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Optimization of Antioxidant Extraction from Bay Leaf (*Syzygium polyanthum* Wight.) in Various Mass Ratios, Solvent Concentrations, and Maceration Times Using Iodometry

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maceration;
optimization;
quercetin.

ABSTRACT. The objective of this study was to determine the optimal extraction conditions for maximizing the antioxidant content of bay leaf (Syzygium polyanthum Wight.) through the evaluation of the effects of the mass of crude plant material, the composition of the solvent, and the time of maceration. Bay leaf extracts were obtained by maceration with 96% ethanol, ethanol–water mixtures (2:1, 1:1, 1:2), and distilled water, using mass material from 10 to 50 g and extraction times from 1 to 11 days. Antioxidant content was determined by iodometric titration using quercetin as standard and expressed as quercetin equivalents (QE). The calibration curve for quercetin revealed a linear relationship between the concentration of quercetin and the volume of iodine reduced ($y = 0.0103x + 0.3735$). Analysis of variance (ANOVA) showed that maceration time, ethanol concentration and crude plant material mass had a significant effect on antioxidant content ($p < 0.05$). The developed quadratic model was in good agreement with the experimental data with R^2 value of 93.46%. The best conditions for the extraction were obtained with 50 g of raw plant material, an ethanol-water ratio of 2:1 and a period of 9 days of maceration which gave the highest antioxidant content of 30.7394 mg QE/g sample.

INTRODUCTION

Syzygium polyanthum Wight. (salam) is an endemic species of *Myrtaceae* that grows the Southeast Asia, including Indonesia. Bay leaves have been used for generations as the kitchen spices and traditional medicines because they are rich in secondary metabolite compounds such as phenolic compounds, flavonoids, tannins, and alkaloids, which are a group of compounds that are widely known to have a variety of important biological activities, including antioxidant activity (Rahman *et al*., 2023). The hydroxyl group found in phenol and flavonoid compounds acts as an antioxidant by providing hydrogen atoms and neutralizing free radicals (Aklimah and Ekayanti, 2022). One of the most prominent flavonoid compounds that plays a crucial role in the biological activity of bay leaves is quercetin.

Quercetin (3,3′,4′, 5,7-pentahydroxyflavone) is a polyphenolic flavonoid compound that has an active phenolic hydroxyl group and a double bond. *B-rings* in the natural flavonoid class are the main active sites for antioxidants and the capture of reactive oxygen species (ROS) by increasing the body's levels of natural antioxidant enzymes such as glutathione (GSH) and superoxide dismutase (SOD), while lowering the level of malondialdehyde (MDA) as a marker of oxidation-induced cell damage (Alharbi *et al*., 2025). Free radicals are unstable reactive species that have one or more unpaired electrons, so they will seek out and snatch electrons from nearby vital components to return to a stable state. Although the body has an endogenous antioxidant defense system, it is sometimes not enough to cope with the excessive production of free radicals (Amin and Assafa, 2025).

Bay leaves are extracted by the maceration process, which is a solid-liquid extraction process in which a solid sample is immersed in an organic solvent at room temperature in order to dissolve the active compound (Huang *et al*., 2025). Maceration has an advantage in its ability to maintain the structural stability of components that are sensitive to heat and chemical degradation impacts, such as flavonoids, which can reduce their bioactivity (Zhu *et al*., 2025). However, this method is time-consuming and only involves natural diffusion to extract the compound

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resulting in low antioxidant activity values due to its limited solvent penetration and slow diffusion rate (Nugrahani *et al*., 2024).

The ethanol–water mixture was chosen as the extraction solvent due to its suitable polarity for the extraction of phenolic and flavonoid compounds . Phenolic compounds have diverse chemical structures and polarities. Therefore, the use of a single solvent often limits the extraction efficiency of some bioactive compounds. Water addition to ethanol increases the polarity of the solvent, which promotes swelling of plant cell walls and makes it easier for the solvent to penetrate into plant tissues, thus resulting in better release of intracellular compounds. Simultaneously, ethanol increases solubility of moderately polar phenolic and flavonoid compounds that cannot be effectively extracted by water alone. Ethanol-water mixture has higher extraction efficiency and antioxidant recovery than pure solvents, because the combined solvent system has an intermediate polarity which can extract a wider range of bioactive compounds. Recent studies have demonstrated that mixed-solvent systems, especially ethanol–water mixtures, may considerably improve the total phenolic content and antioxidant activity due to their better mass transfer properties and wider solvation capacity for phytochemicals (Martínez-Inda *et al*., 2025; Sun *et al*., 2025). Moreover, ethanol is a green solvent in view of its low toxicity, biodegradability and its use in food, nutraceutical and pharmaceutical applications. Thus, ethanol-water mixtures are an attractive medium for the sustainable extraction of natural antioxidants (Radić and Vitali Čepo, 2025).

