

Bioassay Evaluation Antioxidant Activity, Total Phenolic Content, and Total Flavonoid Content in *Blumea balsamifera* Leaves under Different Environmental Conditions

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Abstract

Blumea balsamifera is a medicinal plant widely used in traditional medicine and known for its bioactive compounds with antioxidant potential. This study evaluated the effect of different environmental conditions on the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of *B. balsamifera* leaf extracts collected from coastal (PB-1; 25 m asl) and non-coastal areas (AG-1; 108 m asl) in Mandailing Natal Regency, North Sumatra, Indonesia. Leaf extracts were prepared by methanol maceration. TPC and TFC were determined using the Folin–Ciocalteu and aluminum chloride methods, while antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The results showed that AG-1 had higher TPC and TFC values, reaching 237.9 mg GAE/g extract and 35.89 mg QE/g extract, respectively, whereas PB-1 exhibited stronger antioxidant activity with an IC₅₀ value of 6.839 µg/mL. Environmental analysis indicated that AG-1 had higher light intensity and near-neutral soil pH, while PB-1 had more acidic soil conditions. These findings indicate that environmental factors influence secondary metabolite accumulation and antioxidant activity in *B. balsamifera*. Non-coastal environments promoted higher phenolic and flavonoid accumulation, whereas coastal environments enhanced antioxidant activity.

Keywords: antioxidant activity; *Blumea balsamifera*; environmental conditions; flavonoids; phenolics.

INTRODUCTION

Exogenous antioxidants play a crucial role in protecting the body against oxidative damage caused by free radicals (Gulcin, 2025). These free radicals are generated naturally during normal metabolic processes and through exposure to environmental factors such as pollution, ultraviolet radiation, and chemical substances (Chandimali et al., 2025). Under physiological conditions, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are involved in essential cellular functions, including cell signaling and immune responses (Giulietti et al., 2024). However, excessive accumulation of these reactive species beyond the body's antioxidant defense capacity can induce oxidative stress and damage vital cellular components, including lipids, proteins, and DNA (Chandimali et al., 2025). Such oxidative damage has been associated with the development of various degenerative diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders (Pooja et al., 2025; Russo et al., 2018; Muscolo et al., 2024). Therefore, the exploration of exogenous antioxidants derived from natural sources, particularly medicinal plants rich in phenolic and flavonoid compounds, has

gained considerable scientific attention as a safer and more sustainable strategy for preventing oxidative stress-related diseases (Hossain et al., 2025).

Among medicinal plants with therapeutic potential, *Blumea balsamifera* DC. (Asteraceae) has gained attention for its diverse pharmacological properties and widespread ethnomedicinal use in Southeast Asia. This species, locally known as “Sembung” in Indonesia “Ainaxiang” in China, and “Naat” in Thailand (Pang et al., 2014; Widhiantara & Jawi, 2021), is widely distributed across tropical regions, including Indonesia, Malaysia, Thailand, Vietnam, and the Philippines (Pang et al., 2014). *Blumea balsamifera* is widely adaptable to various habitats, including forest margins, riverbanks, grasslands, secondary forests, and disturbed areas, ranging from coastal lowlands to elevations of approximately 1,000 m above sea level (Pang et al., 2014). Traditionally, the leaves of *B. balsamifera* have been used to treat wounds (L. Chen et al., 2026; Masyudi et al., 2022), while leaf decoctions are commonly utilized to relieve coughs, colds (Omac et al., 2021), and postpartum wounds (Masyudi et al., 2023). Scientific investigations have revealed that *B. balsamifera* leaves

possess various pharmacological activities, including antimicrobial (Ismail et al., 2022), anti-cholesterol (Widhiantara et al., 2021), antioxidant, and antibacterial properties (Kusumawati et al., 2019). These biological activities are closely associated with its phytochemical constituents, particularly phenolics, flavonoids, tannins, saponins, and other bioactive secondary metabolites (Masyudi et al., 2022; J. Wang et al., 2023). However, studies investigating the total phenolic and flavonoid contents of *B. balsamifera* growing under different environmental conditions remain limited.

