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Phytochemical Screening and *In Vitro* DPPH Radical Scavenging Activity of the Crude Methanolic Leaf Extract of *Melastoma malabathricum* L. from Pontianak, West Kalimantan

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ABSTRACT

Oxidative stress contributes to many chronic diseases, and natural antioxidants from local medicinal plants are being explored as alternatives to synthetic additives. *Melastoma malabathricum* L. (senggani) is traditionally used as a medicinal plant in West Kalimantan, Indonesia; however, most previous studies tested solvent fractions or aqueous extracts, and data on the crude methanolic leaf extract of plants from West Kalimantan obtained with a fully documented DPPH procedure remain limited. This study aimed to identify the secondary metabolite classes and to determine the *in vitro* DPPH radical scavenging activity of the crude methanolic leaf extract of *M. malabathricum*. In a laboratory experiment, leaf powder (400 g) was macerated in methanol (1:10 w/v) for three days, and the extract was characterized for moisture content and loss on drying. Qualitative phytochemical screening was performed for alkaloids, flavonoids, steroids, terpenoids, saponins, and tannins. Antioxidant activity was measured using the DPPH radical scavenging assay (final DPPH concentration 0.05 mM) at 517 nm at five concentrations (20–100 µg/mL) in triplicate, and the IC_{50} was estimated by linear regression of the individual replicate data (n = 15), with its 95% confidence interval (CI) calculated by the delta method. The extract yield was 12.5% (w/w). Terpenoids, saponins, and tannins were detected; alkaloids were presumptive (positive with Dragendorff reagent only, not confirmed), and flavonoids and steroids were not detected by the colour tests used. Moisture content and loss on drying were 11.95% and 12.71%, respectively. DPPH inhibition increased with concentration from 8.68 ± 0.52% to 43.56 ± 0.26% (R^2 = 0.9839). Because 50% inhibition was not reached, the IC_{50} was extrapolated: 116.7 µg/mL (95% CI 111.8–121.6 µg/mL), indicating moderate activity. The crude methanolic leaf extract of *M. malabathricum* showed concentration-dependent DPPH radical scavenging of moderate potency *in vitro*. These preliminary findings do not identify the constituents responsible for the activity and do not establish therapeutic efficacy or safety; testing over a wider concentration range with a positive control, chromatographic confirmation of alkaloids, quantitative phenolic and tannin analysis, and complementary assays are needed.

Keywords: *Melastoma malabathricum*; DPPH; IC_{50}; Phytochemical screening; *In vitro* antioxidant activity

INTRODUCTION

Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the body's antioxidant defence capacity. This condition plays an important role in the pathogenesis of chronic diseases such as cardiovascular disease, diabetes, neurodegenerative disorders, and cancer (Sharifi-Rad et al., 2020). Modern lifestyles characterized by the consumption of processed foods, exposure to chemicals, and low physical activity further promote oxidative stress (Sharifi-Rad et al., 2020). For this reason, medicinal plants with antioxidant properties have long been used to prevent or treat diseases triggered by oxidative stress. Dietary polyphenols, for example, can scavenge free radicals, chelate transition metals, and modulate endogenous antioxidant defences in experimental models (Rudrapal et al., 2022). However, these benefits should not be generalized. Most of the evidence derives from *in vitro* and animal studies; clinical trials of antioxidant therapy have produced inconsistent results, partly because of poor bioavailability and limited delivery to target tissues (Ashok et al., 2022; Di Lorenzo et al., 2021); and some antioxidant compounds act as pro-oxidants at high doses (Ouyang et al., 2020; Sharifi-Rad et al., 2020). Conversely, the synthetic phenolic antioxidants butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) are permitted food additives within regulatory limits, and the concerns about them—hepatotoxicity, cytotoxicity, oxidative stress, endocrine disruption, and possible carcinogenicity—arise mainly from experimental studies at high exposure levels and from their widespread presence in foods and the environment (Xu et al., 2021). Natural antioxidants are

therefore not inherently safer than synthetic ones; rather, local plants are worth characterizing as alternative sources, provided that their activity and safety are demonstrated experimentally.