A few studies have been done to extract the bioactive compounds from the leaves of *Syzygium polyanthum*. But their goals and optimization methods are very different. Hidayati *et al*. (2017) evaluated extracts obtained with different solvent systems and reported that the polarity of the solvent greatly affected the antioxidant activity and total phenolic content. Kusumaningrum *et al*. (2020) optimized the ultrasonic-assisted extraction of terpenoids using ethanol, varying the extraction temperature and solvent-to-solid ratio, but the optimization target was the yield of terpenoids, not antioxidant compounds. More recently, Najib *et al*. (2025) compared conventional and green extraction methods of *S. polyanthum* and reported variations in phytochemical profiles and antioxidant activities between extraction techniques.

The antioxidant properties of the extracted bay leaf will be quantified using iodometric titration. This is a redox reaction where the oxidizing sample is first reduced in the presence of KI, and the amount of I2 released is determined by titration with a standard solution of sodium thiosulfate ($Na_2S_2O_4$) (Reactions 1 – 3) (Meyiwa, 2020). This method is useful in both oxidant and antioxidant assessments, with oxidants produce iodine from potassium iodide and antioxidants reduce iodine produced by oxidants (Mir *et al*., 2019). Although spectrophotometric methods such as DPPH and ABTS are widely known for their simplicity and good correlation with phenolic compounds and flavonoids, iodometric titration was chosen in this study because it directly quantifies the electron donor reduction capacity of antioxidant compounds through stoichiometric redox reactions, thus allowing for quantitative expression of antioxidant content in the form of quercetin equivalents (mg QE/g sample) without the need for specialized spectrophotometric instrumentation.

$KIO_3 + 5KI + 6HCl \rightarrow 6KCl + 3H_2O + 6I_2$ (Reaction 1)

$C_{15}H_{10}O_7 + I_2 \rightarrow C_{15}H_8O_7 + 2I^- + 2H^+$ (Reaction 2)

$2Na_2S_2O_3 + I_2 \rightarrow Na_2S_4O_6 + 2NaI$ (Reaction 3)

Although the antioxidant potential of bay leaves has been extensively researched and reported, research on optimizing extraction variables to obtain effective antioxidant compounds still needs to be developed. No previous study has simultaneously optimized dried plant material mass, solvent concentration, and maceration time in a single systematic study using iodometric analysis with quercetin as the standard. Therefore, this study aims to fill this gap by comprehensively examining the interaction of the three variables to determine the optimal maceration conditions to maximize antioxidant extraction from *S. polyanthum* Wight leaves.

RESEARCH METHODS

This study employed an experimental method to optimize the extraction of antioxidant compounds from bay leaves (*S. polyanthum* Wight.) using the maceration technique. The effects of three independent variables, namely ethanol concentration, crude plant material mass, and maceration time, on the antioxidant capacity of the extract were evaluated using the maceration technique. The equipment used included a Kirin KFC-307 chopper, a WANT analytical balance, a Labtech LEV400V rotary vacuum evaporator, a One-Two Cups 200-mesh filter cloth, a Pyrex burette and stative, and Microsoft Excel and Minitab statistical software, as well as standard laboratory glassware.

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The materials used were fresh bay leaves (*Syzygium polyanthum* Wight.), KOHKOL 96% ethanol, distilled water (water) from CV Progo Mulyo Yogyakarta, Alpha Chemika 0.01 N potassium iodate (KIO_3), Merck 10% pure potassium iodide (KI), Merck 1 N hydrochloric acid (HCl), Merck 0.01 N sodium thiosulfate pentahydrate ($Na_2S_2O_3.5H_2O$), Merck sodium carbonate (Na_2CO_3), Merck 1% soluble starch (amylum), and Sigma-Aldrich quercetin standard (3,3',4',5,7-pentahydroxyflavone). The equipment used included a Kirin KFC-307 chopper, a WANT analytical balance, a Labtech LEV400V rotary vacuum evaporator, a One-Two Cups 200-mesh filter cloth, a Pyrex burette and stative, and Microsoft Excel and Minitab statistical software, as well as standard laboratory glassware.