The production and accumulation of secondary metabolites in plants are strongly influenced by environmental conditions, such as soil pH, nutrient availability, salinity, and abiotic stress, which may lead to variations in bioactive compound content and antioxidant activity (Pant et al., 2021; Qaderi et al., 2023; Yang et al., 2018). Therefore, understanding the relationship between environmental conditions and secondary metabolite accumulation is important not only for scientific purposes but also for optimizing the cultivation of medicinal plants (Qaderi et al., 2023). In this study, *Blumea balsamifera* leaves were collected from coastal and non-coastal areas at 25 and 108 m above sea level, respectively, representing different environmental conditions.

This study also considered environmental parameters such as soil pH and nutrient content to better understand their relationship with secondary metabolite composition and antioxidant bioactivity in *B. balsamifera* leaves.

Therefore, this study aimed to evaluate the total phenolic content, total flavonoid content, and antioxidant activity of *B. balsamifera* leaves growing under different environmental conditions. The findings of this study are expected to provide scientific insights that support the development and utilization of *B. balsamifera* as a natural medicinal resource, while also serving as a basis for more efficient cultivation management for pharmaceutical and healthcare applications.

MATERIALS AND METHODS

Study area

Leaves of *Blumea balsamifera* were collected from two different locations in Mandailing Natal Regency, North Sumatra, Indonesia. The first site was a coastal area in Perdamaian Baru Village (25 m above sea level; 99°6'45"–99°9'4" E and 0°29'34"–0°31'53" N). The second site was a non-coastal hilly area in Aek Garingging Village, Lingga Bayu District (108 m above sea level; 99°16'44"–99°16'50" E and 0°36'40"–0°36'43" N) (Figure 1.). Plant samples were cleaned and analyzed at the Laboratory of Universitas Samudra. Plant identification was carried out at Universitas Samudra, and a voucher specimen was deposited under voucher number 056/UN54.42/Km/2026. Soil samples were collected from the subsoil layer at a depth of 5–10 cm by taking samples from three different points at each location as replicates (Akpan-Idiok & Solomon, 2012).

