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ANTIOXIDANT ACTIVITY TEST AND ANALYSIS OF COMPOUNDS CONTAINED IN ANGELS'S TRUMPET FLOWER EXTRACT (*Brugmansia suaveolens*)

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Abstract

Indonesia is a highly biodiverse country, second only to Brazil. This gives Indonesia enormous potential in ethnopharmacology. Previous studies have shown that the leaves of the Brugmansia suaveolens plant, also known as Angel trumpet, have high antioxidant activity. The aim of this study is to test the antioxidant activity of B. suaveolens flowers and conduct quantitative screening to determine which compounds play the greatest role in their antioxidant activity. This research used the DPPH test and Gas Chromatography-Mass Spectrometry (GC-MS) screening. Ascorbic acid was used as the comparator in this study. Before the DPPH and GC-MS testing, the samples were extracted using the cold maceration technique with a 95% ethanol solvent. Then, the samples were concentrated using a rotary evaporator. The IC_{50} value of the ethanol extract of B. suaveolens flowers is 196.16 µg/mL, while the IC_{50} value of ascorbic acid, the comparator, is 3,814 µg/mL. This result shows that the ethanol extract of B. suaveolens flowers has moderate antioxidant activity. Screening bioactive compounds with GC-MS showed that Hexadecenoic acid and Octadecanoic acid play the biggest role in antioxidant activity. However, the compound with the highest content in the ethanol extract of B. suaveolens flowers is scopolamine.

Keywords: Brugmansia suaveolens, Antioxidant, DPPH, GC-MS.

INTRODUCTION

The Solanaceae family is widely distributed and includes several economically important species. The family contains 2,700 species divided into 98 genera (Costa et al., 2022). These plants grow worldwide, particularly in tropical and subtropical regions. Some species can also be used as ornamental plants (Petricevich et al., 2020). According to several articles, these plants are toxic and often cause poisoning in some countries (Petricevich et al., 2020; Tailwal et al., 2023; da Costa et al., 2023). The most common cause of poisoning is the accidental consumption of these plants (Jayawickreme et al., 2019). However, it is also commonly used in traditional medicine in some Latin American countries to treat toothaches, inflammation, wounds, general pain, abscesses, dermatitis, fungal infections, diarrhea,

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coughs, and eye pain (Jayawickreme et al., 2019). Previous studies have also found that this plant contains compounds that act as antioxidants. The primary chemical compounds in plants that function as antioxidants are polyphenols (complex forms of phenolic compounds, which are the main group of antioxidants), alkaloids, scopolamine, glycosides, and flavonoids (Nathania et al., 2020).

Antioxidants are compounds that bind to free radicals and stop oxidative reactions. Free radicals are molecules that can destroy proteins, carbohydrates, fats, and cellular macromolecules, such as DNA. They can also cause other cells to lose their electrons. This occurs because these molecules have one or more unpaired electrons in their outer orbit (Yuri et al., 2023). Indonesia is a highly biodiverse country, ranking second only to Brazil. This makes Indonesia a significant player in the field of ethnopharmacology, meeting both local needs and export demands. Plants with antioxidant activity are promising in preventing and treating chronic diseases associated with oxidative stress. This has led to increased interest in local antioxidant-rich plants, making them a focus of research and development in Indonesia (Harahap & Priawan, 2024). Plants containing phytochemical compounds offer benefits such as antioxidant, antimicrobial, and antipyretic activities. Forest belladonna (B. suaveolens) contains tropane alkaloids (scopolamine and atropine) and various active compounds, such as flavonoids, phenolic compounds, and triterpenoids. This makes forest belladonna one of the plants with potential for use as a free radical scavenger and an effective therapeutic agent (da Costa et al., 2023).