Preparation of Raw Materials

The main ingredients used in this study were fresh bay leaves, 96% ethanol as an organic solvent, and water as a polar solvent. The fresh bay leaves were then washed using running water, separated from the base of the stalk, and mashed using a chopper so that they became crude plant material. Crude plant material was then weighed at 10, 20, 30, 40, and 50 g, and then 200 mL of solvent was added at various variations in solvent concentrations and maceration times.

Maceration Process

In the maceration process, solvents with various variations in solvent concentrations in volume ratio (v/v) were used, namely 96% ethanol, ethanol:water 2:1, 1:1, 1:2, and pure water. Each sample was macerated for various time periods, namely 1, 3, 5, 7, 9, and 11 days. The maceration temperature was maintained at room temperature and stirring was performed regularly every 24 hours. After maceration is complete, the maceration results are separated from the solids using a filter, which is then further purified.

Evaporation process

The evaporation process was carried out to thicken the extracts obtained and recover the solvents that were used. Evaporation was carried out using a rotary vacuum evaporator at a pressure of 250 mbar and a temperature of 65 °C to ensure that no solvent remained, and the rotation of the appliance is maintained at 20 rpm. The evaporation process is stopped when no more solvent evaporates.

Antioxidant Content Analysis

The extract was then analyzed using iodometric titration. 1 mL of extract was diluted with 70% ethanol to 25 mL. 10 mL of this solution was mixed with 10 mL of KIO3 0.01 N, 10 mL of KI 10%, and 10 mL of HCl 1 N. Then 5 drops of 1% amylum indicator were added to produce a dark blue iodine-starch complex. The end of the titration was reached when the dark blue color had completely disappeared and the solution was clear, meaning that all the remaining iodine had reacted with sodium thiosulfate. All titrations were performed in duplicate and results are reported as means.

Experimental Design and Statistical Analysis

The statistical significance of the extraction parameters and their effects on the final antioxidant yield was determined by an analysis of variance (ANOVA) with a second-order polynomial regression model. The model studied the effect of three independent variables: maceration time (A), ethanol concentration (B), and mass of crude botanical material (C) on antioxidant content, expressed as mg QE/g sample.

RESULTS AND DISCUSSION

This study aimed to optimize the extraction conditions of antioxidant compounds by the maceration method by varying extraction time, crude plant material mass, and solvent ratio, and to determine antioxidant content using the iodometric titration method with quercetin as the standard. The iodometric method was chosen because of its ability to measure the capacity of antioxidant compounds in reducing iodine (I_2) to iodide (I^-).

Standard Curve of Quercetin Antioxidant Activity

Quercetin was used as the standard curve because it is one of the flavonoid compounds that has high antioxidant activity and a rich chemical structure of hydroxyl groups as an electron donor. A standard curve was obtained in Figure 1, which shows the relationship between quercetin concentration and iodine volume (I_2) reduced by quercetin, with the line equation y = 0.0103x + 0.3735. The equation of the line will be used to determine the

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antioxidant content contained in the bay leaf sample, expressed as the equivalent of quercetin (mg QE/g sample). The high equivalent value of quercetin indicates that the extract has a high content of antioxidant compounds, especially from the phenolic and flavonoid groups. This also indicates that the extraction conditions used have been able to maintain the reduction activity of bioactive compounds. However, the use of iodometric titration with visual endpoint detection may have some limitations. For example, in the presence of dark colored extracts such as bay leaf extract, it is difficult to accurately detect the color change from dark blue to colorless. This visual subjective assessment carries the risk of human error in the endpoint determination. To minimize this bias, titration was performed 3 times under the same lighting condition and the results were averaged.

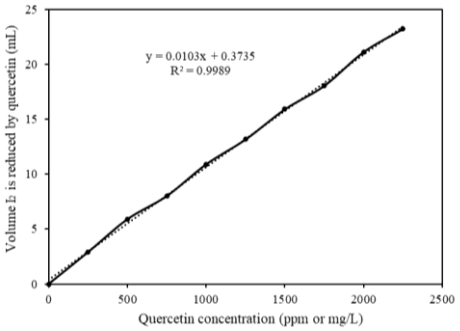


Figure 1. Standard curve of the relationship between quercetin concentration and quercetin-reduced volume of iodine (I_2).