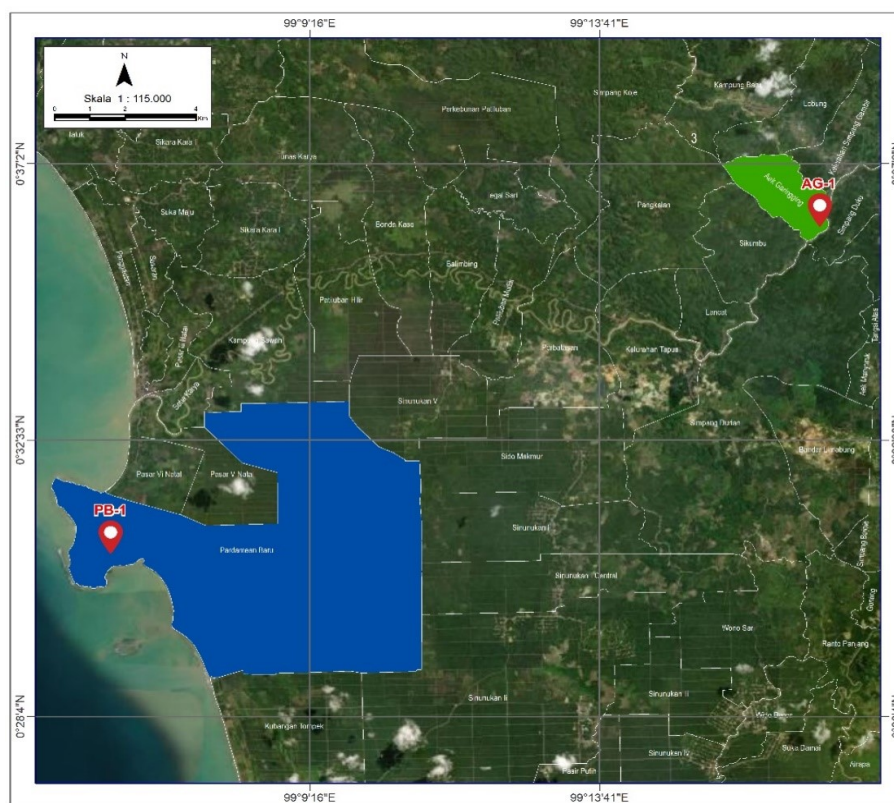


Figure 1. Sampling locations of *B. balsamifera* leaves in Perdamaian Baru (PB-1) and Aek Garingging (AG-1), Mandailing Natal Regency, North Sumatra, Indonesia.

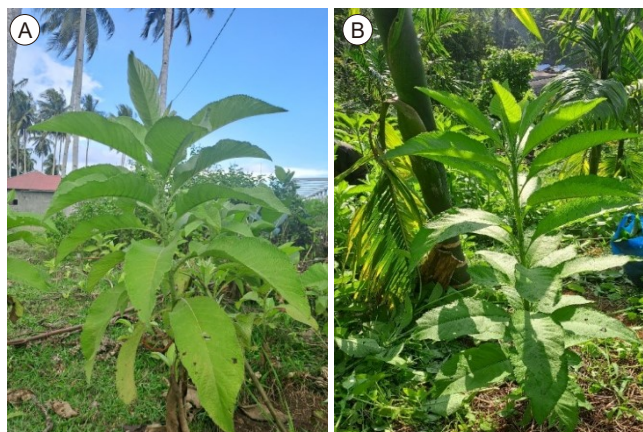


Figure 2. *B. balsamifera* plants at locations; A). Perdamaian Baru (PB-1) and B). Aek Garingging (AG-1).

Tools and materials

This study utilized various instruments and chemicals for sample preparation and laboratory analyses. Field equipment included an altimeter, a three-in-one soil meter, sample containers, jumbo plastic bags, a ruler, and a small shovel. Laboratory equipment consisted of a UV–Vis spectrophotometer, glass funnels, Whatman filter paper, test tubes or vials, droppers, micropipettes, an analytical balance, spatulas, cuvettes, a dark incubator, and volumetric flasks. The chemicals used included 80% methanol, 70% and 96% methanol, distilled water, 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich, St Louis, USA), and ascorbic acid (Vitamin C) (Sigma-Aldrich, St Louis, USA) for antioxidant analysis. Total phenolic content (TPC) and total flavonoid content (TFC) analyses employed Folin–Ciocalteu reagent (Sigma-Aldrich, St Louis, USA), gallic acid ($C_7H_6O_5$) (E. Merck), Na_2CO_3 (E. Merck), $AlCl_3$ (E. Merck), CH_3CO_2K (E. Merck), and quercetin ($C_{15}H_{10}O_7$) (E. Merck).

Soil analysis

Soil analysis was performed at the Laboratory of Universitas Syiah Kuala. Soil chemical and physical characteristics were analyzed using standard methods. The measured parameters included soil pH in water and KCl using a pH meter, organic carbon (C-organic) using the Walkley and Black method, total nitrogen (N-total) using the Kjeldahl method, available phosphorus (P) using the Bray II method, and cation exchange capacity (CEC) determined using ammonium acetate at pH 7 (Rauzatul & Helmi, 2022).

Extraction of *Blumea balsamifera* Leaves

Dried leaves of *Blumea balsamifera* were powdered and extracted by maceration using methanol as the solvent. Methanol was selected due to its high polarity and effectiveness in extracting polyphenolic and antioxidant compounds (Truong et al., 2019). The powdered sample was soaked in methanol for 3 days at room temperature with occasional stirring. The extract was then filtered using a glass funnel and Whatman No. 1 filter paper. The

filtrate was concentrated under reduced pressure using a rotary evaporator (Büchi Labortechnik, Germany) at 40–50 °C, followed by further drying in a water bath at 40 °C until a constant dry extract was obtained. The dried extract was stored in airtight bottles at room temperature until further analysis. The maceration procedure followed the method described by Ginting et al., (2021).

