.04 µg/ml), and Bruguiera sp fruit (60.77 µg/ml). This difference in antioxidant value is caused by differences between species, sampling locations, and the extraction methods and solvents used. Past studies reported antioxidant activity of different parts of *A. marina* like leaves (56.8 µg/mL) and roots (23.7 µg/mL), also indicated that *A. marina* has potent antioxidant activity, and so it is a very promising scavenging agent [12], [13], [30]-[32].

Antioxidants, especially those derived from plants, can be used to reduce the production of reactive oxygen species (ROS), thereby reducing damage to healthy body cells. In addition, antioxidants can also be found in the heartwood because this part has a higher concentration of extractive substances compared to the sapwood [11]. The extractive substances found in heartwood are usually secondary metabolite compounds, most of which are derivatives of phenolic compounds that impart color characteristics and biological defense properties to the heartwood. Several groups of phytoconstituents found in the extractive substances of heartwood include tannins, terpenes, flavonoids, lignin, aromatic compounds, and lecithin [33].

4. Conclusions

Based on the research conducted, it is known that the stem bark of *A. marina* has strong antioxidant activity due to the presence of phenolic derivative phytoconstituents such as flavonoids and tannins. These phytoconstituents contribute as agents that neutralize free radicals, which are the cause of tissue damage in the body. The utilization of the core stem of *A. marina* sourced from Bagan Kuala Village has significant benefits due to the abundance of extractive substances and its potential as a strong antioxidant. The results of this research is the first to report on antioxidant activity of *A. marina* heartwood, these findings are expected to be explored further as an ailment for degenerative disease induced by Reactive Oxidative Stress (ROS), which can be made into an oral supplement, topical preparations, and can be isolated for further research, thus generating a greater economic value expected to serve as a reference for future studies related to the utilization and further development of research and commercial use of the heartwood of *A. marina*.

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