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The Effect of Methanol Concentrations Against Phenolic Total Content and Antioxidant of Balakacida Leaves (*Chromolaena odorata*)

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Abstract

The balakacida plant with the Latin name *Chromolaena odorata* is a plant that lives in a tropical climate. The balakacida plant is a plant of the Asteraceae Family. Phytochemical tests on balakacida leaves showed the presence of secondary metabolites of the tannin, phenol, flavonoid, saponin and steroid groups. This study aims to extract balakacida leaves and test the total phenolic and antioxidant content. Sample extraction was carried out by the maceration method using methanol solvent. Testing of total phenolic content using the Folin Ciocalteu method, while testing of antioxidant content using the DPPH method using a UV-Vis spectrophotometer. The results showed that the concentration of methanol solvent significantly affected the acquisition of total phenolics and antioxidants. The highest total phenolic content was obtained at a methanol extract concentration of 80% of 145.64 mg GAE/g. For antioxidant content, a methanol extract concentration of 60% showed the highest activity with an average DPPH % inhibition of 59.77%. It can be concluded that a methanol extract concentration of 60% is the optimal concentration to produce balakacida leaf extract with the best antioxidant activity, although not with the highest total phenolic content.

Keywords: antioxidant; balakacida; *Chromolaena odorata*.

INTRODUCTION

One of the plants that is interesting to study is *Chromolaena odorata*, commonly known as balakacida leaves. This plant belongs to the Asteraceae family and is widely recognized in various tropical countries as a medicinal plant. Marsella & Saleh (2024) indicate that balakacida leaves contain various bioactive compounds, including flavonoids, alkaloids, and tannins, which have potential as antioxidant agents. This plant is known not only for its health benefits but also for its ability to grow rapidly and adapt to diverse environmental conditions, making it an invasive species in several regions. The utilization of balakacida leaves in the health sector is becoming increasingly important in line with the growing need for sustainable natural resources.

Phenolic compounds are a group of organic compounds commonly found in plants and are known to provide various health benefits, including their ability to act as antioxidants. According to research by Ayumi et al. (2023), phenolic compounds can help reduce the risk of degenerative diseases such as cancer and heart disease. The total phenolic content in an extract can be influenced by various factors, including the type of solvent used, the extraction method, and the extraction time. Therefore, it is important to optimize the extraction process to obtain the maximum phenolic content from *Chromolaena odorata* leaves.

The extraction of phenolic compounds from plants can be carried out using various methods, one of which is maceration. This method involves soaking plant materials in a solvent for a certain period to maximize the extraction of the desired compounds. Solvent concentration plays an important role in the extraction process, as it can affect the solubility of phenolic compounds. Research by Ramayani et al. (2021) shows that the use of solvents with different concentrations can result in varying phenolic contents. Therefore, it is important to test various solvent concentrations in the extraction of *Chromolaena odorata* leaves to determine the optimal conditions that can enhance total phenolic content and antioxidant activity.

The selection of solvent type is also an important factor in the extraction of phenolic compounds. The solvent used can influence its ability to dissolve phenolic compounds, which in turn affects the total phenolic content obtained. Tommy et al. (2022) indicate that polar solvents such as ethanol and methanol are more effective in extracting phenolic compounds compared to non-polar solvents. Therefore, choosing the appropriate solvent is essential to achieve optimal extraction results.

MATERIALS AND METHODS

Material

The sample used in this study was the balakacida leaf. The chemicals used included methanol (pro analysis grade), distilled water, DPPH, Folin-Ciocalteu, Na_2CO_3, ascorbic acid. The equipment used included a macerator, glassware, micropipettes, UV-Visible spectrophotometer.

Sample Extraction

The leaves of balakacida were collected from Cilegon City, Banten, Indonesia. A total of 1.0 kg of fresh samples were collected. The fresh samples were then air-dried at room temperature for two weeks. About 100 g of the dried balakacida leaf samples were extracted using the maceration method with 50, 60, 70, 80 and 90% of methanol. The extract was then concentrated using a rotary evaporator.

