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Phytochemical Profile and Antioxidant Activity of Bandotan (*Ageratum conyzoides* L.) Leaves from East Kalimantan

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Abstract

Ageratum conyzoides L. is a wild plant known to contain secondary metabolites with potential antioxidant activity, such as flavonoids and phenolics. However, the content and activity of active compounds in the leaves of A. conyzoides growing in the Berau region, East Kalimantan, have not been extensively studied. This study aims to evaluate the phytochemical content and antioxidant activity of ethanol extracts from A. conyzoides leaves collected from the Batu Putih area, Berau. The samples, in the form of fresh leaves, were dried and ground before being extracted using the maceration method with 96% ethanol as the solvent. The resulting extract was then subjected to phytochemical screening to identify groups of active compounds and tested for antioxidant activity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, with absorbance measured at a wavelength of 420 nm. Phytochemical screening results indicated the presence of alkaloids, phenolics, and tannins, while flavonoids, terpenoids, and triterpenoids were not detected. The antioxidant assay showed that the extract exhibited strong activity, with an IC50 value of 61.89 ppm, although it was still higher than that of vitamin C, which had an IC50 value of 26.77 ppm. In conclusion, the ethanol extract of A. conyzoides leaves from Batu Putih has potential as a natural antioxidant source and is worthy of further development in the pharmaceutical field.

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INTRODUCTION

Berau is one of the regions in East Kalimantan that has tropical environmental characteristics with high rainfall and humidity, conditions that support the growth of medicinal plants. The Batu Putih area in Berau is known for its relatively fertile soil, rich in organic matter, which can enhance the availability of nutrients for plants. Such soil fertility has been shown to influence the biosynthesis and accumulation of secondary metabolites in medicinal plants for example, the addition of organic matter and improved soil fertility can increase phenolic and flavonoid contents, thereby strengthening the antioxidant properties of tropical medicinal plants (Pant et al., 2021). In bandotan (*Ageratum conyzoides* L.), environmental factors such as soil characteristics are known to play a role in the biosynthesis of secondary metabolites, so plants growing in Batu Putih are estimated to have higher levels of bioactive compounds compared to those from less fertile regions (Dewi et al., 2021).

Ageratum conyzoides L., commonly known as bandotan, is a wild plant widely found in Indonesia that can grow well in plantation areas as well as open land, and is often regarded as a weed (Harefa et al., 2022). Traditionally, this plant has been used by local communities to treat various ailments. Bandotan is known to possess several pharmacological activities,

including antidiabetic, anti-inflammatory, antioxidant, analgesic, anxiolytic, and antibacterial properties (Melissa & Muchtaridi, 2017). Phytochemical tests on the leaves, stems, and roots of this plant have revealed the presence of alkaloids, flavonoids, tannins, saponins, triterpenoids, steroids, and phenolic compounds. In vitro studies have shown that methanol and ethanol extracts of *A. conyzoides* exhibit high free radical scavenging (DPPH) activity, which correlates with significant total phenolic and flavonoid contents (Adetuyi et al., 2018).

Free radicals are highly reactive molecules because they contain unpaired electrons, making them unstable and prone to attacking surrounding molecules to achieve stability (Syahrudin et al., 2022). These attacks can trigger oxidation reactions, especially when involving non-ionic free radicals, which can ultimately damage cellular structures and biological tissues, leading to various degenerative diseases and even cancer (Verawaty, 2018). Antioxidants play an important role in neutralizing free radicals and preventing oxidative damage; however, the effectiveness of natural antioxidants, especially those derived from medicinal plants, may vary depending on environmental and biochemical factors of the plant (Rusli et al., 2022). This study offers a novel contribution by exploring the antioxidant activity of *Ageratum conyzoides* L. leaf extract specifically collected from the Batu Putih area in Berau, East Kalimantan, an understudied region with a unique tropical climate and fertile soil conditions that may influence the biosynthesis of secondary metabolites. To date, scientific studies on the phytochemical profile and antioxidant potential of *A. conyzoides* from this specific ecological zone remain limited. By integrating the local environmental context with antioxidant activity evaluation using the DPPH method, this research provides new insights into the potential of *A. conyzoides* as a distinctive natural antioxidant source from a specific region.