One promising medicinal plant is senggani (*Melastoma malabathricum* L., Melastomataceae), which is also known locally as senduduk or harendong (Lestari et al., 2022; Meilawati et al., 2022). The plant is recorded as a medicinal plant in West Kalimantan and is traditionally used to treat diabetes, diarrhoea, dysentery, and high blood pressure (Lestari et al., 2022; Zheng et al., 2021). Its leaves are commonly boiled in water and drunk after cooling as a herbal remedy (Lestari et al., 2022). Traditional uses also include the treatment of wounds, stomach ache, and toothache (Mayasari et al., 2021). Many medicinal plants contain antioxidants such as polyphenols and flavonoids that act as active agents (Mayasari et al., 2021), making senggani relevant for phytochemical and antioxidant investigation. A recent narrative review concluded that *M. malabathricum* extracts show consistent anti-inflammatory activity and provide a scientific basis for developing the plant as a herbal medicine for oxidative stress-related diseases (Apridamayanti et al., 2026). Scientific evidence of the antioxidant activity of senggani is therefore an important step toward its rational use.

Several studies have investigated the chemical composition and antioxidant activity of senggani leaves. Mayasari et al. (2021) reported that the ethyl acetate extract had the highest antioxidant activity, with an IC_{50} of 43.30 µg/mL in the DPPH assay, presumably related to phenolic compounds. Meilawati et al. (2022) found that the methanolic extract and ethyl acetate fraction contained flavonoids, tannins, and saponins; the ethyl acetate fraction showed the strongest antioxidant activity (IC_{50} 23.08 µg/mL) and α-glucosidase inhibitory activity (IC_{50} 3.835 µg/mL), with low toxicity to *Artemia salina* larvae (LC_{50} > 1000 µg/mL). A study of aqueous extracts showed that extraction conditions influenced antioxidant activity, with IC_{50} values of approximately 2.13–19.20 ppm, and LC-MS/MS analysis identified quercetin as the dominant compound; the best aqueous extract was more active than vitamin C, suggesting its potential as a functional food (Lestari et al., 2022). LC-MS-based metabolomics combined with molecular docking has also been used to identify antihyperglycaemic and antioxidant compounds in senggani leaves (Lestari et al., 2024), and ultrasound-assisted extraction optimized by Box–Behnken design has been applied to recover its antioxidants (Hosni et al., 2023). In addition, the anticarcinogenic activity of the methanolic leaf extract has been attributed to phenolic compounds and activation of the endogenous antioxidant system (Zakaria et al., 2022). These studies consistently used the DPPH method as the main antioxidant assay (Lestari et al., 2022; Mayasari et al., 2021; Meilawati et al., 2022). Collectively, they indicate that senggani leaves are a promising antioxidant source, although the magnitude of activity depends on the type of extract, fraction, and extraction conditions.

Solvent selection is a key determinant of the recovery of secondary metabolites and their biological activities. Studies of other medicinal plants show that differences in solvent polarity produce marked differences in antioxidant potential and that polar solvents are suitable for extracting many phytochemicals (Ounis et al., 2023). In *Thymelaea hirsuta*, for example, methanolic macerates and aqueous decoctions both showed strong radical scavenging activity, yet the activity still differed significantly according to solvent polarity (Ounis et al., 2023). Methanol, as a polar solvent, has yielded senggani leaf extracts containing flavonoids, tannins, and saponins (Meilawati et al., 2022), with biological activities associated with phenolic compounds (Zakaria et al., 2022). Qualitative phytochemical screening is commonly combined with antioxidant assays to relate classes of secondary metabolites to the observed activity (Mayasari et al., 2021; Meilawati et al., 2022). Among antioxidant assays, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method is one of the most popular because it is simple: the extract is mixed with DPPH solution and absorbance is measured after a defined period (Gulcin & Alwasel, 2023). Polyphenols can scavenge the DPPH radical through hydrogen-atom donation (Lestari et al., 2022). Results are usually expressed as IC_{50}, but standardized reaction conditions are needed so that results can be compared between studies (Gulcin & Alwasel, 2023). Moreover, *in vitro* antioxidant activity still requires confirmation by *in vivo* testing (Lestari et al., 2022).

This study differs from previous investigations in three respects. First, the strongest activities reported for senggani leaves were obtained with fractionated or optimized preparations, such as the ethyl acetate extract (Mayasari et al., 2021), the ethyl acetate fraction (Meilawati et al., 2022), and optimized aqueous or ultrasound-assisted extracts (Hosni et al., 2023; Lestari et al., 2022). The crude methanolic extract—the unfractionated material from which such fractions are prepared—has received less attention, yet its activity under documented conditions provides the baseline against which the gain from fractionation can be judged. Second, the samples originate from Pontianak, West Kalimantan; to the authors' knowledge, no DPPH data on senggani leaves from this area have been reported, and because growing location and leaf age significantly affect the phytochemical content and antioxidant activity of senggani (Mayasari et al., 2025), data from new geographical origins are needed. Third, reported IC_{50} values vary widely partly because studies differ in DPPH concentration, incubation time, and whether extract concentrations are expressed in the test solution or in the final reaction mixture (Gulcin & Alwasel, 2023; Wołosiak et al., 2022). This study therefore reports the final DPPH concentration, the concentration basis, the regression parameters, and the confidence interval of the IC_{50}, so that the result can be compared directly with future studies. Reporting both the classes of secondary metabolites and the radical scavenging potential of the same extract within one series of tests also facilitates

interpretation of the relationship between chemical composition and antioxidant activity (Mayasari et al., 2021; Meilawati et al., 2022).