Antioxidant Activity Test Using DPPH

An amount of 50 mg of the *T. procumbens* flower extract sample was dissolved in methanol and transferred into a 50 mL volumetric flask to obtain a stock solution with a concentration of 1000 ppm. From this 1000 ppm stock solution, a series of concentrations (10, 20, 30, 40, and 50 ppm) was prepared. DPPH was weighed (10 mg) and dissolved in 62.5 mL of methanol to obtain a DPPH solution with a concentration of 0.05 µM. For the assay, 2.4 mL of each extract concentration was mixed with 0.6 mL of the DPPH solution, then incubated at room temperature in the dark for 30 minutes. The absorbance was measured at a wavelength of 517 nm using a UV-Visible spectrophotometer (Situmeang, Swasono, et al., 2025). All sample tests were performed in triplicate.

Total Phenolic

The TPC was determined using the Folin-Ciocalteu method previously described by Kabri et al. (2023), with certain modifications. The ekstrak were prepared at a concentration of 1000 µg/ml. In summary, approximately 1 mL of Folin-Ciocalteu reagent was combined with 200 µL of fractions and 0.8 mL of a 7.5% (m/v) Na_2CO_3 solution. Following a 60-minute incubation period at room temperature, the absorbance was measured at a wavelength of 765 nm. A calibration curve was prepared using a series of gallic acid solutions with varying concentrations. The results of the total phenolic content (TPC) were expressed as mg GAE/g of the dried extract. All tests were conducted in triplicate.

Preparation of Gallic Acid Standard Curve

The preparation of a 1000 ppm gallic acid stock solution was carried out by weighing 10 mg of gallic acid and dissolving it in pro analysis (p.a.) methanol until the volume reached 10 mL (Hidayatullah et al., 2023). From the 1000 ppm stock solution, serial dilutions of 20, 40, 60, 80, and 100 ppm were prepared. A total of 0.5 mL from each concentration of the gallic acid standard solution was taken and then added with 2.5 mL of Folin–Ciocalteu reagent (previously diluted with distilled water at a ratio of 1:10). The solution was homogenized and allowed to stand for 2 minutes. Subsequently, 2 mL of 7.5% Na_2CO_3 was added and the mixture was left in the dark for 30 minutes. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 765 nm.

Data analysis

The one-way ANOVA test was used in statistical evaluation and data representation using Microsoft exel and origin 9 software. The data were reported as the mean ± standard deviation.

RESULTS AND DISCUSSION

Sample Preparation

The sample used in this study was balakacida leaves (*Chromolaena odorata*) obtained from Pengarengan Village, Bojonegara District, Serang Regency, Banten. The collected balakacida leaves were then cleaned, washed, and dried for 3 × 24 hours. This drying process was carried out in the laboratory of Sekolah Tinggi Analis Kimia Cilegon. The samples were cut into smaller pieces to increase the surface area, allowing the solvent to more effectively extract the chemical compounds contained in the sample. Meanwhile, the drying process aimed to reduce the moisture content in the balakacida leaves. Moisture content can affect the extraction process, as it may promote microbial growth and interfere with the interaction between the solvent and the desired compounds.

The balakacida leaves that had been dried for 3 × 24 hours experienced a weight reduction from 1000 grams to 726 grams, with a moisture content of 27.4% (attachment 2). Drying was carried out in a room by air-drying without heating to prevent chemical changes. After the drying process, the next step was grinding. The grinding process was performed using a blender until balakacida leaf powder was obtained. This powder was then used in the extraction process.

Sampel Extraction

The extraction process was carried out in the laboratory of Sekolah Tinggi Analis Kimia Cilegon. The extraction was performed by weighing 2 grams of sample for each treatment and using 98% methanol as the solvent, which was then diluted to concentrations of 50%, 60%, 70%, 80%, and 90%. The maceration method was chosen because it is simple to perform and does not require heating, thereby preventing the chemical compounds in the sample from being damaged or degraded. The use of methanol aims to extract polar compounds.

The extract obtained from the maceration process was then evaporated and concentrated using an oven. This evaporation and concentration process was carried out in the laboratory of Sekolah Tinggi Analis Kimia Cilegon.