METHOD

Tools and Materials

The tools used includes a stirring rod, blender, suction bulb, petri dish, ring clamp, filter funnel, 50 mL beaker, 100 mL beaker, 1000 mL beaker, hot plate, watch glass, cuvette, 10 mL volumetric flask, 25 mL volumetric flask, 100 mL volumetric flask, analytical balance, dropper pipette, 5 mL graduated pipette, 25 mL graduated pipette, test tube rack, rotary evaporator, spatula, UV-Vis Single Beam Spectrophotometer (1000 nm), stand & clamp, test tubes. The materials used include aluminum foil, distilled water, dried bandotan leaves (*Ageratum conyzoides* L.), DPPH (2,2-diphenyl-1-picrylhydrazyl), 70% ethanol, 96% ethanol, 5% $FeCl_3$, 2% HCl, concentrated HCl, H_2SO_4, filter paper, Dragendorff reagent, Liebermann-Burchard reagent, magnesium solid, and vitamin C (Annisa et al., 2022).

Sample Preparation

Bandotan leaves were collected in Berau, East Kalimantan. Then, wet sorted using running water, then dried by airing, not exposed to direct sunlight. Refined using a blender, sifted bandotan leaves powder. Extracted the sample using ethanol solvent for 3×24 hours. Filtered with filter paper and evaporated the solvent using a vacuum rotary evaporator until a thick extract was obtained. Stored the thick extract obtained from the process in a desiccator with a temperature of approximately 4ºC (Sari et al., 2024)

Phytochemical Test

Phytochemical screening includes examination of alkaloid, terpenoid, triterpenoid, flavonoid, phenolic, and tannin compound groups. First alkaloid examination, 2 mL of ethanol extract of bandotan leaves was put into a test tube, and 2 mL of 2% HCl was then heated for 5 minutes and filtered. The filtrate obtained is dripped with Dragendorff reagent as many as 3 drops. The presence of alkaloids can be seen by the appearance of an orange precipitate. The second

examination of terpenoids, ethanol extract of bandotan leaves, as much as 2 mL, coupled with Liebermann-Burchard reagent, as much as 1 mL. Terpenoids are considered positive if a purple color is formed (Mengkido et al., 2019). The third examination of triterpenoids, taking as much as 1 mL of ethanol extract of bandotan leaves and putting it into a test tube. After that, it is added with 3 drops of Liebermann-Burchard reagent; if a brownish ring color is formed, it means that the ethanol extract of bandotan leaves is positive for triterpenoids (Putri et al., 2022). Fourth, flavonoid examination, flavonoid testing was carried out by Wilstatter test, namely, ethanol extract of bandotan leaves was pipetted as much as 2 mL and put into a test tube. The extract was added 0.1 gram of magnesium powder and 3 drops of concentrated HCl, then the mixture was shaken until homogeneous. The orange-yellow color formed indicates the presence of flavonoids (Arifin et al., 2019). Fifth Phenolic examination, taking as much as 1 mL of ethanol extract of bandotan leaves and putting it into a test tube, and then adding $FeCl_3$ 5%, as many as 3 drops. Phenol compounds are indicated if there is a blue-black color change (Arifin et al., 2019). In the sixth examination of tannin, as much as 2 mL of concentrated extract was put into a test tube and then dissolved with ethanol. Added 1 mL $FeC1_3$ 5%, positive results are indicated by colors such as bluish black or green, then the ethanol extract of bandotan leaves (*Ageratum conyzoides* L.) in the study contains tannins (Putri et al., 2022).

