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Antioxidant and Antibacterial Activity of Ethanol Extract of Senggani Roots (*Melastoma malabathricum* L.)

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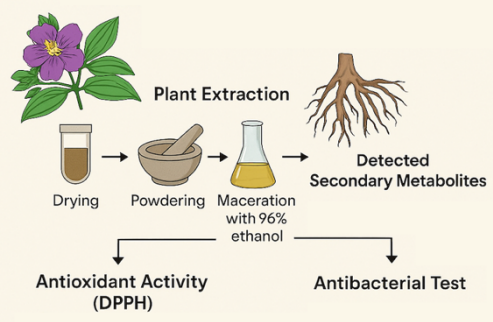

Abstract: This study investigated the antioxidant and antibacterial activities of the ethanol extract of senggani root (*Melastoma malabathricum* L.), aiming to assess its free radical scavenging potential and its ability to inhibit *Escherichia coli*. The research involved several stages, including sample preparation, maceration using 96% ethanol, antioxidant testing using the DPPH method at 517 nm, and antibacterial evaluation using the agar diffusion method. Five treatment groups were used in the antibacterial assay: a negative control (sterile distilled water), a positive control (100 µg nystatin), and three extract concentrations (75, 100, and 125 ppm), each tested in triplicate. The results showed that the extract had an IC_{50} value of 4.67 ppm, which was comparable to that of vitamin C (4.01 ppm), indicating strong antioxidant activity. In antibacterial testing, the extract produced inhibition zones of 11.16 mm, 11.66 mm, and 12.16 mm at the respective concentrations, while the positive control showed a 9.5 mm inhibition zone. These findings suggest that the ethanol extract of senggani root possesses potent antioxidant properties and demonstrates promising antibacterial activity against *Escherichia coli*.

Keywords: maceration, antioxidant, antibacterial, senggani root

1. Introduction

The increasing global interest in plant-based medicine has heightened the focus on medicinal plants such as *Melastoma malabathricum* L., commonly known as senggani. This plant has been integral to Indonesian ethnomedicine, particularly its roots, which have traditionally been employed to treat various ailments including diarrhea, boils, bleeding wounds, burns, and bacillary dysentery [1]. Recent phytochemical investigations have identified various bioactive compounds in *M. malabathricum*, such as flavonoids, saponins, tannins, steroids, and triterpenoids. These compounds are recognized for their antimicrobial and antioxidant properties, providing a strong rationale for academic inquiry into this plant's therapeutic potential [2–4]. Antioxidants play a crucial role in mitigating oxidative stress, which is implicated in chronic diseases such as cancer, cardiovascular disease, diabetes, and neurodegenerative disorders [5]. Studies utilizing the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay have demonstrated that various extracts of *M. malabathricum* exhibit notable antioxidant activity. In one study, the methanolic extracts from the roots, leaves, and stems displayed significant DPPH and ABTS radical scavenging activity, with enhanced effects observed under specific growth conditions, further highlighting the plant's strong antioxidant potential [2,6]. The quantitative evaluation of antioxidant capacity is typically reported in terms of IC_{50} values derived from UV-Vis spectrophotometric analysis [5].

The escalating problem of antibiotic resistance has intensified the search for alternative antibacterial agents. *Escherichia coli*, a common Gram-negative bacterium, is frequently associated with infections such as diarrhea and sepsis. The need for effective natural antibacterial solutions has become increasingly urgent, particularly as conventional antibiotics lose their efficacy [4,7]. Research has shown that *M. malabathricum* possesses antibacterial activity against *E. coli* and

other pathogenic bacteria, supporting its traditional use as a remedy for infectious diseases [3,4,8]. Investigating the root's antibacterial potential is especially significant given the alarming rise in bacterial resistance worldwide [5,9].

This study therefore aims to evaluate both the antioxidant potential and antibacterial activity of ethanol extracts derived from the roots of *M. malabathricum*. By examining the extract's free radical scavenging capacity and inhibitory effects against E. coli, this research seeks to validate the plant's traditional medicinal uses while contributing to the ongoing search for effective natural therapeutic agents [1,3,5].

2. Results

2.1. Preparation of sample

The senggani roots are thoroughly washed and dried before being ground into a fine powder. This powder undergoes maceration in 96% ethanol for 24 hours, yielding a concentrated extract characterized by distinct phytochemicals, including flavonoids, saponins, and tannins [1,2]. Following extraction, phenolic compounds have been shown to exhibit significant antioxidant activities, which can be quantitatively assessed using methods such as the DPPH radical scavenging assay [3,4]. In assessing the antioxidant capability of the senggani root extract, the DPPH radical scavenging assay is a widely utilized method due to its simplicity, speed, and minimal sample requirements for evaluating free radical scavenging activity. The IC_{50} values obtained from this method effectively illustrate the extract's capacity to neutralize DPPH radicals. Literature reports indicate that extracts of senggani roots display competitive IC_{50} values, with some findings showing values as low as 4.67 ppm, comparable to that of vitamin C [1,3]. This high efficacy highlights the potential of senggani roots in combating oxidative stress associated with various chronic diseases, aligning with traditional claims of their therapeutic benefits [2,3].