Forest tuber plants contain compounds that can be analyzed using gas chromatography-mass spectrometry (GC-MS). This technique is highly sensitive and detailed, making it suitable for identifying and measuring various complex compounds present in a sample. Ethanol is considered safer and more environmentally friendly than other solvents and is widely used for extraction because the bioactive and organic compounds in plants dissolve in ethanol (Harahap & Priawan, 2024). Previous research on B. suaveolens has focused solely on measuring the IC50 of ethanol leaf extracts using the DPPH method, despite the possibility that the flower parts contain higher levels of antioxidant compounds (Costa et al., 2022). A similar study by Nathania et al. (2020) focused on measuring the IC50 of ethanol extracts from B. suaveolens leaves in Tomohon and qualitatively identifying biochemical compounds. This study is the first to measure the IC_{50} using the DPPH method with B. suaveolens flower ethanol extracts and perform quantitative identification of biochemical compounds with GC-MS. GC-MS identifies the presence of compounds and can describe approximately 120 compounds, determining the relative percentage of each compound in the B. suaveolens flower extract (Jayawickreme et al., 2019).

Data obtained from IC50 testing and the quantitative identification of biochemical compounds in the ethanol extracts of B. suaveolens flowers from Tomohon can be used to compare their profile to that of B. suaveolens species from other regions. This is also the first study to identify the most influential markers or compounds responsible for the flowers' antioxidant effects. B. suaveolens leaves exhibit significantly stronger antioxidant activity than leaves from other Indonesian plants (Nadia et al., 2024). According to Nathania et al. (2020), the ethanol extract of B. suaveolens leaves has strong antioxidant activity, with an IC_{50} value of 56,640.3 ppm. Through qualitative testing of phytochemical compounds, it was also found

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that B. suaveolens leaf extract contains phenolic compounds, saponins, tannins, triterpenoids, flavonoids, and alkaloids (Nathania et al., 2020). Unlike previous studies, this study aimed to determine the antioxidant activity of the ethanol extract of forest jasmine flowers (B. suaveolens) using the DPPH method and identify biochemical compounds quantitatively using gas chromatography-mass spectrometry (GC-MS). The results of this study provide new information about the antioxidant activity of B. suaveolens flowers and identify the compounds responsible for this activity. Furthermore, the results contribute new data on the specific compounds contained in the local B. suaveolens plant found in Tomohon, which can serve as a foundation for further development and research in the field of biopharmaceuticals.

RESEARCH METHODS

The B. suaveolens flower samples used in this study were collected in Talete Satu village in Tomohon City, North Sulawesi. The collection location is shown in Figure 1. The flowers used in this study were orange and neither too young nor too old. After collection, the samples were washed and cleaned with running water. Then, they were cut into small pieces and weighed to a total of 500 grams. The maceration process was conducted at the Unima Microbiology Laboratory. The concentration of the ethanol extract of the forest angel's trumpet flower was performed at the North Sulawesi Police Forensic Laboratory. The antioxidant testing using the DPPH method and the screening of compounds present in the samples were carried out at the Integrated Research and Testing Laboratory of Gadjah Mada University.