Antioxidant Test with DPPH method

DPPH solution was made by weighing 15.77 mg of DPPH, then dissolved with 96% ethanol and vortexed until dissolved, after which the DPPH solution was diluted to 1 mL, and added ethanol was added to 10 mL and then let stand for 30 minutes. Next is the determination of the maximum absorption wavelength of DPPH, where the solution is taken using a pipette as much as 0.7 mL, then ethanol 96% until the limit mark, left for 30 minutes in a dark place, and the absorption is measured at a wavelength of 420 nm. Furthermore, the examination of the antioxidant concentration of ethanol extract of bandotan leaves, where the test solution of bandotan leaf extract with various concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm was allowed to stand for 30 minutes and then read the maximum wavelength of 420 nm with a comparison solution of vitamin C concentration of 1.25 ppm, 2.5 ppm, 5 ppm 10 ppm and 20 ppm with the same treatment. The results of the reading then made the curve equation $y = ax + b$.

RESULTS AND DISCUSSION

Extraction serves as a technique for separating active compounds soluble in a specific solvent from simplicial materials (Purwanti, 2022). The primary objective of this process is to isolate chemical components or secondary metabolites contained within the sample. Its principle relies on the solvent's capacity to dissolve target compounds from the source material (Asworo & Widwiastuti, 2023). In this study, bandotan leaf extraction was performed using the maceration method with ethanol as the solvent. This method was selected due to its straightforward procedure, minimal equipment requirements, suitability for heat-sensitive compounds, and ability to yield high extract volumes through repeated solvent extraction (Hidayati & Harjono, 2017). For maceration, 143 grams of powdered bandotan leaves were weighed and soaked in ethanol for 3×24 hours. Following immersion, the mixture was filtered, and the solvent was evaporated to obtain a thick extract. The ethanol-macerated extract exhibited a dark green color. Subsequently, the filtrate was evaporated using a rotary evaporator below the solvent's boiling point to remove residual ethanol. This evaporation was conducted at 50°C to prevent degradation of anthocyanin compounds, which occurs above 60°C. The extraction results of Bandotan leaves are presented in Table 1.

Table 1. Bandotan leaves extract yield

Solvents	Weight of Powder	Weight of Extract	Shape	Color	Smell
Ethanol	143,522 grams	289,7 grams	Liquid	Dark green	Distinctive

The extraction process using ethanol as a solvent for 143.522 grams of dried *Ageratum conyzoides* L. leaf powder yielded 289.7 grams of extract, indicating high solvent efficiency. Ethanol is well recognized for its ability to dissolve polar to semi-polar compounds such as flavonoids, phenolics, and tannins, which are commonly found in leaf simplicia. The dark green color of the extract suggests the presence of chlorophyll and conjugated phenolic compounds, both of which are known to exhibit strong antioxidant potential (Mokrani & Madani, 2016). The characteristic odor detected in the extract further indicates the presence of ethanol-soluble volatile aromatic compounds, including light terpenoids and esters (Salehi et al., 2022). In the context of natural product extraction, ethanol is also regarded as a safe, non-toxic, and food-grade solvent, suitable for pharmaceutical and nutraceutical applications (Azwanida, 2015).

The relatively high yield also reflects the selectivity and efficiency of ethanol in extracting target bioactive compounds. These findings are in agreement with the report by Nile et al. (2019), which demonstrated that ethanol extraction of *Euphorbia hirta* leaves resulted in high levels of phenolic and flavonoid contents along with significant biological activity. On the other hand, it should be noted that an extract yield exceeding the initial powder weight may indicate residual solvent or water that has not been completely removed during evaporation. Therefore, further drying is recommended to obtain a concentrated extract that is truly solvent-free.