Determination of total phenolic content (TPC)

Total phenolic content of the extracts was determined using the Folin–Ciocalteu (Indriaty et al., 2023; Mwamatope et al., 2020). A total of 5 mg of extract was dissolved in methanol pa and diluted with deionized water to 5 mL. An aliquot of 0.2 mL sample solution was reacted with Folin–Ciocalteu reagent and 10% (b/v) Na_2CO_3 , followed by incubation for 120 min at room temperature. Gallic acid at various concentrations was used to construct the calibration curve. Absorbance was measured using a UV–Vis spectrophotometer (Shimadzu UVmini-1240, Kyoto, Japan) at 765 nm. Total phenolic content was expressed as mg gallic acid equivalent per g extract (mg GAE/g). All analyses were performed in triplicate.

Determination of total flavonoid content (TFC)

Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric (Phuyal et al., 2020), with quercetin as the reference standard. A total of 5 mg of extract was dissolved in methanol pa to a final volume of 5 mL, and 1 mL of the sample solution was reacted with methanol, $AlCl_3$, potassium acetate, and deionized water. Quercetin standard solutions at various concentrations were prepared to construct the calibration curve. After incubation for 30 min at room temperature, absorbance was measured using a UV–Vis spectrophotometer at 440 nm. Total flavonoid content was expressed as mg quercetin equivalent per g extract (mg QE/g). All analyses were performed in triplicate.

Antioxidant activity assay

Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method (Yahya et al., 2021). Briefly, 2.5 mg of extract was treated with two drops of 2% dimethyl sulfoxide (DMSO, Merck, Germany) and then allowed to stand for 24 h. The extract was then prepared at concentrations of 1.56, 3.125, and 6.25 μ g/mL in methanol pa and sonicated. Subsequently, 4 mL of each solution was mixed with 1 mL of DPPH solution prepared by dissolving 7.9 mg of DPPH powder (molecular weight 394.32 g/mol) in methanol pa to a final volume of 50 mL. The mixtures were homogenized and incubated in a dark incubator for 30 min at 37°C. Absorbance was measured at 517 nm using a UV–Vis spectrophotometer, with pa methanol used as the blank. A similar procedure was performed using ascorbic acid as the positive control at concentrations of 1, 3, 6, 9, 12, and 15 μ g/mL. The percentage of DPPH radical

scavenging activity was calculated to determine the IC₅₀ value, defined as the concentration required to inhibit 50% of DPPH radicals.

RESULTS AND DISCUSSION

Environmental and Soil Characteristics of Sampling Sites

Environmental parameters, including temperature, humidity, and light intensity at the two sampling locations, are presented in Table 1. PB-1, located in the coastal area at 25 m above sea level, exhibited a slightly higher temperature (35.1 °C), higher humidity, and lower light intensity (1,500 Cd) compared to AG-1, which was located in a non-coastal hilly area at 108 m above sea level with a lower temperature (33.7 °C) and higher light intensity (2,000 Cd). These differences indicate that PB-1 had a warmer and more humid environment with lower light exposure due to the presence of surrounding shrubs and canopy cover. In contrast, AG-1 had relatively cooler and more open environmental conditions, resulting in higher light intensity. In addition, PB-1 was dominated by sandy soil typical of coastal areas, whereas AG-1 was

characterized by red clay soil commonly found in hilly regions.

Table 1. Environmental Conditions of the Sampling Locations.

Location	Temperature (°C)	Humidity	Light Intensity (Cd)
PB-1	35.1	8	1,500
AG-1	33.7	4	2,000

Soil characteristics were also evaluated as important environmental factors in this study. The soil properties of both sampling locations are presented in Table 2. The analysis revealed clear differences in the physical and chemical characteristics of the soils. PB-1 was dominated by sand fraction (61%), with 27% silt and 12% clay, whereas AG-1 was dominated by silt fraction (81%), with only 6% sand and 13% clay. These findings are consistent with the geographical characteristics of each location, where PB-1 represents a coastal area characterized by sandy soil, while AG-1 represents a hilly area dominated by silt and red clay soil. These differences in soil texture indicate variations in water retention, aeration, and nutrient availability, which may influence plant growth and metabolic processes (Mohammadi et al., 2011; Vaghela & Gohel, 2023).

