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RESEARCH ARTICLE

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

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Abstract

This study evaluated the antioxidant and antibacterial activities of the ethanol extract of senggani root (*Melastoma malabathricum* L.). The extract was obtained through maceration using 96% ethanol and tested for antioxidant activity using the DPPH method at 517 nm. Antibacterial activity against *Escherichia coli* was assessed using the agar diffusion method with five groups: a negative control (sterile distilled water), a positive control (100 µg nystatin), and extract concentrations of 75, 100, and 125 ppm, tested in triplicate. The extract showed a strong antioxidant effect with an IC value of 4.67 ppm, comparable to vitamin C (4.01 ppm). It also demonstrated antibacterial activity, producing inhibition zones ranging from 11.16 to 12.16 mm.

Keywords: *Melastoma malabathricum*, Antioxidant, Antibacterial, Senggani Root.

1 Introduction

The increasing global interest in plant-based medicine has intensified the focus on medicinal plants such as *Melastoma malabathricum* L., commonly known as senggani. This plant has long been integrated into Indonesian ethnomedicine, particularly its roots, which have traditionally been used to treat various ailments, including diarrhea, boils, bleeding wounds, burns, and bacillary dysentery [1]. Recent phytochemical studies have identified several bioactive compounds in *M. malabathricum*, including flavonoids, saponins, tannins, steroids, and triterpenoids. These compounds are well recognized for their antimicrobial and antioxidant properties, providing a strong scientific basis for further investigation of the therapeutic potential of the plant [2–4].

Antioxidants play a crucial role in reducing oxidative stress, which is implicated in chronic diseases such as cancer, cardiovascular disease, diabetes, and neurodegenerative disorders [5]. Studies using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay have demonstrated that various extracts of *M. malabathricum* exhibit significant antioxidant activity. For example, methanolic extracts of roots, leaves, and stems have shown strong DPPH and ABTS radical scavenging activity, with enhanced effects observed under specific growth conditions, further highlighting the antioxidant potential of the plant [2,6]. The antioxidant capacity is commonly quantified using IC values obtained through UV–Vis spectrophotometric analysis [5]. The growing 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 agents

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has become increasingly urgent as the efficacy of conventional antibiotics continues to decline [4,7].

Previous studies have demonstrated that *M. malabathricum* exhibits antibacterial activity against *E. coli* and other pathogenic bacteria, supporting its traditional use in the treatment of infectious diseases [3,4,8]. Investigating the antibacterial potential of the root is particularly important in light of the alarming global rise in bacterial resistance [5,9]. Therefore, this study aims to evaluate the antioxidant and antibacterial activities of ethanol extracts derived from the roots of *M. malabathricum*. By assessing the extract's free radical scavenging capacity and its inhibitory effects against *E. coli*, this research seeks to validate the plant's traditional medicinal applications while contributing to the development of effective natural therapeutic agents [1,3,5].

2 Methodology

2.1 General

Laboratory equipment comprised standard glassware, a vacuum rotary evaporator, an analytical balance, an incubator, and a UV–Vis spectrophotometer (Shimadzu UVmini-1240). The chemicals and culture media used in this study included 96% ethanol, Mueller–Hinton agar, nystatin (100 µg), sterile distilled water (aquadest), vitamin C, and 1,1-diphenyl-2-picrylhydrazyl (DPPH).

2.2 Biomaterial

The plant material used in this study was senggani root (*Melastoma malabathricum* L.). The roots were selected based on their traditional use in Indonesian ethnomedicine and their reported bioactive properties, particularly antioxidant and antibacterial activities. Fresh roots were collected and carefully separated from the stems to ensure sample purity. Prior to extraction, the plant material was cleaned to remove adhering soil and impurities, then air-dried under ambient conditions to reduce moisture content. The dried roots were subsequently processed into powder form to facilitate efficient extraction of bioactive compounds. The prepared plant material was then used for further phytochemical extraction and biological activity assays.

2.3 Bacterial Strain and Culture Conditions

The bacterial strain used in this study was *Escherichia coli*, a Gram-negative bacterium commonly associated with gastrointestinal infections. The strain was obtained from a laboratory stock culture and maintained under standard microbiological conditions. Prior to antibacterial testing, the bacterial culture was subcultured on Mueller–Hinton agar and incubated at 37°C for 24 hours to ensure optimal growth and viability. A bacterial suspension was then prepared and adjusted to the McFarland turbidity standard to achieve a uniform cell density suitable for the disc diffusion assay [5].