Table 2. Phytochemical test results of Bandotan leaves extracts

Chemical compounds	Reagents	Results	Description
Alkaloid	HCl + Dragendorff	+ (orange precipitate)	(+) If an orange precipitate formed
Terpenoid	Liebermann-Burchard	- (translucent green)	(+) If purple color is formed
Triterpenoid	Liebermann-Burchard	- (translucent green)	(+) If a brown ring is formed
Flavonoid	Mg + HCl	- (translucent green)	(+) If an orange-yellow color is formed
Phenolic	$FeCl_3$	+ (bluish-black)	(+) If a bluish-black color is formed
Tannin	$FeCl_3$	+ (greenish-black)	(+) If a bluish-black or greenish-black color is formed

Phytochemical screening of *Ageratum conyzoides* L. leaves extract (collected from Batu Putih, Berau Regency) obtained via ethanol maceration revealed significant alkaloid, phenolic, and tannin content. For alkaloid detection, Dragendorff's reagent was added, producing an orange precipitate that indicates interaction between alkaloids and tetraiodobismuthate (III) ions (Sulistyarini et al., 2019). Terpenoid/triterpenoid tests using Liebermann-Burchard reagent showed no color change, suggesting undetectably low concentrations potentially attributable to regional phytochemical variation or extraction efficacy limitations (Jungjunan et al., 2023; Cahyani & Mita, 2018).

Flavonoid analysis yielded no positive reaction, consistent with reported low concentrations (0.22% b/v in macerated extracts) compared to Soxhlet extraction (0.32% b/v) (Wardhani et al., 2023). According to Kumar and Pandey (2013), flavonoid content in plants is strongly influenced by environmental factors such as soil type, light intensity, rainfall, and abiotic stress. *Ageratum conyzoides* plants growing in the Batu Putih region, Berau Regency, have been reported to contain low levels of flavonoids, as supported by previous studies showing that the total flavonoid content in ethanol extracts obtained through maceration reached only 0.22% b/v. This concentration is below the detection limit of the Wilstätter method, which is qualitative in nature. In addition, the chemical stability of flavonoids also affects detection results. Flavonoids are known to be labile compounds that readily degrade when exposed to high temperatures, light, oxygen, or pH fluctuations during extraction and storage.

If the extraction process is not carried out under controlled conditions, the chemical structure of flavonoids may degrade, making them unrecognizable in qualitative testing. In this study, extraction was performed using the maceration method with ethanol as the solvent, which, although commonly used for phenolic compound extraction, has been shown to have lower efficiency compared to other methods. Do et al. (2014) demonstrated that Soxhlet or ultrasonic extraction techniques are capable of yielding higher flavonoid content. Therefore, the undetectable flavonoid levels observed in this study are attributable to a combination of factors, including low flavonoid concentrations in the sample due to environmental conditions, compound degradation during extraction, and the limitations of both the maceration method and the Wilstätter test in detecting flavonoids at low concentrations or in degraded forms.

Conversely, tannin detection with 1% $FeCl_3$ produced a greenish-black color, confirming iron-hydroxyl group interactions characteristic of polar tannin (Purwanti, 2022). Phenolic compounds were identified through bluish-black complex formation with $FeCl_3$, indicating substantial phenolic content correlated with antioxidant activity (Syamsudin et al., 2022). The selective extraction profile demonstrates ethanol's compliance with the 'like dissolves like' principle, where semi-polar solvent affinity facilitates dissolution of alkaloid, phenolic, and tannin (Purwanti, 2022)."