Table 2. Soil Nutrient Analysis.

Type of Analysis	PB-1	Criteria	AG-1	Criteria
Sand %	61	-	6	-
Silt %	27	-	81	-
Clay %	12	-	13	-
pH (H ₂ O) (1:2.5) Electrometric	4,21	Acidic	7,31	normal
Organic Carbon (Walkley & Black) %	2,80	Moderate	1,71	Low
Total N (Kjeldahl) %	0,26	Moderate	0,12	Low
P Bray II (mg/kg)	1,20	Very Low	0,75	Very Low
Exchangeable K (exch. K)	0,26	Low	0,15	Very Low
Cation Exchange Capacity (CEC)	14,40	Low	15,20	Low
Electrical Conductivity (EC) mS/cm	0,14	Moderate/Marginal	0,17	Moderate/Marginal
Moisture (%)	8	Wet	4	Dry

Total Phenolic and Flavonoid Contents of *B. Balsamifera*

The methanolic extracts obtained from the maceration of *B. balsamifera* leaves are presented in Figure 3. The extracts from both sampling locations showed differences in color and extract appearance, indicating possible variations in phytochemical composition influenced by environmental conditions.

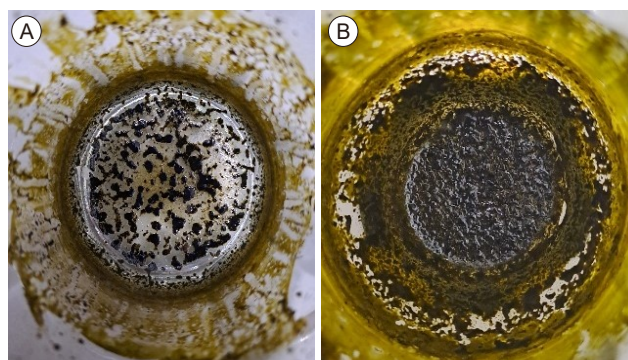


Figure 3. Methanol extract of *B. balsamifera* leaves from area A). PB-1 and B). AG-1.

Polyphenols are one of the primary secondary metabolites found in plants and are naturally synthesized compounds characterized by their phenolic structure (Singla et al., 2019). Polyphenols are broadly categorized into flavonoids and phenolic acids, both of which play significant biological roles in various physiological processes in plants (Górniak et al., 2019). In this study, TPC was determined using the Folin-Ciocalteu method with gallic acid as the standard compound, while TFC was measured using the colorimetric method with quercetin as the standard. TPC and TFC in *B. balsamifera* leaf extracts were measured using a calibration curve of gallic acid and quercetin (Table 3).

From two different sampling locations, PB-1 had a TPC of 182.7 mg GAE/g extract, while AG-1 showed a higher TPC of 237.9 mg GAE/g extract in methanol. These results indicate that TPC at location PB-1 is lower compared to AG-1 (Figure 1), but overall the phenolic value obtained is higher than the results of the study by Kusumawati et al., (2022). Likewise, the total flavonoid content showed comparable variations between the two locations. At PB-1, the flavonoid content was recorded at 21.61 mg QE/g extract, while AG-1 had a higher value of 35.89 mg QE/g extract in methanol (Figure 4). This result is lower than the flavonoid levels reported by Kusumawati et al., (2022). The differences observed can be attributed to variations in environmental and soil conditions (Table 2). Soil plays an important role in plant growth and secondary metabolite synthesis by functioning as the main medium for absorbing essential nutrients, which in turn affects the phytochemical profile responsible for the production of bioactive compounds

(Nazari et al., 2023; Vaghela & Gohel, 2022). Soil nutrients come from decomposed organic matter, minerals, and water-soluble chemical compounds, all of which contribute to plant development (Du et al., 2025; K. Mohammadi et al., 2011). The differences in phytochemical content of TPC and TFC were reinforced by environmental factors, based on soil analysis.