The evaporation process aimed to obtain a concentrated extract quickly and effectively. Evaporation was performed at a temperature of 45°C to prevent the decomposition of the compounds contained in the extract. Decomposition is the process of breaking down a substance into simpler forms. In the evaporation process, the final yield obtained from the methanol extract of *Chromolaena odorata* leaves was an average of 0.8 grams, with an average extract yield percentage of 41%.

Galac Acid Standar Curve

The preparation of a gallic acid standard curve is a crucial step in the quantitative analysis of polyphenol compounds, particularly those measured as gallic acid equivalents. This standard curve serves to determine the relationship between the concentration of an analyte (gallic acid) and the measured absorbance value. In this study, the gallic acid standard curve was prepared using several series of gallic acid standard concentrations (20, 40, 60, 80, and 100 ppm), whose absorbances were measured at the maximum wavelength using a UV-Vis spectrophotometer. The absorbance of sample shown in Table 1.

Table 1. The absorbance value of galat acid in various concentration.

Concentrations (ppm)	Absorbances
20	0.825
40	1.219
60	1.951
80	2.285
100	2.904

The data obtained from the absorbance measurements of the gallic acid standards are presented in Table 1 and visualized in Figure 1. From these data, it can be observed that the higher the concentration of gallic acid, the greater the absorbance value. This relationship indicates a positive correlation between concentration and absorbance, which is consistent with the Beer-Lambert Law. The Beer-Lambert Law states that the absorbance of a solution is directly proportional to the concentration of the solute and the path length of the light passing through it (Lia Agustin & Rilla Agustina, 2021).

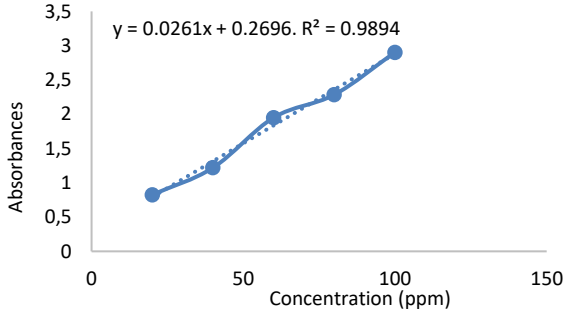

Figure 1. Regresion linear curve of galat acid.

Based on Figure 1, the linear regression equation obtained was y = 0.0261x + 0.2696, with a coefficient of determination (R^2) of 0.9894. This equation represents the mathematical relationship of the standard curve, where *y* is the absorbance and *x* is the concentration of gallic acid in ppm. An R^2 value of 0.9894 indicates that the linear regression model is highly effective in explaining the variation in absorbance based on concentration. An R^2 value close to 1 signifies a strong linear relationship between the two variables, meaning that the resulting standard curve can be accurately used to estimate the concentration of gallic acid in the samples based on their absorbance values (Tulnisa et al., 2025).

This gallic acid standard curve will serve as the basis for calculating the total phenolic content in the test samples. By using the obtained regression equation, the concentration of gallic acid in the samples can be determined after measuring their absorbance values. The successful construction of the standard curve with a high correlation coefficient is crucial to ensure the accuracy and precision of the subsequent quantitative analysis.

Total Phenolic Content Result

The determination of total phenolic content was carried out on five samples (A1–A5) using the spectrophotometric method. The results of the total phenolic content analysis for samples A1–A5 are presented in Table 2.

Table 2. Determination of total phenolic content result.

Sampel code	TPC (mg GAE/g)
A1	60.84
A2	86.54
A3	13.99
A4	145.64
A5	126.29

Based on the results presented in Table 2, variations in the total phenolic content can be observed among the five samples (A1–A5). The highest total phenolic content was recorded in sample A4 with a value of 145.64 mg GAE/g sample, followed by sample A3 (139.99 mg GAE/g sample), A5 (126.29 mg GAE/g sample), A2 (86.54 mg GAE/g sample), and the lowest was sample A1 with a value of 60.84 mg GAE/g sample.

Phenolic compounds are a group of plant secondary metabolites that possess various biological functions, including antioxidant activity (Sukma et al., 2022). The presence of phenolic compounds in natural materials is strongly influenced by several factors such as plant species, the plant part used, environmental growing conditions, extraction methods, and the type of solvent employed (Firdiyansyah et al., 2023). The differences in phenolic content among samples A1 to A5 indicate variations in the composition or concentration of phenolic compounds within these samples.