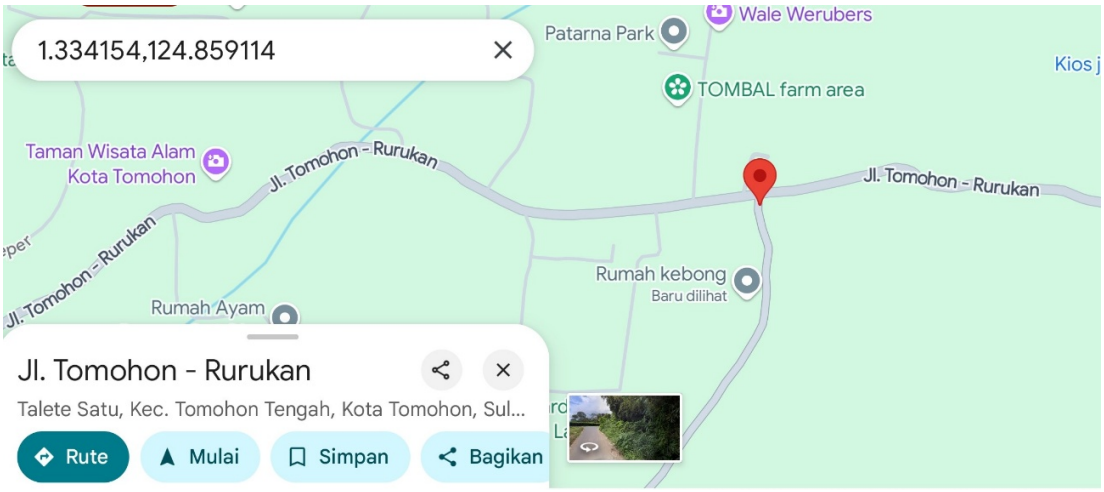

Figure 1. Sample Collection Location Map

Tools and Materials

Identification of antioxidant values in Brugmansia suaveolens using the following equipment: vials, vortex, mask, beaker, measuring flask, dry tissue, filter paper, lab spatula, jar, analytical balance, test tube, aluminum foil, burette, measuring cup, micropipette, plant scissors, paper scissors, funnel, gloves, pencil, labels, cuvette, petri dish, Butchi V-300 Switzerland rotary evaporator, Shimadzu UV-1800 spectrophotometer (Nathania et al., 2020). To identify the compounds contained within, the following are required: pipettes, syringes, amber bottles, microcentrifuge tubes, centrifuge, vial glasses, Thermo

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Scientific Trace 1310 Gas Chromatograph, Thermo Scientific ISQLT Single Quadropole Mass Spectrometer. The materials required are forest orchid flowers collected from the village of Talete Satu Tomohon in North Sulawesi, 95% ethanol, DPPH, and Vitamin C.

Flower Extraction

This extraction process uses a modified version of the method described by Nathania et al. (2020). Brugmansia suaveolens flowers are extracted using the maceration method. First, a 500 g sample of B. suaveolens flowers is weighed. Then, 1.5 L of 95% ethanol solvent is added to the sample, and the mixture is macerated for three 24-hour periods. The filtrate is separated every 24 hours, and the process is repeated. Then, the filtrate is evaporated using a Butchi V-300 Switzerland rotary evaporator at 45°C. This temperature was chosen because using a higher temperature could damage the sample or decrease its phenolic content (da Costa et al., 2023). The sample is evaporated until it forms a thick extract resembling a paste or cream. The yield of the thick extract is measured using the following formula:

$$\%\text{Yield} \frac{\text{Thick extract weight}}{\text{Extracted simplisia weight}} \text{ x } 100\%$$

Next, 120.8 mg of the sample was weighed and dissolved in 10 mL of solvent. Then, it was diluted with a dilution factor of 10x. The sample was then centrifuged to obtain the supernatant, which was tested for antioxidant activity. Testing antioxidant activity is important for evaluating a plant extract's potential to scavenge free radicals.

Antioxidant Activity Test Method

Antioxidant activity can be tested using the DPPH method. It is one of the most common quantitative approaches used for this type of testing (Nursifa et al., 2025). The DPPH method was chosen because it is simple, quick, and does not require large samples (Pakaya et al., 2023).

Preparation of Vitamin C Control Solvent

A total of 1 mL of 0.4 mM DPPH solution was added to 4 mL of ethanol. The control solution was then determined for its maximum wavelength using a UV-Vis spectrophotometer. The wavelength obtained was 517 nm.

Determination of Absorbance From Vitamin C Control Solution

The control solution that has been prepared is then measured for absorbance at the maximum wavelength to determine the control absorbance.

Determination of Vitamin C Sample Absorbance

A sample of a certain volume was added with 1 mL of 0.4 mM DPPH solution and ethanol was added to a total volume of 5 mL. The solution was incubated for 30 minutes in a dark room. The sample solution was then measured for absorbance at the maximum wavelength to determine the absorbance of the sample.