Based on soil analysis, it was found that the PB-1 location had a more acidic pH compared to AG-1. The higher acidity at PB-1 is attributed to the coastal characteristics of the area, where soils often form from deposits of mud or sand rich in sulfide compounds (VanZomeren et al., 2020). When these soils are exposed to oxygen, oxidation of sulfides occurs, producing sulfuric acid and consequently increasing soil acidity (Starkey, 1966). This acidic soil condition can influence the production of secondary metabolites in plants. Zargoosh et al. (2019) demonstrated that acidic soil pH can reduce phenolic content in *Scrophularia striata*. This finding highlights the significant role of soil pH in regulating plant secondary metabolism, including the production of polyphenols in *B. balsamifera*.

Table 3. Calibration curve and R² values for Gallic Acid and Quercetin standards.

Standard	Regression Equation	R ²
Gallic Acid	$y = 0,134x - 0,0489$	0,9306
Quercetin	$y = 0,1519x + 0,2317$	0,9616

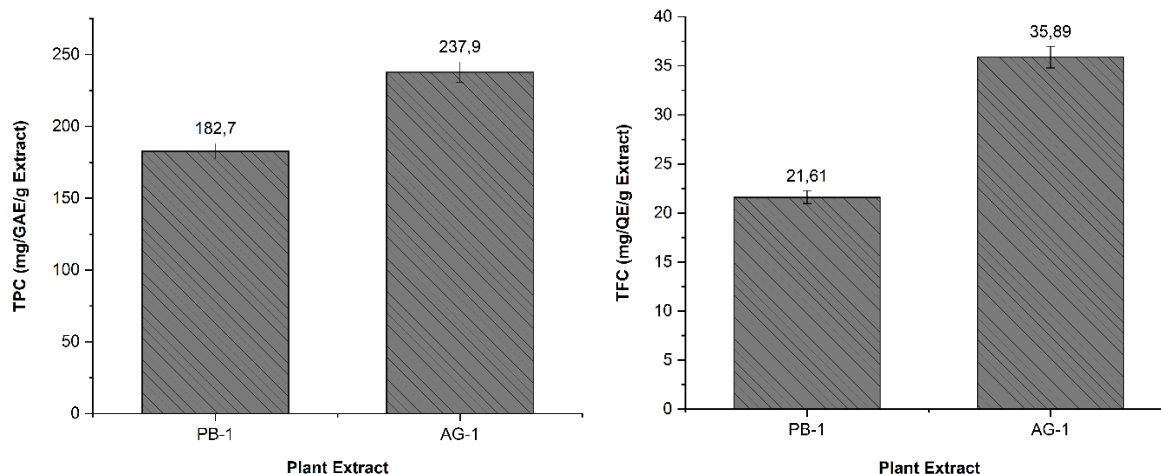


Figure 4. TPC & TFC content of *B. balsamifera* from location PB-1 (25 m asl) and location AG-1 (108 m asl).

Based on the results, the total phenolic content (TPC) and total flavonoid content (TFC) of *B. balsamifera* were higher in the non-coastal area (AG-1) than in the coastal area (PB-1). The TPC value in AG-1 reached 237.9 mg GAE/g extract, whereas PB-1 exhibited a lower value of

182.7 mg GAE/g extract. Similarly, the TFC value in AG-1 was 35.89 mg QE/g extract, compared to 21.61 mg QE/g extract in PB-1. Overall, the phenolic content obtained in this study was higher than that reported by Kusumawati et al., (2022), whereas the flavonoid content

was relatively lower. These variations were likely associated with differences in environmental conditions and habitat characteristics.