The chemical composition of the extract not only reinforces its antioxidant properties but also confirms its phytochemical profile. Previous studies have identified the presence of flavonoids, tannins, and saponins in senggani root extract, which contribute significantly to its pharmacological potential [2,3]. These bioactive compounds enhance the plant's medicinal value, supporting its long-standing use in ethnomedicine and emphasizing the need for continued research into its natural therapeutic applications [1,3]. Overall, the preparation and analysis of senggani root extract, as described, demonstrate its promising antioxidant potential supported by robust phytochemical characterization. The integration of traditional preparation methods with modern scientific techniques provides a foundation for further exploration of this plant's extensive medicinal benefits.

2.2. Regression analysis and IC_{50} value interpretation

According to Zuhra et al. [1], higher antioxidant activity corresponds to a lower IC_{50} value. In this study, the IC_{50} values of the ethanol extract of senggani root and vitamin C were 4.67 ppm and 4.01 ppm, respectively, classifying both as very strong antioxidants. Based on the classification by Setha et al. [2], antioxidant compounds are categorized as follows: weak (>150 ppm), moderate (100–150 ppm), strong (50–100 ppm), and very strong (<50 ppm). The high antioxidant activity of the extract can be attributed to the presence of secondary metabolites such as flavonoids, alkaloids, quinones, phenolics, tannins, steroids, triterpenoids, and saponins. These compounds possess hydroxyl functional groups that contribute to their ability to donate electrons or hydrogen atoms to neutralize free radicals [3,4]. Specifically, the oxygen atoms in the hydroxyl groups possess lone electron pairs that can stabilize reactive free radical species by interrupting radical chain reactions [3].

On the other hand, Beer–Lambert's Law suggest that when light passes through a solution at a specific wavelength, part of the light is absorbed and part is transmitted, producing a linear relationship between absorbance and concentration [5]. In this case, the anomaly may result from the redox interaction between the oxidizing agent (DPPH) and the reducing agent (bioactive compounds in the extract). As the concentration increases, more reduction occurs, potentially altering the light absorption and leading to a non-linear trend where absorbance decreases with increasing concentration (Fig.1) [6–10].

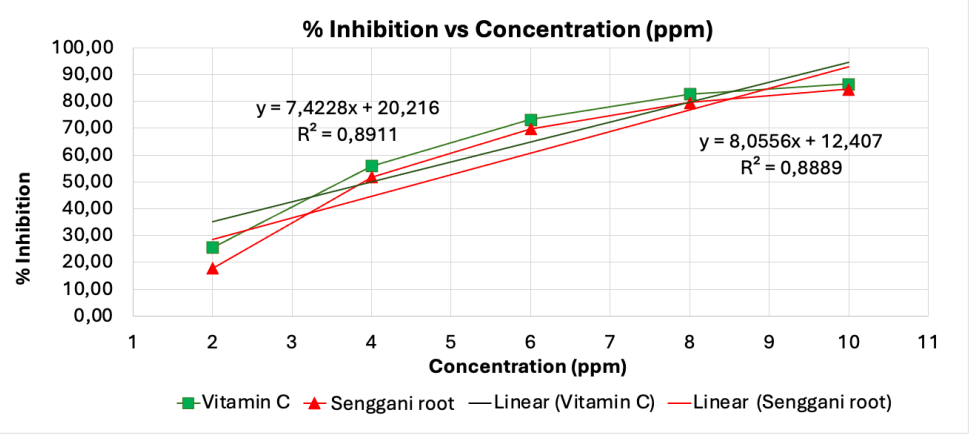

Figure 1. Regression analysis

2.3. Antibacterial activity of ethanol extract of senggani root (Melastoma malabathricum L.)

The antibacterial activity of the ethanol extract was tested against *E. coli* using the disc diffusion method to measure inhibition zones. Five treatment groups were used: a negative control (sterile distilled water), a positive control (100 µg nystatin), and three extract concentrations (75 ppm, 100 ppm, and 125 ppm), each tested in triplicate. The inhibition zone diameters observed are presented in Table 1.