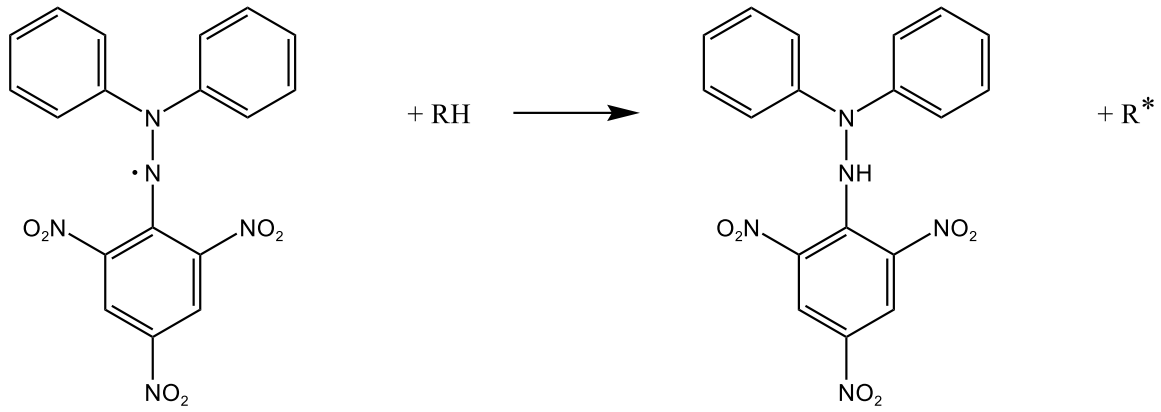

Figure 1. The reaction between DPPH and the hydrogen atom from the antioxidant compound

Compared to other approaches, the DPPH method offered several advantages. This method was straightforward, easy to use, and required only a small quantity of sample and testing reagent. DPPH (1,1-diphenyl-2-picrylhydrazyl) was a purple synthetic free radical with an unpaired nitrogen atom. The basic principle of antioxidant testing using the DPPH method was based on a chemical reaction between antioxidant compounds and DPPH free radicals through a hydrogen atom transfer mechanism or by donating an electron or hydrogen atom. This reaction caused a color change in the solution, typically from dark purple to pale purple or

yellow, indicating a reduction in radical activity. As a result, the absorbance of the sample decreased, which was measured spectrophotometrically (Ngibad & Lestari, 2020).

Table 3. Antioxidant Activity Test Results of Bandotan Leaves Extract

Replication	Concentration (ppm)	Absorbance	Average Absorbance	% Antioxidant Activity	Linear Equation	IC_{50} (ppm)
1		0,399				
2	10	0,378	0,404	67,37		
3		0,437				
1		0,446				
2	20	0.474	0,470	58,57		
3		0,490				
1		0,490			y = -02709x+ 66,767	61,89
2	30	0,5006	0,498	56,27		
3		0,502				
1		0,506				
2	40	0,5013	0,503	55,76		
3		0,502				
1		0,502				
2	50	0,503	0,504	55,23		
3		0,503				

The antioxidant activity test was conducted on the ethanol extract of bandotan leaves (*Ageratum conyzoides* L.) using a DPPH solution, with each concentration tested three times to obtain optimal results from the sample. The concentrations varied at 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm, and were measured using a UV-Vis spectrophotometer at a wavelength of 420 nm. The resulting percentages of antioxidant activity were 67.37%, 58.57%, 56.27%, 55.76%, and 55.23%, respectively. The following curve shows the relationship between the concentration of the ethanol extract of Bandotan leaves and the percentage of antioxidant activity.

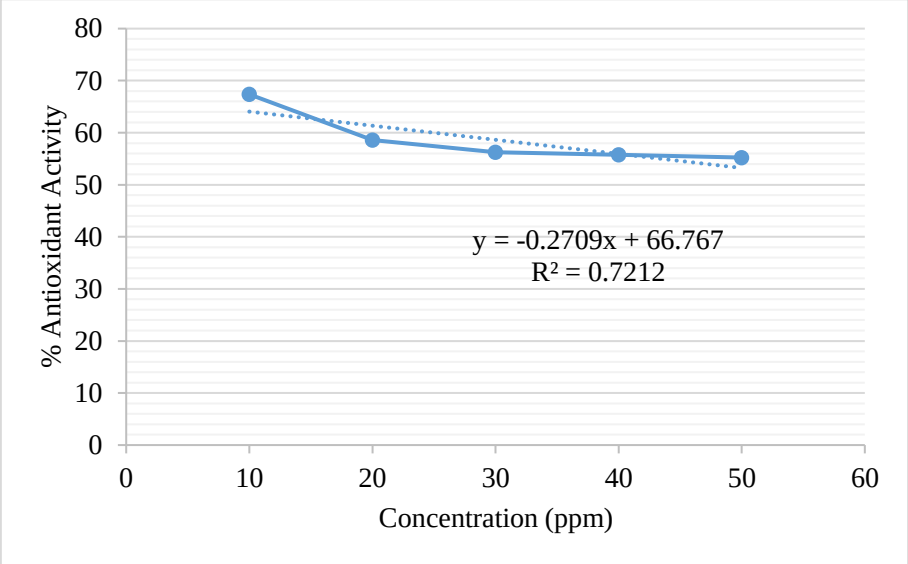

Figure 2. Linear regression curve of the antioxidant activity of bandotan leaf extract