Effect of Maceration Time on Antioxidant Content

The time of maceration is an important parameter in the extraction process because it is related to the kinetics of mass transfer between the material matrix and the solvent. Based on the results of the study, the increase in maceration time led to an increase in the measurable antioxidant content until it reached an optimal point, namely the maceration time of 9 days, as in Figure 2. This phenomenon can be explained through the diffusion mechanism, in which the longer the contact time between the solvent and the material, the more bioactive compounds such as flavonoids and phenolics diffuse into the solvent. In the early stages of maceration, the gradient takes place quickly. Over time, the concentration gradient decreases until it is close to equilibrium.

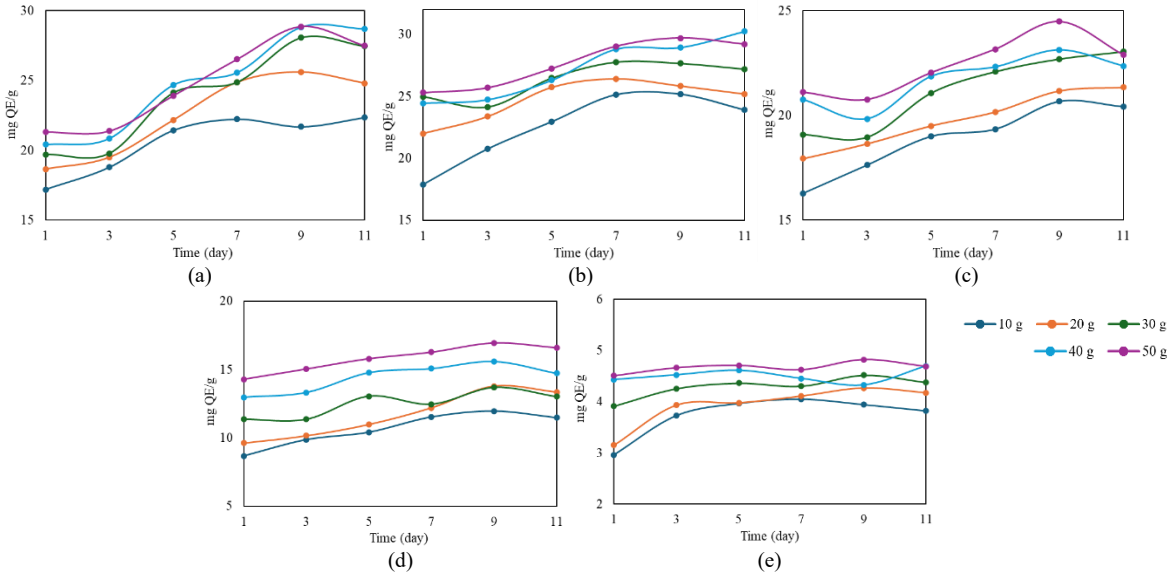


Figure 2. The relationship between maceration time and antioxidant content at various masses and solvent concentrations: (a) 96% ethanol, (b) ethanol:water 2:1, (c) ethanol:water 1:1, (d) ethanol:water 1:2, and (e) water.

However, after reaching the optimal time, the increase in maceration time no longer provides a significant increase in antioxidant content, and tends to decrease. This is in line with research conducted by Johanes *et al.* (2025) which showed the highest antioxidant activity on day 3 and decreased on day 7. This can be caused by several factors, including the degradation of phenolic compounds due to oxidation, hydrolysis, as well as exposure

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to light and environmental temperature. In addition, the possibility of an enzymatic reaction due to decay that damages the structure of the active compound can also occur during maceration that lasts too long.

Effect of Mass to Solvent Ratio on Extraction Efficiency

The solvent-to-ingredient ratio was varied by a fixed solvent volume of 200 mL and a crude plant material mass of 10 – 50 g, so the ratio decrease from 1:20 to 1:4 (m/v). Figure 3 shows that the antioxidant content increased linearly as the mass of the material increased. This linear relationship indicates that the extraction system has not yet reached a saturation state; therefore, the solvent is still able to dissolve antioxidant compounds in proportion to the amount of ingredients. This phenomenon occurs because the concentration gradient is maintained and the number of available target compounds increases.