2.4 Extraction and Isolation

Fresh senggani roots (1 kg) were separated from the stems and air-dried for approximately three days. The dried roots were ground using a blender and sieved through a 36-mesh sieve, yielding 500 g of dry powder. The powder was macerated in 5 L of 96% ethanol for 24 hours. The mixture was filtered to separate the filtrate from the residue, and the filtrate was concentrated using a vacuum rotary evaporator, followed by further thickening with a hair dryer (modified from Musman, 2013).

2.5 Antioxidant Activity and IC Determination

Antioxidant activity was evaluated using the DPPH free radical scavenging method. A stock solution was prepared by dissolving 1 mg of ethanol extract in 10 mL of 96% ethanol (100 ppm), followed by serial dilutions to obtain concentrations of 2, 4, 6, 8, and 10 ppm. Vitamin C solutions at equivalent concentrations were used as the 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 served as the blank, while the control consisted of 1 mL DPPH mixed with 3 mL ethanol. For the assay, 3 mL of each extract solution was mixed with 1 mL DPPH solution, homogenized, and incubated in the dark for 30 minutes. Absorbance was measured at 517 nm using a UV–Vis spectrophotometer. The percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \frac{A_0 - A_1}{A_0} \times 100 \qquad (1)$$

where A0 represents the absorbance of the control and A1 represents the absorbance of the sample. The IC value was determined using linear regression analysis between extract concentration (ppm) and percentage inhibition [1].

2.6 Antibacterial Activity Assay

Antibacterial activity was assessed using the disc diffusion method against *Escherichia coli*. Five treatment groups were prepared: a negative control

(sterile distilled water), a positive control (nystatin 100 µg), and extract concentrations of 75, 100, and 125 ppm. Each treatment was performed in triplicate. Mueller–Hinton agar was sterilized and poured into Petri dishes (approximately 20 mL per plate) and allowed to solidify. A standardized *E. coli* suspension adjusted to the McFarland turbidity standard was spread evenly onto the agar surface using a sterile cotton swab. Sterile paper discs soaked in each extract concentration were placed on the agar surface under aseptic conditions in a laminar airflow cabinet. The plates were incubated at 37°C for 24 hours. After incubation, the diameter of the inhibition zones was measured using a ruler.

3 Results

3.1 Preparation of Sample

The senggani roots are thoroughly washed and dried before being ground into a fine powder. The powdered material 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, the phenolic compounds present in the extract have been shown to exhibit significant antioxidant activity, which can be quantitatively assessed using methods such as the DPPH radical scavenging assay [3,4].

In evaluating the antioxidant capacity of the senggani root extract, the DPPH radical scavenging assay is widely employed due to its simplicity, rapidity, and minimal sample requirements for assessing free radical scavenging activity. The IC values obtained from this assay effectively reflect the extract's ability to neutralize DPPH radicals. Literature reports indicate that senggani root extracts exhibit competitive IC values, with some studies reporting 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, consistent with traditional claims regarding their therapeutic benefits [2,3].

The chemical composition of the extract further supports its antioxidant properties and 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 above, demonstrate its promising antioxidant potential supported by comprehensive phytochemical characterization. The integration of traditional preparation methods with modern scientific techniques provides a strong foundation for further exploration of the plant's extensive medicinal benefits.

3.2 Regression Analysis and IC Interpretation

According to Zuhra et al. [1], higher antioxidant activity corresponds to a lower IC value. In this study, the IC 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 proposed by Setha et al. [2], antioxidant compounds are categorized as weak (>150 ppm), moderate (100–150 ppm), strong (50–100 ppm), and very strong (<50 ppm). Therefore, both the extract and vitamin C fall within the very strong antioxidant category.

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 contain hydroxyl functional groups that contribute to their ability to donate electrons or hydrogen atoms, thereby neutralizing 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 suggests that when light passes through a solution at a specific wavelength, a portion of the light is absorbed while the remainder is transmitted, resulting in a linear relationship between absorbance and concentration [5]. In this study, the observed anomaly may be attributed to redox interactions between the oxidizing agent (DPPH) and the reducing agents (bioactive compounds in the extract). As the concentration of the extract increases, greater reduction of DPPH occurs, which may alter the absorption characteristics and lead to a deviation from linearity, where absorbance decreases with increasing concentration (Figure 1) [6–10].