Based on Figure 2, the linear regression equation obtained is y = -0.2709x + 66.767 with a correlation coefficient of $R^2 = 0.7212$. The IC_{50} value was determined by substituting y = 50 into the regression equation, resulting in an IC_{50} value of 61.89 ppm for the ethanol extract of bandotan leaves.

Table 4. Antioxidant Test Results of Vitamin C

Replication	Concentration (ppm)	Absorbance	Average Absorbance	% Antioxidant Activity	Linear Equation	IC_{50} (ppm)
1		0,581				
2	10	0,581	0,580	48,98		
3		0,579				
1		0,574				
2	20	0,573	0,573	49,60		
3		0,573				
1		0,566			y =	
2	30	0,565	0,566	50,21	0,0608x +	26,77
3		0,567			48,372	
1		0,560				
2	40	0,561	0,560	50,74		
3		0,559				
1		0,551				
2	50	0,553	0,552	51,45		
3		0,554				

In the determination of the antioxidant content of bandotan leaf extract, a vitamin C solution was used as the standard. The standard solution was used to compare the antioxidant activity between vitamin C and the bandotan leaf extract. Using the same concentration variations as those used for the ethanol extract of bandotan leaves, the antioxidant activity percentages of vitamin C were 48.98%, 49.60%, 50.21%, 50.74%, and 51.45%. The regression equation obtained from the linearity test using Microsoft Excel is y = 0.0608x + 48.372, with a correlation coefficient of $R^2 = 0.9984$. The IC_{50} value was determined by substituting y = 50 into the regression equation. Based on the calculation, the IC_{50} value of vitamin C was found to be 26.77 ppm. The following is the linear regression curve of the antioxidant activity of vitamin C.

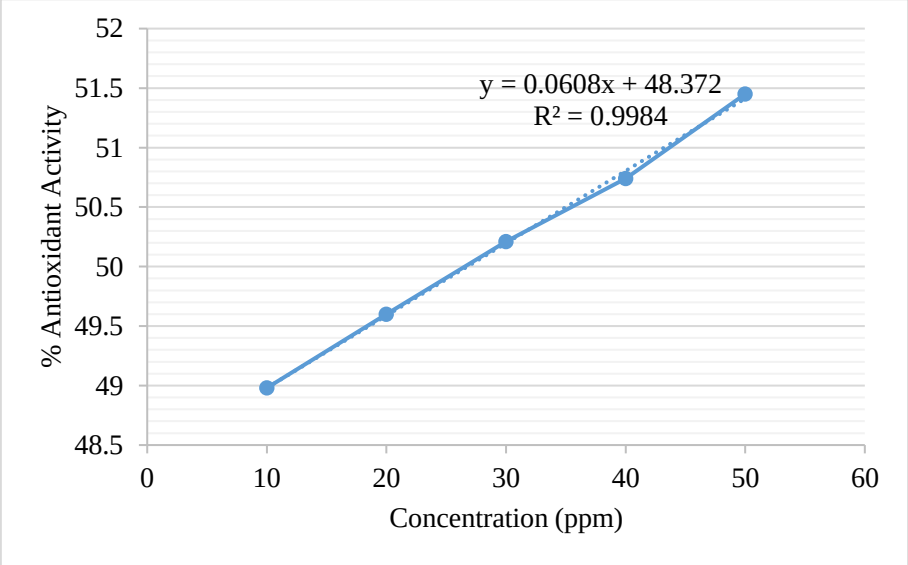

Figure 3. Linear regression curve of the antioxidant activity of vitamin C

Based on the results of antioxidant activity testing using the DPPH method, the ethanol extract of *Ageratum conyzoides* L. leaves exhibited strong antioxidant activity, with an IC_{50} value of 61.89 ppm. According to commonly used criteria for determining antioxidant strength, a compound is considered to have very strong antioxidant activity if the IC_{50} value is less than