However, this relationship only applies to a certain range. If the mass of the material is continuously increased, the system will be close to saturation, and the extraction efficiency decreases. In addition, an increase in the mass can increase the viscosity and extract non-specific compounds that could potentially affect the iodometric results. The results showed that the ratio of the solvent used was still in the unsaturated condition, where a 200 mL solvent was still effective in extracting antioxidant compounds up to 50 grams of ingredients.

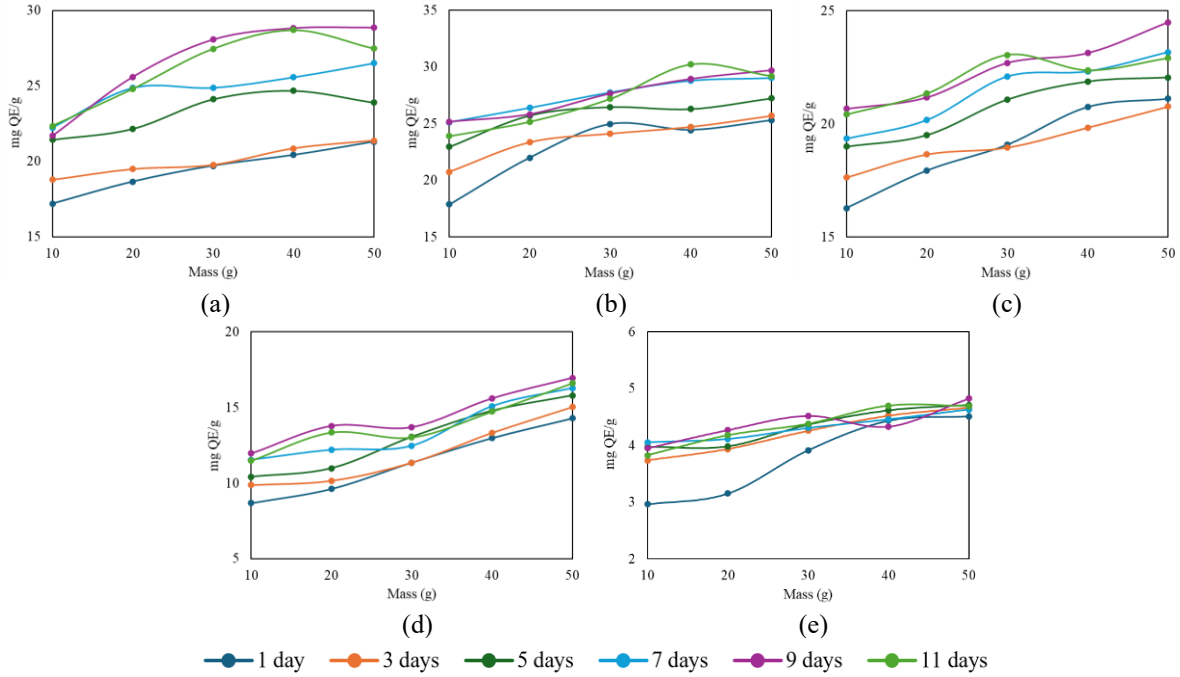


Figure 3. Relationship between crude plant material mass and antioxidant content at different maceration times and solvent concentrations: (a) 96% ethanol, (b) ethanol:water 2:1, (c) ethanol:water 1:1, (d) ethanol:water 1:2, and (e) water.

Effect of Ethanol Concentration on Antioxidant Content

The concentration of ethanol as solvent plays an important role in determining the type and amount of compounds extracted. This is mainly because it can influence the polarity of the solvent system. Ethanol is a semipolar solvent that can be mixed with water in various ratios, allowing polarity adjustment depending on the properties of the target compound. The maximum antioxidant content was obtained when the volume concentration of ethanol (v/v) was 67% (ethanol:water= 2:1), as shown in Figure 4. This is due to the synergy between ethanol and water, where water helps in the swelling of the material cell matrix, making it easier to release bioactive compounds, while ethanol dissolves phenolic compounds and flavonoids effectively.

Conversely, at too low ethanol concentrations, the solvent becomes too polar and is less effective at dissolving semipolar compounds. Meanwhile, at a concentration of 96% ethanol, the solvent's ability to extract polar compounds decreases; therefore, the antioxidant content obtained is also lower. This is in line with research that has been conducted by Islamiyati and Saputri (2018) show that extraction with 70% ethanol solvent actually provides a higher antioxidant content extract than with 96% solvent.