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Determination of IC50

Radical scavenging activity is expressed as a percentage of inhibition, which can be calculated using the following formula:

$$\% \text{ Inhibition} = \frac{Control\ abs - Sample\ abs}{Control\ abs} \times 100\%$$

The regression curve was created by plotting sample concentration vs. % inhibition. The IC50 value was calculated using the regression equation that had been created.

Analysis of Compounds Using GC-MS

Paste or cream samples are dissolved with the solvent specified in the notes (EtOH) in a microtube, then vortexed and centrifuged at 9500 rpm for 5 minutes. The supernatant is then collected and the sample is ready for injection.

RESULT AND DISCUSSION

The extract obtained from rotary evaporation has a very thick consistency, similar to paste or cream, as shown in Figure 2. The yield of the thick extract was measured and centrifuged to obtain the supernatant, as shown in Table 1 and Table 2.

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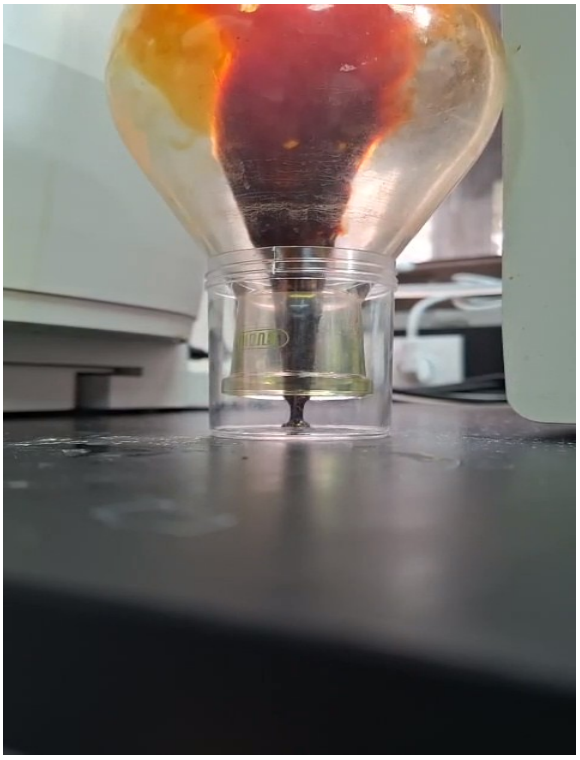

Figure 2. Thick Extract from Rotary Evaporation

Table 2. Extract Yield Measurement

Sample name	Simplisia weight (gr)	Extract weight (gr)	Yield extract (gr)
B. suaveolens ethanol flower extract	500	11, 319	2,2638

Table 2. Extraction of Supernatant from B. suaveolens Flower Extract

	weighing (mg)	solution volume (mL)	Level (mg/mL)	Dilution factor	Level (mg/mL)
Sample	120,8	10	12,08	10	1,21

The supernatant obtained was then injected into the GCMS machine to analyze the compounds contained in the sample. The screening results can be seen in Figure 3 and Table 3.

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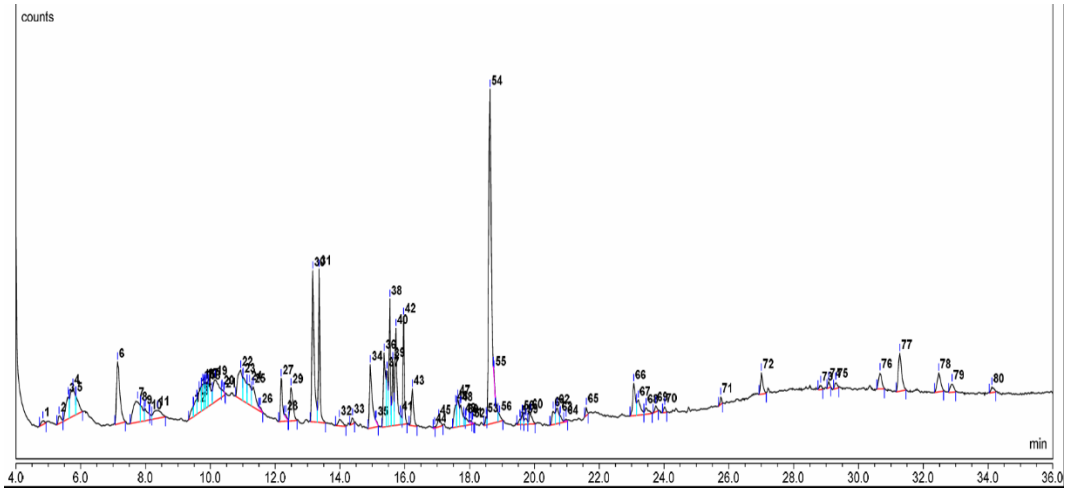