50 ppm, strong if the IC_{50} value ranges from 50 to 100 ppm, moderate if between 101 and 150 ppm, and weak if it falls within 151 to 200 ppm (Sumarni et al., 2019). The highest activity was observed at a concentration of 10 ppm, with an inhibition percentage of 67.37%, but this value decreased to 55.23% at 50 ppm. The decline in effectiveness at higher concentrations can be explained by two mechanisms previously reported in the literature. First, the agglomeration of phenolic compounds at high concentrations may reduce the reactive surface area, thereby limiting the interaction between active compounds and free radicals. Second, competitive reactions among bioactive compounds in the complex extract matrix can lower radical-scavenging efficiency due to inhibitory or competitive intermolecular interactions. According to Blois' classification, the IC_{50} value obtained categorizes the extract as having strong antioxidant activity (IC_{50} between 50–100 ppm) (Huang & Prior, 2016).

As a comparison, pure vitamin C exhibited higher antioxidant activity than the ethanol extract of bandotan leaves, with an IC_{50} value of 26.77 ppm, while the extract showed an IC_{50} of 61.89 ppm. This difference can be explained by the fact that vitamin C is a single compound with a simple structure and high purity, allowing it to scavenge free radicals more efficiently. In contrast, the bandotan leaf extract contains a mixture of various secondary metabolites, such as phenolics, tannins, and alkaloids, which may interact with one another and influence their overall radical-scavenging effectiveness. Additionally, the presence of other non-antioxidant compounds in the extract may inhibit the activity of the active components (Melinda et al., 2024). Therefore, although the extract has a higher IC_{50} value, the result still indicates that it possesses potential as a natural antioxidant (Hasanuddin et al., 2023).

Thus, the results of this study strengthen the evidence that the ethanol extract of bandotan leaves possesses considerable potential as a natural antioxidant agent. Although its activity is still lower than that of vitamin C, its potential for development in phytopharmaceutical formulations or herbal-based health products remains promising, especially considering the plant's abundant availability and the relatively simple extraction process (Ghasemzadeh et al., 2018).

CONCLUSION

Based on the findings of this study, the ethanol extract of *Ageratum conyzoides* L. leaves collected from the Batu Putih region, Berau Regency, demonstrated strong antioxidant activity with an IC_{50} value of 61.89 ppm, despite the absence of flavonoid detection through the Wilstatter test. This result highlights the predominant role of phenolic compounds and tannins in contributing to the extract’s antioxidant activity. The specific environmental conditions of the plant's growing site were shown to influence its phytochemical profile, as evidenced by the contrast between the present findings and previous studies that reported higher flavonoid content in samples from other regions. Furthermore, the reduced antioxidant efficacy at higher concentrations is understood to result from compound agglomeration and competitive interactions among active molecules within the complex extract matrix. Therefore, this study offers a scientific contribution to the understanding of how local environmental factors shape the bioactivity potential of medicinal plants, as well as the underlying mechanisms of antioxidant compound interactions.

RECOMMENDATIONS

Based on the research results, it is recommended to optimize the extraction method, such as using Soxhlet or ultrasonic techniques, to enhance the content of active compounds, particularly flavonoids that were not detected in the previous extraction. Further analysis using

instruments such as TLC or HPLC is also necessary to specifically identify the active compounds. In addition, it is important to conduct toxicity tests, extract stability tests, and other biological activity assays such as antibacterial or anti-inflammatory tests. To support its development as a phytopharmaceutical, further in vivo studies or preclinical trials are highly recommended.

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