Based on the above, this study aimed to (1) identify the classes of secondary metabolites (alkaloids, flavonoids, steroids, terpenoids, saponins, and tannins) in the crude methanolic leaf extract of senggani through qualitative phytochemical screening; (2) determine the percentage of DPPH radical inhibition by the extract at five concentrations (20, 40, 60, 80, and 100 µg/mL) in triplicate using a UV-Vis spectrophotometer at 517 nm; and (3) calculate the IC_{50} by linear regression and classify the strength of the extract's *in vitro* antioxidant activity. The six classes were selected on the basis of previous studies, which reported them in senggani leaves (Idris et al., 2024; Mayasari et al., 2021; Meilawati et al., 2022) and which link phenolics, terpenoids, and, to a lesser extent, alkaloids and saponins with the antioxidant activity of plant extracts (Câmara et al., 2024; Huynh et al., 2020); they were not selected from a preliminary investigation. The results are expected to provide baseline scientific data for developing senggani leaves as a natural antioxidant source, while adding Indonesian evidence on the use of local medicinal plants.

METHODS

Study design

This was a laboratory experimental study with a descriptive qualitative and quantitative approach. The qualitative approach was used to identify secondary metabolite classes through phytochemical screening, whereas the quantitative approach was used to characterize the extract and determine its antioxidant activity. The study material was the crude methanolic extract of senggani (*Melastoma malabathricum* L.) leaves obtained by maceration. The independent variable was extract concentration (20, 40, 60, 80, and 100 µg/mL), and the dependent variable was the percentage inhibition of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Each concentration was tested in three replicates, and the relationship between the two variables was analysed by linear regression to obtain the IC_{50} value.

Time and location

The study was conducted in January 2025. Sample preparation and extraction were carried out at the Research and Chemical Biotechnology Laboratory, Universitas Tanjungpura, Pontianak. Extract characterization, phytochemical screening, and antioxidant testing were carried out at the Integrated Laboratory of Poltekkes Kemenkes Pontianak.

Equipment and materials

The equipment comprised a closed glass maceration vessel, rotary evaporator, analytical balance, drying oven (Binder), desiccator, porcelain crucibles, UV-Vis spectrophotometer (Inscienspro 110), cuvettes, volumetric flasks, volumetric pipettes, and test tubes. The materials were senggani leaves, analytical-grade methanol, DPPH powder, and phytochemical screening reagents, namely Dragendorff, Mayer, Wagner, Wilstätter, and Liebermann–Burchard reagents, concentrated H_2SO_4, 2 M HCl, and 1% $FeCl_3$.

Plant material and sample preparation

Fresh senggani leaves were collected in January 2025 from Pontianak, West Kalimantan, Indonesia. The plant was identified at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Tanjungpura, Pontianak. A total of 2 kg of fresh leaves underwent wet sorting. Only mature, fully expanded, green leaves that were free from disease, insect damage, and mechanical injury were selected; young shoots, yellowing (senescent) leaves, stems, and foreign matter were discarded. The sorted leaves were gently washed under running water and drained. The leaves were then dried in an oven at 50 °C for 3 h. A moderate temperature of 50 °C was chosen to shorten drying time and limit microbial and enzymatic degradation while reducing the loss of thermolabile constituents expected at higher temperatures; nevertheless, drying and storage conditions can alter the content of phenolics and other bioactive compounds (Anamaria et al., 2020), so an effect of this treatment on heat-sensitive metabolites cannot be excluded. The dried leaves were ground into simplicia powder and sieved through a 40-mesh sieve.

Extraction

Extraction was performed by maceration. Simplicia powder (400 g) was placed in a closed glass maceration vessel with 4 L of analytical-grade methanol (1:10 w/v). Maceration lasted three days. Every 24 h the macerate was filtered and the residue was re-macerated with a fresh 4 L of methanol (total solvent volume 12 L). The mixture was stirred periodically and kept at room temperature in the closed vessel throughout maceration. The filtrates from each solvent replacement were pooled and concentrated using a rotary evaporator at 40 °C under reduced pressure to obtain a thick extract. Yield was calculated as: Yield (%) = (weight of thick extract / weight of simplicia powder) × 100.