Table 1. Bioactivity of senggani root

Bioindicator	Inhibitor Zone (mm)				
	Control	Control	Extract (ppm)		
	(+)	(-)	75	100	125
E. coli	9.5	-	11	11.5	12
E. coli	9.5	-	11	11.5	12
			11.5	12	12.5
Average	9.5	-	11.6	11.6	12.1

The antioxidant compounds present in the senggani root ethanol extract were shown to exhibit antibacterial potential, as demonstrated by their inhibitory effects on *E. coli*. The appearance of clear zones surrounding the discs placed on Mueller-Hinton Agar plates indicated bacterial growth inhibition. The tested concentrations as 75 ppm, 100 ppm, and 125 ppm, produced inhibition zones measuring 11.16 mm, 11.66 mm, and 12.16 mm, respectively. The positive control (nystatin 100 µg) produced a 9.5 mm inhibition zone, while the negative control (sterile distilled water) showed no inhibition (0 mm). These results indicate that all tested concentrations of the ethanol extract exhibited antibacterial activity against *E. coli*, with inhibition zones increasing proportionally with concentration. This concentration-dependent activity further supports the extract's potential as a natural antibacterial agent. Two types of control groups were used in this study as the positive control (nystatin) was intended to validate the antibacterial activity under standard conditions, while the negative control (sterile distilled water) was used to confirm that the solvent alone had no inhibitory effect on bacterial growth.

The antibacterial activity of the extract is likely attributed to the presence of active secondary metabolites such as flavonoids, saponins, tannins, and triterpenoids [11]. Flavonoids, in particular, may exert antibacterial effects by interacting with proteins in the bacterial cell wall and cytoplasmic membrane to form complexes that disrupt bacterial metabolism and structural integrity [11].

3. Discussion

The results of this study indicate that the ethanol extract of *Melastoma malabathricum* L. (commonly known as senggani) root exhibits significant antioxidant and antibacterial properties, thus reinforcing its traditional medicinal use. The antioxidant activity, measured using the DPPH radical scavenging assay, produced an IC_{50} value of 4.67 ppm, which is comparable to that of vitamin C (4.01 ppm). According to Citrariana et al. [10], antioxidant compounds with IC_{50} values below 50 ppm are classified as "very strong," placing the ethanol extract of senggani root in this highly active category. This potent antioxidant activity is likely attributed to the presence of bioactive secondary metabolites such as flavonoids, tannins, saponins, steroids, and triterpenoids. These compounds can neutralize free radicals through mechanisms involving electron or hydrogen donation, with hydroxyl groups playing a central role. The oxygen atoms in these hydroxyl groups contain lone

electron pairs that contribute to the interruption of radical propagation, thereby helping to reduce oxidative stress in biological systems.

An anomaly was noted during the DPPH assay, in which the relationship between concentration and absorbance deviated from the linearity expected under the Beer–Lambert law. While absorbance is typically directly proportional to concentration, a decrease in absorbance with increased concentration was observed in this study. This non-linear behavior may be caused by redox interactions between the oxidizing agent (DPPH) and reducing agents in the extract, which could influence the optical properties of the solution at higher concentrations [11]. The antibacterial activity of the extract was assessed using the disc diffusion method against Escherichia coli. The inhibition zones measured at extract concentrations of 75 ppm, 100 ppm, and 125 ppm were 11.16 mm, 11.66 mm, and 12.16 mm, respectively, which were significantly larger than that of the positive control (nystatin, 100 µg), which produced a 9.5 mm zone. The negative control (sterile distilled water) did not show any inhibition. These results confirm the dose-dependent antibacterial effect of the ethanol extract of senggani root [12].

The antibacterial effects observed are likely due to the presence of flavonoids, saponins, tannins, and triterpenoids. Flavonoids, for example, can disrupt bacterial cell walls by forming complexes with extracellular proteins, thereby compromising membrane integrity. Saponins and tannins may further inhibit microbial enzymatic activity and promote cell lysis. Such findings are consistent with earlier studies that highlighted the antimicrobial properties of senggani root against a variety of pathogens [13]. In conclusion, the findings of this study validate the traditional application of senggani root in treating infections and oxidative stress. Furthermore, they suggest the plant's potential as a natural source for the development of therapeutic agents. Future studies should focus on isolating and characterizing individual active compounds and exploring their pharmacological mechanisms in detail [14–16].

4. Materials and Methods

4.1. Study Location and Period

This research was conducted from March to June 2018 at the Chemistry Laboratory, Faculty of Teacher Training and Education (FKIP), and the Research Laboratory of Chemistry, Faculty of Mathematics and Natural Sciences (MIPA), Syiah Kuala University (Unsyiah), as well as at the Laboratory of the Health Analyst Academy, Banda Aceh.

4.2. Instruments

The instruments used in this study included: glass jars, vacuum rotary evaporator, analytical balance, spatula, stirring rod, beakers, suction flasks, volumetric pipettes, dropper pipettes, hair dryer, test tubes, tweezers, spirit lamp, Erlenmeyer flasks, volumetric flasks, Petri dishes, glass funnels, incubator, ruler, and a UV-Vis spectrophotometer (Shimadzu UVmini-1240).