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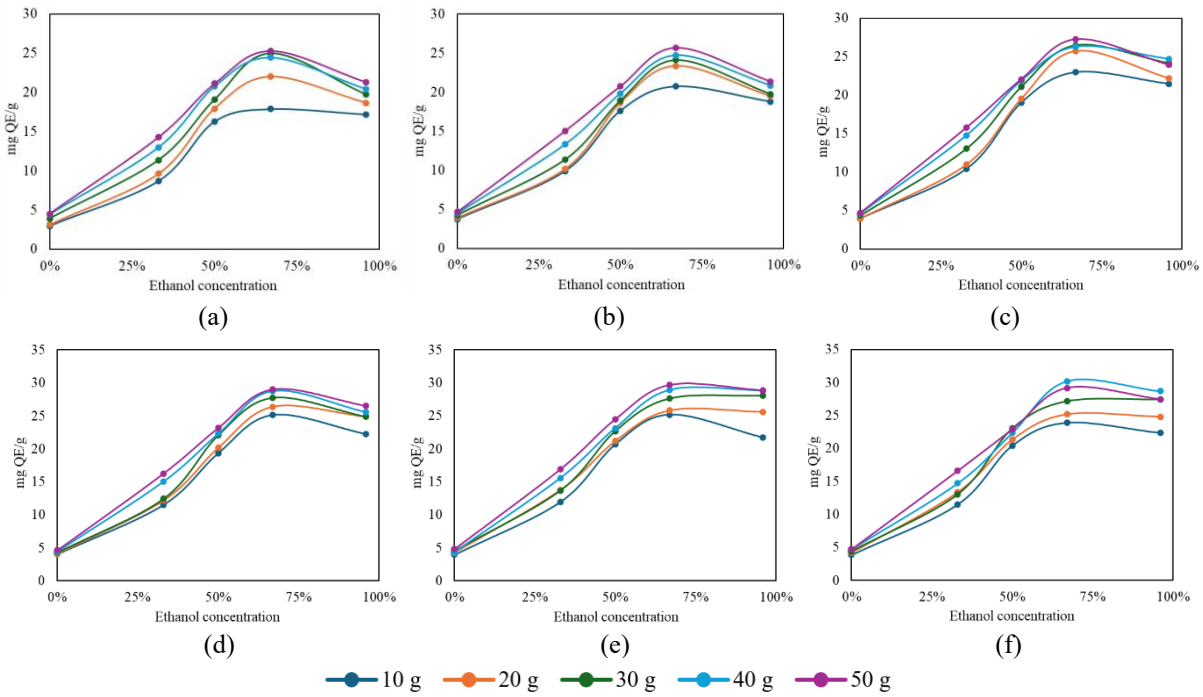


Figure 4. Relationship between solvent concentration and antioxidant content at various masses and maceration times: (a) 1 day, (b) 3 days, (c) 5 days, (d) 7 days, (e) 9 days, and (f) 11 days.

In this study, the antioxidant capacity of the bay leaf extract was evaluated by the iodometric titration method and the extract showed an antioxidant capacity of about 30.7394 mg QE/g sample, meaning that the extract had a relatively high reducing power against iodine. The value given as Quercetin Equivalent (QE) is the total antioxidant activity of the extract due to the presence of antioxidant compounds mainly phenolics and flavonoids. The high obtained value of QE points out that the maceration conditions used in this study were effective in extracting bioactive compounds from bay leaves.

The results of this study are in line with the research of Bahriul *et al*. (2014) and Wilapangga and Sari (2018) who used the DPPH method in testing the antioxidant activity of bay leaf extract. Bahriul *et al*. (2014) reported an IC_{50} value of 11,001 ppm in old leaves, while Wilapangga and Sari (2018) obtained an IC_{50} value of 19.97 ppm, both of which belong to the category of very strong antioxidants. The low IC_{50} value indicates that bay leaf extract has a high ability to capture free radicals. In the DPPH method, antioxidant activity is shown by a decrease in absorbance due to the radical reduction of DPPH from purple to yellow after reacting with antioxidant compounds. Bahriul et al. stated that the higher the concentration of the extract, the greater the decrease in DPPH absorbance that occurs. These results are in accordance with this study, where the optimization of maceration time and solvent ratio increases the antioxidant content of bay leaf extract. This shows that the increase in extraction efficiency affects the number of phenolic compounds and flavonoids extracted, so that the antioxidant activity is higher.