Extract characterization

Two related quality parameters were determined. Moisture content quantifies only the water in the extract, whereas loss on drying quantifies the total mass lost on heating, that is, water plus other volatile substances such as residual solvent or volatile oils; loss on drying is therefore expected to be equal to or slightly higher than moisture content. Both parameters indicate the susceptibility of an extract to microbial growth and hydrolytic degradation during storage. Moisture content was determined gravimetrically on 1 g of accurately weighed extract and calculated as: Moisture content (%) = (mass of water determined / initial mass of extract) × 100. Loss on drying was determined by weighing 1 g of extract into a crucible previously dried at 105 °C and tared, heating it in an oven at 105 °C, cooling it in a desiccator, and weighing; heating and weighing were repeated until constant weight was reached. Loss on drying was

calculated as: LOD (%) = (W_1 − W_2) / W_1 × 100, where W_1 is the initial weight of the extract and W_2 its weight after drying to constant weight.

Phytochemical screening

Qualitative phytochemical screening was performed on the thick methanolic extract to detect alkaloids, flavonoids, steroids, terpenoids, saponins, and tannins. Each test was compared with a control consisting of the reagent without extract so that colour changes, precipitates, or foam could be assessed objectively. Alkaloids were tested by dissolving 0.5 g of extract in 2 M HCl, filtering, and adding Dragendorff, Mayer, and Wagner reagents to separate portions of the filtrate; results were positive when an orange precipitate (Dragendorff), a yellowish-white precipitate (Mayer), or a brown precipitate (Wagner) formed. Flavonoids were tested with the Wilstätter reaction by adding Mg powder and concentrated HCl to the extract solution; a red to orange colour relative to the control indicated a positive result. Steroids were tested with the Liebermann–Burchard reagent by adding acetic anhydride and concentrated H_2SO_4; a blue or green colour different from the control indicated a positive result. Terpenoids were tested by adding concentrated H_2SO_4; a reddish-brown colour indicated a positive result. Saponins were tested by dissolving the extract in hot water, shaking vigorously, and adding one drop of 2 M HCl; foam that remained stable for 10 min indicated a positive result. Tannins were tested by adding a few drops of 1% $FeCl_3$ to the extract solution; a blackish-green colour indicated a positive result. Because these colour and precipitation tests are qualitative and their detection limits were not determined, a negative result was interpreted as "not detected under the test conditions" rather than as absence. A class was recorded as presumptive when the results of different reagents for the same class disagreed.

DPPH antioxidant assay

Antioxidant activity was determined with the DPPH radical scavenging method using UV-Vis spectrophotometry. A 0.1 mM DPPH solution was prepared by dissolving 3.94 mg of DPPH (molecular weight 394.32 g/mol) in methanol to 100 mL in a volumetric flask; the solution was protected from light with aluminium foil and used on the day of preparation. First, the maximum wavelength was determined by scanning this DPPH solution over 400–600 nm, and the wavelength of highest absorbance was used for all measurements. Second, a stock solution of the extract (1000 µg/mL) in methanol was diluted to five concentrations (20, 40, 60, 80, and 100 µg/mL), each prepared in three replicates (R1, R2, and R3). Third, 4 mL of each test solution was mixed with 4 mL of the DPPH solution, giving a final DPPH concentration of 0.05 mM (50 µM) in the reaction mixture, homogenized, and incubated for 30 min at room temperature in the dark. The absorbance of the mixture was then measured at the maximum wavelength. The control (A_{blank}) consisted of 4 mL of DPPH solution and 4 mL of methanol. Throughout this paper, extract concentrations refer to the test solutions before mixing; the corresponding final concentrations in the reaction mixture were half these values (10–50 µg/mL). Sample blanks (extract solution with methanol in place of DPPH) were not measured, so no correction was made for the intrinsic absorbance of the extract at 517 nm; any such absorbance would lead to an underestimation of inhibition. A positive control (ascorbic acid or quercetin) was not tested in this study. Both omissions are acknowledged as limitations. The procedure followed commonly used DPPH methods (Gulcin & Alwasel, 2023; Lestari et al., 2022).