The higher phenolic and flavonoid contents observed in AG-1 may be attributed to environmental conditions that are more favorable for secondary metabolite biosynthesis, particularly higher light intensity and near-neutral soil pH. High light intensity has been reported to enhance the activity of phenylalanine ammonia-lyase (PAL), a key enzyme in the phenylpropanoid pathway involved in the biosynthesis of phenolic and flavonoid compounds (Lee et al., 2022; Yang et al., 2022). In addition, near-neutral soil pH may improve nutrient availability and support plant metabolic processes, thereby indirectly promoting the accumulation of bioactive compounds (Zargoosh et al., 2019). Soil analysis revealed that PB-1 had more acidic soil conditions than AG-1. Acidic soils are commonly associated with coastal environments and may affect plant metabolic processes as well as secondary metabolite biosynthesis (Anwar et al., 2024). Zargoosh et al., (2019) reported that acidic soil conditions reduced

phenolic content in *Scrophularia striata*, which is consistent with the findings of the present study. Therefore, environmental factors such as light intensity and soil pH play important roles in determining the bioactive compound content of *B. balsamifera*. Higher light intensity tends to enhance the biosynthesis of phenolic and flavonoid compounds (Wang et al., 2022), whereas acidic soil conditions may inhibit their accumulation (Chen et al., 2020).

Antioxidant activity

Antioxidant activity is an important parameter in determining the biological potential of plant extracts, particularly in inhibiting free radicals that may cause cellular damage (Nwozo et al., 2023). In this study, the antioxidant activity of *B. balsamifera* leaf extracts was evaluated using the DPPH method to assess the ability of active compounds to scavenge free radicals. Figure 5 presents the percentage of DPPH radical inhibition as well as the IC_{50} values of extracts obtained from different growing locations, with ascorbic acid used as the positive control.

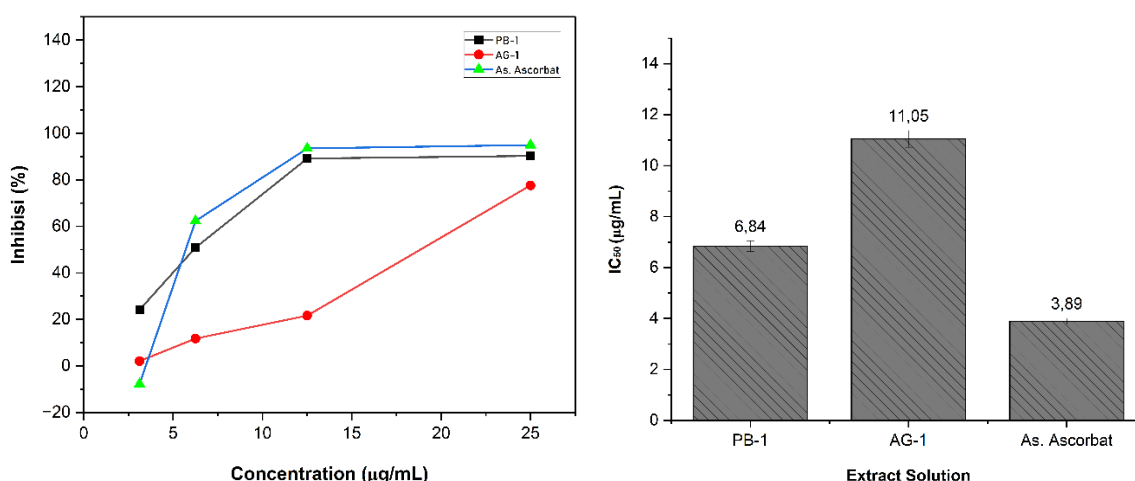


Figure 5. Inhibition of 2,2-diphenyl-1-picrylhydrazyl (a) and median inhibitory concentration (b) of *B. balsamifera* leaf extracts from the PB-1 (25 m asl) and AG-1 (108 m asl) locations.