The high phenolic content in samples M4 and M3 indicates that these samples may possess greater antioxidant potential compared to the others. Phenolic compounds contain hydroxyl groups that play an important role in scavenging free radicals; therefore, the higher the phenolic content, the stronger the antioxidant capacity (Sukma et al., 2022). These differences may also result from variations in the origin or treatment of the samples. For instance, differences in plant varieties or maturity levels can influence the biosynthesis of phenolic compounds within the plant (Safitri et al., 2023).

Antioxidant test Result

The antioxidant activity test of balakacida leaf extract using the DPPH method is presented in Table 3. This assay measures the ability of the extract to inhibit DPPH free radicals, expressed as a percentage of inhibition.

Table 3. The antioxidant activity test of balakacida leaf extract using the DPPH method.

Sample	Absorbances		Inhibition (%)		Average
	1)	2)	1)	2)	(%)
Blank	1.33	1.042	0	0	0
A1	0.542	0.546	47.531	47.500	47,516
A2	0.418	0.416	59.535	60.000	59,768
A3	0.562	0.566	45.595	45.577	45,586
A4	0.653	0.649	36.786	37.596	37,191
A5	0.497	0.494	51.888	52.500	52,194

Based on Table 3, it can be observed that the highest average DPPH inhibition percentage was shown by sample A2 (60% concentration) at 59.768%, followed by A5 (90% concentration) at 52.194%, A1 (50% concentration) at 47.516%, A3 (70% concentration) at 45.586%, and the lowest was A4 (80% concentration) at 37.191%.

The antioxidant activity of balakacida leaf extract was evaluated using the DPPH method, which is a commonly used technique to assess free radical scavenging capacity (Marsella & Saleh, 2024). The results indicate that the concentration of methanol solvent has a significant effect on the antioxidant activity of the extract. Sample A2 (60% methanol) exhibited the highest antioxidant activity, with an inhibition percentage of 59.768%. This suggests that using 60% methanol is the optimal concentration for extracting antioxidant compounds from balakacida leaves that are effective in scavenging DPPH radicals.

An increase in the concentration of polar solvents such as methanol often enhances the solubility of polar compounds like phenolics and flavonoids, which are natural antioxidants (Amalia Rachmawati et al., 2020). However, in this study, it was observed that increasing the methanol concentration above 60% (A3 and A4) actually resulted in a decrease in antioxidant activity. This reduction may be attributed to several factors. First, at excessively high solvent concentrations, unwanted non-polar compounds may also be extracted, potentially affecting the purity and effectiveness of the antioxidant constituents (Fatah et al., 2024). Second, certain antioxidant compounds that are sensitive to very high solvent concentrations or extreme extraction conditions may undergo denaturation or degradation (Amin et al., 2024).

Interestingly, sample A5 (90% concentration) showed a renewed increase in antioxidant activity compared to M3 and M4, although it was still lower than A2. This phenomenon may indicate the presence of other antioxidant compounds that are more soluble at higher methanol concentrations, or the occurrence of complex interactions among components extracted at different solvent concentrations. The optimal solvent concentration is crucial in the extraction of bioactive compounds. These findings are consistent with previous studies showing that the highest solvent concentration does not always yield the best antioxidant activity; rather, there is an optimal concentration that can efficiently dissolve active compounds without co-extracting interfering substances (Fatah et al., 2024).

In general, phenolic compounds are known as a class of secondary metabolites that greatly contribute to the antioxidant activity of plant extracts. Phenolic compounds possess hydroxyl groups that can donate hydrogen atoms to stabilize free radicals, thereby exhibiting antioxidant activity. Therefore, a positive correlation often exists between total phenolic content and antioxidant activity.

CONCLUSIONS

The highest phenolic content was obtained at a methanol solvent concentration of 80%, while the highest antioxidant activity was observed at a methanol concentration of 60%. The concentration of methanol solvent influenced both the total phenolic content and the antioxidant activity of balakacida (Chromolaena odorata) leaves. The optimum conditions based on solvent concentration were achieved at 80% methanol for total phenolic content (145.64 mg GAE/g) and at 60% methanol for antioxidant activity (59.768%).

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