Data analysis

Percentage inhibition was calculated as ((A_{blank} − A_{sample}) / A_{blank}) × 100% for each replicate and summarized as mean ± standard deviation (SD) for each concentration. The relationship between concentration (x) and percentage inhibition (y) was analysed by ordinary least-squares linear regression, y = ax + b, fitted to the individual replicate observations (n = 15; five concentrations × three replicates) using Microsoft Excel 2019 (LINEST function). The slope (a), the intercept (b), their standard errors (SE) and 95% CIs, the coefficient of determination (R^2), and the F-test of the regression were reported. The IC_{50} was obtained by substituting y = 50, giving IC_{50} = (50 − b) / a. Because the IC_{50} lay outside the tested concentration range, it was obtained by extrapolation. Its SE was estimated with the delta method: $SE(IC_{50}) = (s / |a|) \times \sqrt{[1/n + (IC_{50} - \bar{x})^2 / S_{xx}]}$, where s is the residual standard deviation of the regression, n = 15, $\bar{x}$ = 60 µg/mL is the mean concentration, and $S_{xx} = \Sigma(x_i - \bar{x})^2$ = 12,000 (µg/mL)^2. The 95% CI was calculated as $IC_{50} \pm t_{0.975;\,13} \times SE(IC_{50})$, with $t_{0.975;\,13}$ = 2.160. As a sensitivity check, the 95% CI was also calculated with Fieller's theorem, which accounts for the uncertainty of the slope in a ratio estimate. Antioxidant strength was classified according to Wilapangga and Sari (2018) as very strong (IC_{50} < 50 µg/mL), strong (50–100 µg/mL), moderate (101–150 µg/mL), weak (151–500 µg/mL), or inactive (> 500 µg/mL).

RESULTS AND DISCUSSION

Extract characteristics

Maceration of senggani leaf powder with methanol produced a thick extract (50 g) with a yield of 12.5% (w/w) (Table 1). The moisture content of the extract was 11.95%, and the loss on drying was 12.71%. Loss on drying exceeded moisture content by 0.76 percentage points, which is consistent with the loss of small amounts of volatile substances other than water, such as residual methanol. Because water content governs the risk of microbial growth and hydrolytic degradation of constituents such as tannins during storage, these values provide a reference for standardizing future batches.

Table 1. Characteristics of the methanolic leaf extract of *Melastoma malabathricum* L.

Parameter	Result
Weight of simplicia powder (g)	400
Weight of thick extract (g)	50

Parameter	Result
Yield (% w/w)	12.5
Moisture content (%)	11.95
Loss on drying (%)	12.71

Phytochemical screening

Phytochemical screening showed that the methanolic extract of senggani leaves contained terpenoids, saponins, and tannins, whereas flavonoids and steroids were not detected under the test conditions (Table 2). Alkaloids were positive with Dragendorff reagent but negative with Mayer and Wagner reagents; this result was therefore classified as presumptive rather than positive.

Table 2. Phytochemical screening of the methanolic leaf extract of *M. malabathricum*

Compound class	Reagent	Observation	Result
Alkaloids	Dragendorff	Orange precipitate	+
	Mayer	Green colour, no precipitate	–
	Wagner	Light brown colour, no precipitate	–
	Overall	Disagreement between reagents	±
Flavonoids	Wilstätter	Light brown colour (same as control)	–
Steroids	Liebermann–Burchard	Green colour (same as control)	–
Terpenoids	Concentrated H_2SO_4	Brown colour	+
Saponins	Hot water + 2 M HCl	Foam stable for 10 min	+
Tannins	1% $FeCl_3$	Blackish-green colour	+

Note: (+) detected; (–) not detected under the test conditions; (±) presumptive: positive with one of three reagents only and not confirmed by a chromatographic method.

Maximum wavelength of DPPH

Scanning the DPPH solution over 400–600 nm produced a maximum absorbance at 517 nm, and this wavelength was used for all absorbance measurements.

Antioxidant activity

As extract concentration increased, DPPH absorbance consistently decreased and percentage inhibition increased, from 8.68 ± 0.52% at 20 µg/mL to 43.56 ± 0.26% at 100 µg/mL (Table 3). The absorbance of the DPPH control (A_{blank}) was 0.776. Inhibition did not reach 50% at the highest concentration tested.

Table 3. Absorbance and percentage inhibition of the DPPH radical by the methanolic leaf extract of *M. malabathricum* (Mean ± SD, n = 3)

Concentration (µg/mL)[a]	Final concentration in reaction mixture (µg/mL)	Absorbance (517 nm)	Inhibition (%)
20	10	0.709 ± 0.004	8.68 ± 0.52
40	20	0.647 ± 0.003	16.58 ± 0.39
60	30	0.588 ± 0.029	24.18 ± 3.73
80	40	0.514 ± 0.004	33.76 ± 0.47
100	50	0.438 ± 0.002	43.56 ± 0.26

Note: [a]Concentration of the extract test solution before mixing 1:1 (v/v) with 0.1 mM DPPH. A_{blank} = 0.776. Sample blanks were not measured.