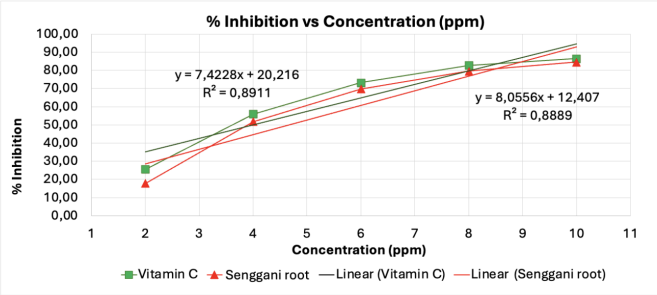

Figure 1. Regression analysis

3.3 Antibacterial Activity

The antibacterial activity of the ethanol extract was evaluated against *E. coli* using the disc diffusion method to measure inhibition zones. Five treatment groups were included: 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 observed inhibition zone diameters are presented in Table 1.

Table 1. Inhibition zone diameter (mm) of senggani root ethanol extract against *E. coli*.

Bioindicator	Control		Extract (ppm)		
	(+)	(−)	75	100	125
E. coli (Rep 1)	9.5	0	11.0	11.5	12.0
E. coli (Rep 2)	9.5	0	11.0	11.5	12.0
E. coli (Rep 3)	9.5	0	11.5	12.0	12.5
Average	**9.5**	**0**	**11.6**	**11.6**	**12.1**

The ethanol extract of senggani root demonstrated antibacterial potential, as indicated by its inhibitory effects on *E. coli*. The formation of clear zones surrounding the disks in Mueller-Hinton Agar plates confirmed inhibition of bacterial growth. The tested concentrations of 75 ppm, 100 ppm, and 125 ppm produced inhibition zones measuring 11.16 mm, 11.66 mm, and 12.16 mm, respectively. In comparison, the positive control (nystatin, 100 µg) produced an inhibition zone of 9.5 mm, while the negative control (sterile distilled water) showed no inhibition (0 mm).

These findings indicate that all concentrations of the ethanol extract tested exhibited antibacterial activity against *E. coli*, with the inhibition zones increasing proportionally with concentration. This concentration-dependent effect further supports the potential of the extract as a natural antibacterial agent. The use of two control groups strengthened the validity of the results: the positive control (nystatin) confirmed antibacterial activity under standard conditions, while the negative control (sterile distilled water) verified that the solvent alone did not inhibit bacterial growth.

The antibacterial activity of the extract is likely attributed to the presence of bioactive 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, forming complexes that disrupt bacterial metabolism and compromise structural integrity [11].

4 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, thereby reinforcing its traditional medicinal use. The antioxidant activity, measured using the DPPH radical scavenging assay, yielded an IC 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 values below 50 ppm are classified as "very strong," placing the ethanol extract of senggani root within 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 neutralize free radicals through mechanisms involving electron or hydrogen donation, with hydroxyl groups playing a central role. The oxygen atoms within these hydroxyl groups possess lone electron pairs that help interrupt radical propagation, thereby reducing oxidative stress in biological systems.

An anomaly was observed during the DPPH assay, in which the relationship between concentration and absorbance deviated from the linearity predicted by Beer–Lambert's law. While absorbance is typically directly proportional to concentration, a decrease in absorbance with increasing concentration was observed in this study. This non-linear behavior may result from redox interactions between the oxidizing agent (DPPH) and the reducing agents present in the extract, which could alter 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 larger than that of the positive control (nystatin, 100 µg), which produced a 9.5 mm inhibition zone. The negative control (sterile distilled water) showed no inhibition. These findings confirm the dose-dependent antibacterial effect of the ethanol extract of senggani root [12].

The observed antibacterial effects 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. These findings are consistent with previous studies highlighting the antimicrobial properties of senggani root against various pathogens [13].

In conclusion, the findings of this study validate the traditional use of senggani root in managing infections and oxidative stress. Furthermore, they suggest the plant's potential as a natural source for the development of therapeutic agents. Future research should focus on the isolation and characterization of individual active compounds, as well as the detailed elucidation of their pharmacological mechanisms [14–16].

5 Conclusion

The findings of this study demonstrate that the ethanol extract of senggani root possesses significant antioxidant and antibacterial activities, supporting its potential as a natural therapeutic agent. The extract exhibited a very strong antioxidant effect, with an IC value of 4.67 ppm, closely comparable to that of vitamin C (4.01 ppm). This high antioxidant capacity is likely attributable to the presence of key secondary metabolites such as flavonoids, tannins, and saponins, which are well known for their free radical scavenging properties.

In addition to its antioxidant potential, the extract also demonstrated 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 effectiveness 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 shows promise for development as a natural antioxidant and antibacterial agent with potential pharmaceutical or nutraceutical applications.

Data Availability Statement

Data will be made available on request.

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 the results, manuscript preparation, and critical revision of the final version of the paper. All authors have read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Ethical Approval and Consent to Participate

Not applicable.

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