Figure 3. GC-MS Screening Results

Table 3. Compounds Identified by GC-MS

No	Retention Time (min)	Compounds name	chemical formula	molecule Weight	Peak Area (%)
1	13.16	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	5,88
2	13.36	Hexadecenoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	3,69
3	15.72	Octadecanoic acid	$C_{18}H_{36}O_2$	284	3,75
4	15.96	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	2,53
5	18.63	Scopolamine	$C_{17}H_{21}NO_4$	303	17,73

In this study, antioxidant activity was expressed as IC50 values. The IC50 value of the ethanol extract of B. suaveolens flowers was 196.160 µg/mL, while the IC50 antioxidant activity of the reference, ascorbic acid, was 3.814 µg/mL. The antioxidant activity test results are shown in Tables 4 and Table 5. The regression equation formula was used to calculate the IC50 values, which can be seen in Figures 4 and Figure 5.

Table 4. Antioxidant Activity Test of *B. suaveolens* Flowers Result

Level (µg/mL)	Absorbance			Free radical scavenging (%)			Average (%)
	I	II	III	I	II	III	
Kontrol		**0,900**					
24,160	0,813	0,814	0,813	9,6	9,5	9,6	9,6
48,320	0,785	0,784	0,784	12,7	12,8	12,8	12,8
96,640	0,664	0,664	0,664	26,2	26,2	26,2	26,2
144,960	0,554	0,554	0,554	38,4	38,4	38,4	38,4
193,280	0,442	0,441	0,441	50,8	51,0	51,0	50,9
241,600	0,341	0,340	0,340	62,1	62,2	62,2	62,1
289,920	0,234	0,234	0,234	74,0	74,0	74,0	74,0
338,240	0,178	0,178	0,178	80,2	80,2	80,2	80,2

Table 5. Ascorbic Acid Antioxidant Activity Test Result

Level (µg/mL)	Absorbance			Free radical scavenging (%)			Average (%)
	I	II	III	I	II	III	
Kontrol		**0,947**					
1,005	0,790	0,790	0,790	16,5	16,5	16,5	16,5
2,010	0,724	0,725	0,726	23,5	23,4	23,3	23,4
3,015	0,578	0,578	0,578	38,9	38,9	38,9	38,9
4,020	0,448	0,448	0,448	52,6	52,6	52,6	52,6
5,025	0,322	0,322	0,322	65,9	65,9	65,9	65,9
6,030	0,191	0,191	0,191	79,8	79,8	79,8	79,8
7,035	0,084	0,084	0,084	91,1	91,1	91,1	91,1