The relationship between concentration and percentage inhibition was linear (Figure 1). Regression on the 15 individual observations gave y = 0.4347x – 0.7303 (slope SE 0.0154, 95% CI 0.401–0.468; intercept SE 1.024, 95% CI –2.94 to 1.48; R^2 = 0.9839; F(1, 13) = 792.7, p < 0.001). Fitting the five mean values yields the same slope and intercept because the design is balanced (R^2 = 0.9965), but the replicate-level fit was used because it incorporates within-concentration variability into the uncertainty estimates. Substituting y = 50 into the regression equation gave an IC_{50} of 116.7 µg/mL (SE 2.25 µg/mL; 95% CI 111.8–121.6 µg/mL by the delta method and 112.1–121.9 µg/mL by Fieller's theorem), which falls into the moderate antioxidant category (Table 4). Because 50% inhibition was not reached within the tested range, this IC_{50} was obtained by extrapolating the regression line 16.7 µg/mL (about 17%) beyond the highest concentration. The estimate assumes that the response remains linear above 100 µg/mL, which cannot be verified with the present data, and the CI reflects only the statistical uncertainty of the fitted line, not this model assumption; the value should therefore be interpreted with caution.

Table 4. Linear regression parameters and IC_{50} of the methanolic leaf extract of *M. malabathricum*

Parameter	Value
Number of observations (n)	15 (5 concentrations × 3 replicates)
Regression equation	y = 0.4347x – 0.7303
Slope (a) ± SE; 95% CI	0.4347 ± 0.0154; 0.401–0.468
Intercept (b) ± SE; 95% CI	–0.730 ± 1.024; –2.94 to 1.48
Coefficient of determination (R^2)	0.9839
Residual standard deviation (s)	1.691
F-test of regression	F(1, 13) = 792.7; p < 0.001
IC_{50} (µg/mL)	116.7 (extrapolated)
SE of IC_{50} (µg/mL), delta method	2.25
95% CI of IC_{50} (µg/mL), delta method	111.8–121.6
95% CI of IC_{50} (µg/mL), Fieller's theorem	112.1–121.9

Parameter	Value
Antioxidant activity category	Moderate (IC_{50} 101–150 µg/mL)

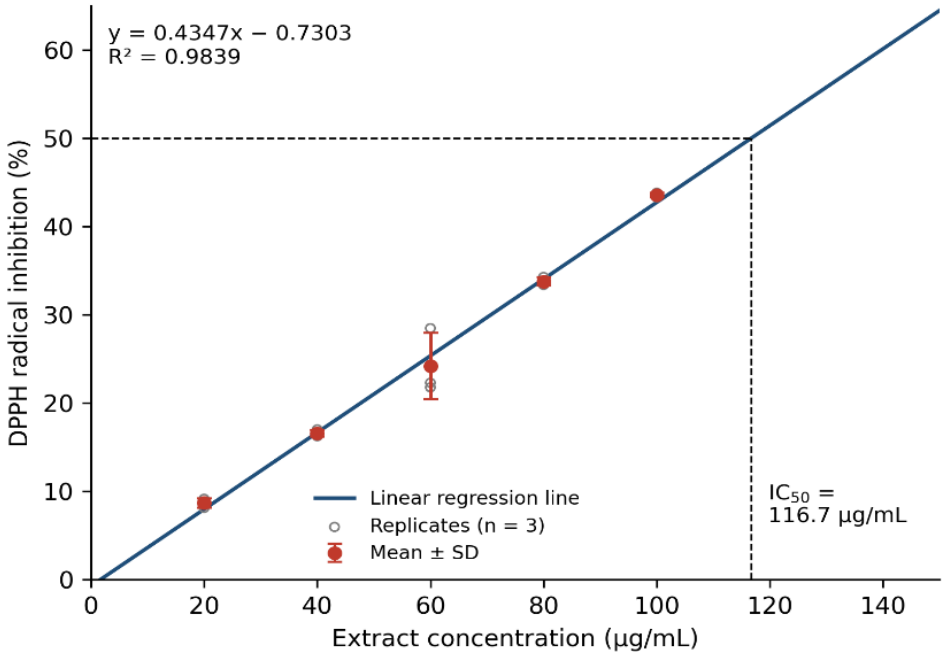


Figure 1. Relationship between extract concentration and DPPH radical inhibition

Note: Open circles show individual replicates (n = 3), red circles show mean ± SD, the solid line is the linear regression fitted to the 15 individual observations, and the dashed lines indicate IC_{50} determination at 50% inhibition. The part of the regression line above 100 µg/mL is an extrapolation beyond the tested range.