4.3. Materials

The materials used were: senggani root (*Melastoma malabathricum* L.), 96% ethanol, filter paper, Escherichia coli culture, Mueller-Hinton agar, nystatin (100 µg), sterile distilled water (aquadest), vitamin C, and 1,1-diphenyl-2-picrylhydrazyl (DPPH).

4.4. Sample Preparation

Fresh senggani roots were separated from the stems and weighed (1 kg). They were then air-dried for approximately three days. Once dried, the roots were ground using a blender and sieved through a 36-mesh sieve. The final dry weight obtained was 500 grams. A total of 500 grams of dry senggani root powder was soaked in 5 liters of 96% ethanol for 24 hours. After maceration, the mixture was filtered to separate the extract from the solid residue. The filtrate was concentrated using a vacuum rotary evaporator and further thickened using a hair dryer (modified method from Musman, 2013).

4.5. Antioxidant Activity Assay Using the DPPH Method

The antioxidant activity was assessed using the DPPH free radical scavenging method. A stock solution of the extract was prepared by dissolving 1 mg of ethanol extract in 10 mL of 96% ethanol, yielding a 100 ppm solution. Serial dilutions were then prepared to obtain test solutions of 2, 4, 6, 8, and 10 ppm. Vitamin C solutions of equivalent concentrations were prepared as a standard. The DPPH solution was prepared by dissolving 0.001 g of DPPH in 25 mL of 96% ethanol to obtain a 0.1 mM solution [1]. Ethanol was used as the blank. The control consisted of 1 mL of DPPH mixed with 3 mL of 96% ethanol. Absorbance was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer. To measure the percentage of inhibition, 3 mL of each extract solution was mixed with 1 mL of DPPH solution. The mixtures were homogenized and incubated in the dark for 30 minutes, after which absorbance was recorded.

4.6. IC_{50} Determination

The IC_{50} value (the concentration of extract required to inhibit 50% of DPPH radicals) was calculated using linear regression analysis between the percentage inhibition and extract concentration. The formula used was:

$$\%\text{Inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where:

- A_0 = Absorbance of control (DPPH only)
- A_1 = Absorbance of sample

The X-axis represented extract concentrations (ppm) and the Y-axis represented the percent inhibition [1].

4.7. Antibacterial Activity Assay

The antibacterial activity of the ethanol extract was evaluated using the disc diffusion method against Escherichia coli. Five treatment groups were prepared:

(a) Group 1: Negative control (sterile distilled water)
(b) Group 2: Positive control (nystatin 100 µg)
(c) Groups 3–5: Extract treatments at concentrations of 75, 100, and 125 ppm

Each treatment was performed in triplicate. Mueller-Hinton agar was sterilized and poured into Petri dishes (~20 mL per plate) and allowed to solidify. A bacterial suspension of E. coli was standardized using the McFarland turbidity standard and evenly spread onto the surface of the agar using a sterile cotton swab. Blank sterile paper discs were soaked in each concentration of the ethanol extract and placed on the agar surface inside a laminar airflow cabinet. The Petri dishes were incubated at 37°C for 24 hours. After incubation, the diameter of the clear inhibition zones around each disc was measured using a ruler (modified from Nuryanti, 2017).

5. Conclusions

The findings of this study demonstrate that the ethanol extract of senggani root possesses significant antioxidant and antibacterial activities, affirming its potential as a natural therapeutic agent. The extract exhibited a very strong antioxidant effect, with an IC_{50} value of 4.67 ppm, closely comparable to that of vitamin C (4.01 ppm). This high antioxidant capacity is likely due to the presence of key secondary metabolites such as flavonoids, tannins, and saponins, which are known for their free radical scavenging properties. In addition to its antioxidant potential, the extract also showed antibacterial activity against *E.coli*, with inhibition zones increasing proportionally with concentration. At 125 ppm, the extract produced a larger inhibition zone than the positive control (nystatin 100 µg), suggesting its efficacy in inhibiting the growth of pathogenic bacteria.

These results validate the traditional use of senggani root in ethnomedicine and highlight the importance of further pharmacological studies to isolate, characterize, and evaluate the bioactive compounds responsible for its therapeutic effects. Overall, senggani root extract holds promise for development into natural antioxidant and antibacterial agents for potential pharmaceutical or nutraceutical applications.

Author Contributions: M.M. contributed to the conceptualization, supervision, and overall coordination of the study. S.N. was responsible for data collection, formal analysis, and methodological design, while E.E. contributed to data processing, visualization, and software development. All authors participated in the interpretation of results, manuscript preparation, and critical revision of the final paper. Each author has read and approved the final version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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