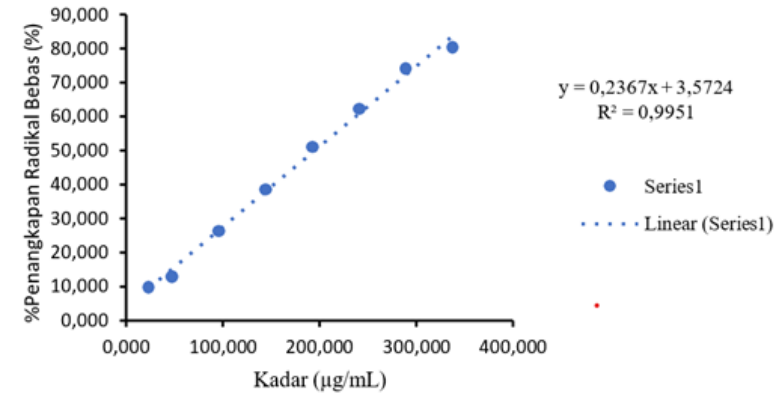

Figure 4. % Inhibition Curve of Ethanol Extract of B. suaveolens Flowers

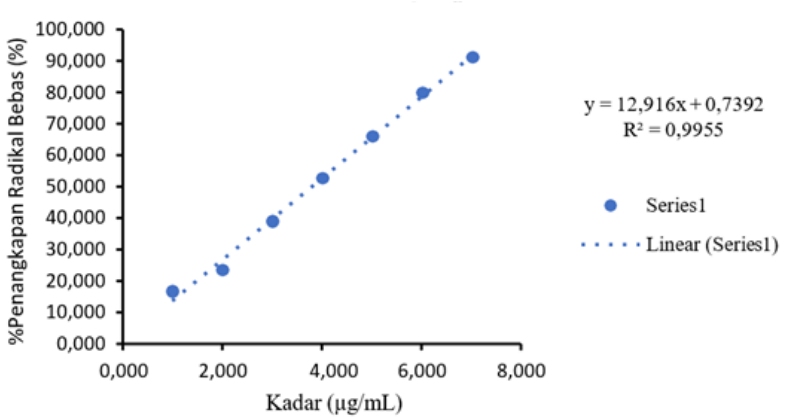

Figure 5. Ascorbic Acid Inhibition Curve

Discussion

According to previous research, the DPPH test of B. suaveolens leaves showed strong antioxidant activity and contained bioactive compounds classified as antioxidants, such as phenolic compounds and

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flavonoids (Nathania et al., 2020). According to Petricevich et al., 2020, in the case of cut flowers, the content of volatile compounds decreases more rapidly. Some factors causing the loss of volatile compounds in B. suaveolens flowers are pathogen attacks. In this study, antioxidant activity was expressed as IC50, which is the concentration of the sample solution required to inhibit 50% of DPPH free radicals (Nathania et al., 2020). The high IC50 value of the ethanol extract of B. suaveolens flowers indicates that its antioxidant activity is still low. The results of phytochemical compound screening using GC-MS also show a correlation with the DPPH test. The compound with the highest content is scopolamine. Meanwhile, several compounds are indicated as being responsible for the antioxidant activity of the ethanol extract from B. suaveolens flowers. Compounds such as hexadecenoic acid, also known as palmitic acid, are natural compounds produced by animals, plants, and microorganisms and act as natural antioxidants (Purushothaman et al., 2024). Another compound involved in the antioxidant activity of the ethanol extract of B. suaveolens flowers is octadecanoic acid, which, in addition to its antioxidant activity, can also act as an antibacterial and antimicrobial agent (Muflihunna et al., 2021). Octadecanoic acid plays an important role in the defense response to specific ultraviolet radiation wavelengths (Luhata P et al., 2023). According to Achika et al., 2023, this compound also has numerous biological activities, one of which is free radical scavenging.

CONCLUSION

The DPPH assay of ethanol extracts of B. suaveolens flowers collected in Talete Satu Village, Tomohon City, North Sulawesi, showed antioxidant activity with an IC50 value of 196.160 µg/mL, while the ascorbic acid used as a reference in this study had an IC50 value of 3.814 µg/mL. Screening of bioactive compounds using GCMS revealed the presence of several compounds with antioxidant properties in the ethanol extract of B. suaveolens flowers, these compounds are Hexadecenoic acid (palmitic acid) and Octadecanoic acid, while the compound with the highest peak area in the GCMS screening is a tropane alkaloid, namely scopolamine, which can be utilized as an antidepressant and anticholinergic agent.

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