Phytochemical profile of the extract

The methanolic extract of senggani leaves in this study contained several classes of secondary metabolites and scavenged the DPPH radical in a concentration-dependent manner, with moderate antioxidant strength. Because these two findings are related, the chemical composition of the extract is discussed first as the basis for interpreting its antioxidant activity. The detection of tannins is consistent with reports that polyphenols and tannins are major constituents of senggani (Tiwari et al., 2023), and tannins and polyphenols have also been found in senggani from the Philippines (Hipol et al., 2023). Both condensed and hydrolysable tannins possess intrinsic antioxidant activity through radical scavenging, metal chelation, and activation of endogenous defences (Camarda et al., 2026; Soldado et al., 2021). The terpenoids detected here have also been reported in senggani leaf extract from South Kalimantan (Idris et al., 2024), and this class is known to have antioxidant potential (Câmara et al., 2024). Saponins, in contrast, have been reported not to contribute to the antioxidant activity of senggani leaves, whereas their polyphenol content correlates with that activity (Huynh et al., 2020). These reports make phenolic constituents, including tannins, plausible contributors to the activity observed here. Because neither total phenolic nor tannin content was quantified in this study, however, the present data cannot establish which constituents are responsible for the activity; the role of tannins remains a hypothesis to be tested by quantitative assays and activity-guided fractionation.

This interpretation must also be weighed against a finding that differs from the literature, namely that flavonoids were not detected, although other studies have reported them in senggani leaves (Idris et al., 2024; Tiwari et al., 2023). The discrepancy may stem from growing location and leaf age, which have been shown to affect the phytochemical content of senggani (Mayasari et al., 2025). Environmental factors such as light, temperature, and altitude also regulate the formation of secondary metabolites (Alami et al., 2024), and post-harvest drying and storage of simplicia can reduce bioactive components (Anamaria et al., 2020). Methodological limitations are equally important. Colour reactions such as the Wilstätter and Liebermann–Burchard tests have limited sensitivity and specificity, their response depends on the structural subclass present, and the intense colour of a crude leaf extract can mask weak colour changes. A negative result therefore means that flavonoids and steroids were not detected under the test conditions, not that they are absent from the extract. Quantitative determinations such as total polyphenol assays can demonstrate the relationship between phenolic content and activity (Huynh et al., 2020).

The alkaloid result requires particular caution. Dragendorff's reagent forms precipitates not only with alkaloids but also with a range of other nitrogen-containing and non-alkaloid substances, so a positive reaction with this reagent alone may be a false positive (Raal et al., 2020). Conversely, the negative Mayer and Wagner tests

may reflect lower sensitivity of these reagents to the alkaloids present or a low alkaloid concentration. Because the three reagents disagreed, alkaloids were recorded as presumptive. Confirmation by thin-layer chromatography with Dragendorff spray reagent and a reference alkaloid, an approach that has been used to characterize senggani constituents (Hipol et al., 2023), was not performed in this study and is recommended, particularly because alkaloids have been reported in other senggani leaf extracts (Idris et al., 2024).

Antioxidant activity in context

A concentration-dependent increase in radical scavenging is consistent with the nature of the DPPH assay, although the linearity of the response depends on the type of antioxidant and the reaction conditions (Wołosiak et al., 2022). For phenolic compounds, the DPPH response is determined by structure, for example the number of hydroxyl groups (Platzer et al., 2021). The phenolic constituents indicated by the positive tannin test may therefore contribute to the measured response, but this cannot be confirmed without quantitative analysis.

The IC_{50} of 116.7 µg/mL indicates weaker activity than values reported for senggani leaves elsewhere, such as 43.30 µg/mL for an ethyl acetate extract (Mayasari et al., 2021), 23.08 µg/mL for an ethyl acetate fraction (Meilawati et al., 2022), and approximately 2–19 ppm for optimized aqueous extracts (Lestari et al., 2022). At least four factors may explain this difference. (1) Extract type: partitioning with ethyl acetate concentrates medium-polarity phenolics and removes constituents such as sugars and pigments that dilute the activity of a crude methanolic extract, and the activity of senggani differs markedly between fractions (Huynh et al., 2020; Meilawati et al., 2022). Within a crude extract, compounds may also interact synergistically, additively, or antagonistically, so the response of the extract is not simply the sum of its components (Nguyen & Karboune, 2023). (2) Plant material: geographical origin, leaf age, and post-harvest drying affect the phenolic content and antioxidant activity of senggani (Anamaria et al., 2020; Mayasari et al., 2025); the failure to detect flavonoids in our extract, in contrast with the quercetin-rich profile reported by Lestari et al. (2022), suggests a lower phenolic load in our sample. (3) Solvent and assay conditions: the solvent determines which compounds are extracted, and in several medicinal plants aqueous extracts showed the strongest DPPH activity (Çakmak Sancar et al., 2026); moreover, IC_{50} values depend on DPPH concentration, incubation time, and the concentration basis (Gulcin & Alwasel, 2023; Wołosiak et al., 2022). Expressed as final concentrations in the reaction mixture, the IC_{50} of our extract would be approximately 58 µg/mL, so part of the apparent difference—and the activity category assigned—depends on this convention. (4) Data analysis: our IC_{50} was extrapolated, whereas several reported values lie within the tested ranges. Because no positive control was tested under identical conditions, these comparisons remain indicative.