The results showed that *B. balsamifera* extracts from the coastal area (PB-1) exhibited stronger antioxidant activity than those from the non-coastal area (AG-1). This was indicated by the lower IC_{50} value of PB-1, which reached 6.839 µg/mL, classifying it as a very strong antioxidant since antioxidant activity is considered very strong when the IC_{50} value is below 50 µg/mL. In general, phenolic and flavonoid compounds are closely associated with antioxidant activity due to the presence of hydroxyl (-OH) groups capable of donating electrons or hydrogen atoms to neutralize free radicals (Górniak et al., 2019; Rice-Evans et al., 1997). However, in the present study, the TPC and TFC values of PB-1 were lower than those of AG-1, whereas its antioxidant activity was comparatively higher.

These findings indicate that antioxidant activity is influenced not only by the total phenolic and flavonoid contents, but also by the types, structures, and composition of bioactive compounds present in the extracts (Mohammadi et al., 2024). Certain phenolic compounds may exhibit stronger antioxidant capacities than others despite being present in lower amounts (Gulcin, 2025; Lisjak et al., 2026). In addition, the more extreme environmental conditions in the coastal area, such as higher temperature, more acidic soil, and greater environmental stress, may induce oxidative stress in plants. Such conditions can stimulate the synthesis of specific antioxidant compounds as a defense mechanism against reactive oxygen species (ROS) (Alam et al., 2025; Hasanuzzaman et al., 2020).

Soil analysis revealed that PB-1 had more acidic soil conditions than AG-1. Acidic soil conditions are known to influence plant metabolism and secondary metabolite biosynthesis (Chaouqi et al., 2023). Previous studies have reported that environments with higher abiotic stress may enhance antioxidant activity in several plant species, even when the total phenolic content is not necessarily higher (Šamec et al., 2021; Zargoosh et al., 2019). Therefore, the stronger antioxidant activity observed in PB-1 is likely associated with the adaptive response of plants to coastal environmental stress, which may stimulate the production of antioxidant compounds with higher biological activity (Alam et al., 2025).

The findings of this study indicate that antioxidant activity does not always increase proportionally with higher phenolic and flavonoid contents. Antioxidant activity is also influenced by the types of bioactive compounds produced as well as the environmental conditions in which the plants grow (Mohammadi et al., 2024). Differences in habitat may affect secondary metabolite accumulation and antioxidant activity in *B. balsamifera*. Non-coastal environments with higher light intensity and near-neutral soil pH were more favorable for the accumulation of phenolic and flavonoid compounds. In contrast, the more stressful coastal environment, characterized by higher temperature and more acidic soil conditions, tended to enhance the antioxidant activity of the plants (Šamec et al., 2021). These findings further suggest that cultivation sites may be selected according to the intended utilization of the plant. Cultivation in non-coastal areas may promote higher phenolic and flavonoid contents, whereas cultivation in coastal areas may produce plants with stronger antioxidant activity. Therefore, environmental management, particularly light intensity and soil conditions, plays an important role in optimizing the bioactive quality of *B. balsamifera* for pharmaceutical and natural healthcare applications.

CONCLUSIONS

The present study demonstrated that differences in habitat influenced the phytochemical content and antioxidant activity of *Blumea balsamifera*. Leaves collected from the non-coastal area (AG-1) showed higher total phenolic and flavonoid contents, whereas extracts from the coastal area (PB-1) exhibited stronger antioxidant activity with a lower IC₅₀ value. These differences were likely associated with variations in environmental factors, particularly light intensity and soil pH. The findings indicate that antioxidant activity was not solely dependent on total phenolic and flavonoid contents, but was also influenced by environmental conditions and the composition of bioactive compounds. Overall, this study highlights the important role of habitat conditions in determining the bioactive potential of *B.*

balsamifera and provides useful information for optimizing its cultivation and pharmaceutical utilization.

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Authors' Contributions: Indriaty designed the study, analyzed the data, and prepared the manuscript. Guswarni pranata conducted field sampling, contributed to laboratory analysis and data interpretation, and participated in manuscript writing. Ekariana S. Pandia contributed to methodology development and manuscript revision. Hasbi asidiqi nasution assisted with data analysis and the preparation of maps and graphical designs. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare no conflicts of interest related to this study.

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