DPPH results must nonetheless be interpreted with care. The assay uses a stable radical that does not occur in nature and reacts differently from radicals *in vivo* (Wołosiak et al., 2022), and the *in vivo* effects of phenolic compounds are further determined by their bioavailability and metabolism (Di Lorenzo et al., 2021). These findings are therefore better regarded as preliminary *in vitro* evidence of the antioxidant potential of senggani than as evidence of biological benefit, therapeutic efficacy, or safety.

Implications

Although preliminary, these results have implications for the development of senggani. The data add a point of comparison for the phytochemical profile and antioxidant potential of senggani from West Kalimantan and support further study of its phenolic constituents, including tannins (Tiwari et al., 2023). The use of tannins requires more detailed characterization because their efficacy is influenced by tannin class, degree of polymerization, and dose (Camarda et al., 2026). Variation due to location and leaf age calls for standardization of raw materials, including specification of geographical origin and harvest stage (Mayasari et al., 2025), and post-harvest treatments such as drying also need to be standardized (Anamaria et al., 2020). Further development must also be accompanied by dose and safety studies, because antioxidant compounds can act as pro-oxidants at high doses (Ouyang et al., 2020).

Limitations

These implications should be read together with the limitations of this study. Only a crude extract obtained with a single solvent and without fractionation was tested, although the activity of senggani differs between fractions (Huynh et al., 2020). Screening was qualitative, with limited sensitivity and specificity, the alkaloid result was not confirmed chromatographically, and phenolics and tannins were not quantified, so the relationship between composition and activity remains presumptive. The DPPH assay did not include a positive control or sample blanks, which limits comparison with other studies and may have led to a slight underestimation of inhibition. Activity was assessed with a single *in vitro* method, whereas complementary methods such as ABTS deserve consideration because some phenolic groups react differently toward the two radicals (Platzer et al., 2021), and standardized reaction conditions are needed for comparability (Wołosiak et al., 2022). The highest concentration tested did not reach 50% inhibition, so the IC_{50} was extrapolated under an assumption of continued linearity. The samples came from a single location and a single drying treatment, limiting generalizability (Alami et al., 2024; Mayasari et al., 2025). Finally, *in vivo* testing was not performed, although bioavailability determines the biological effects of phenolic compounds (Di Lorenzo et al., 2021).

CONCLUSION

Qualitative screening showed that the crude methanolic leaf extract of *Melastoma malabathricum* L.

from Pontianak contained terpenoids, saponins, and tannins; alkaloids were presumptive (positive with Dragendorff reagent only and not confirmed), and flavonoids and steroids were not detected by the colour tests used. *In vitro*, the extract scavenged the DPPH radical in a concentration-dependent manner, with an extrapolated IC_{50} of 116.7 µg/mL (95% CI 111.8–121.6 µg/mL) that corresponds to moderate activity. These findings are preliminary: they derive from a single crude extract, a single sampling location, qualitative screening, and a single *in vitro* assay without a positive control; they do not identify the constituents responsible for the activity and do not establish therapeutic efficacy or safety.

Further studies should compare several solvents and fractions, use samples from multiple locations and leaf ages, quantify total phenolic and tannin contents, confirm the presence of alkaloids by thin-layer chromatography, test a wider concentration range (for example, up to 200 µg/mL) so that the IC_{50} falls within the measured data, include a positive control (ascorbic acid or quercetin) and sample blanks in the DPPH assay, complement the DPPH assay with other antioxidant methods such as ABTS or FRAP, and conduct *in vivo* efficacy and safety studies.

DECLARATIONS

Ethics Approval and Consent to Participate. Not applicable. This in vitro study used plant material only and did not involve human participants or animals.

Availability of Data and Materials. The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest. The authors declare no conflict of interest.

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