Statistical Analysis of Extraction Parameters

The statistical significance of the extraction parameters and their interactions on the final antioxidant yield was determined by analysis of variance (ANOVA). The summary of this statistical model is shown in Table 1. Results showed that the linear terms of maceration time (A), ethanol concentration (B), and crude plant material mass (C) significantly affected the antioxidant capacity ($p < 0.05$). Moreover, highly significant quadratic terms and some binary interactions, especially between ethanol concentration and maceration time, were found, confirming strong non-linear relationships within the optimization space.

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Table 1. Analysis of Variance (ANOVA) for the regression model.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	9	18923.8	2102.65	460.62	0.000
A – Maceration Time	1	27.2	27.22	5.96	0.015
B – Ethanol Concentration	1	2902.7	2902.66	635.88	0.000
C – Crude Plant Material Mass	1	28.1	28.15	6.17	0.014
A^2	1	34.4	34.40	7.54	0.006
B^2	1	2203.2	2203.25	482.66	0.000
C^2	1	9.9	9.88	2.16	0.142
AB	1	201.4	201.44	44.13	0.000
AC	1	0.0	0.01	0.00	0.966
BC	1	40.2	40.20	8.81	0.003
Error	290	1323.8	4.56		
Lack-of-Fit	140	1307.5	9.34	86.24	0.000
Pure Error	150	16.2	0.11		
Total	299	20247.6			

The experimental data were used to develop a mathematical model to predict the correlation between the independent variables and the response. The second-order polynomial regression equation (Equation 1) describes the experimental relationship between the maceration time (A), ethanol concentration (B), the mass of crude plant material (C), and the predicted antioxidant capacity (Y, mg QE/g sample).

$$\text{mg QE/g} = -0.19 + 0.438A + 40.22B + 0.1228C - 0.0339A^2 - 26.10B^2 - 0.001084C^2 + 0.745AB - 0.00011AC + 0.0803BC \quad (1)$$

The coefficient of determination (R^2) was used to assess the quality and goodness of fit of the developed regression model. The model had a high R^2 value of 93.46% which showed that 93.46% of the variability in the antioxidant extraction content could be accurately explained by the independent variables evaluated and only 6.54% of the total variations due to unexplainable factors or pure error. The adjusted R^2 (93.26%) and predicted R^2 (93.08%) were in good agreement with R^2 value (difference less than 20%). This closeness shows the high reliability of the model, not overfitted, and has good predictive capability to determine the antioxidant capacity within the experimental range.

The coefficients of this regression model are positively signed, indicating a synergistic effect, i.e., the increase in these parameters improves the efficiency of antioxidant extraction. In contrast, the negative coefficients show an antagonistic effect, which means that the output decreases when the values increase. The relatively high positive coefficient of ethanol concentration indicated its dominant role in governing the solubility and mass transfer of bioactive compounds from the *Syzygium polyanthum* matrix. In contrast, the negative quadratic coefficients indicate a different curvature in the response surface, mathematically proving the effect of operating beyond the optimum thresholds, inducing solvent saturation or the potential degradation of the target antioxidant compounds.

Economic Outlook

Economically, the maceration method has benefits in capital expenditure (CAPEX) and operational expenditure (OPEX). The process requires only simple equipment, such as extraction tanks and stirrers, so the investment cost is much lower than advanced methods such as Ultrasound-Assisted Extraction (UAE) and Microwave-Assisted Extraction (MAE), which require specialized ultrasonic or microwave systems (Chemat *et al*., 2012). In addition, maceration is performed at room temperature and atmospheric pressure, thus consuming less energy and maintenance costs than modern extraction technologies (Soquetta *et al*., 2018).

Maceration is simple and has low operational cost, although with a longer extraction time, making it economically suitable for small- to medium-scale production. The results of this study also showed that the antioxidant capacity of the crude plant material mass was successfully enhanced without the need for additional sophisticated equipment. Therefore, the combination of maceration extraction and iodometric analysis provides a simple, economic, and applicable method for antioxidant evaluation of natural products.

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CONCLUSION

The optimal conditions for the extraction of antioxidants from bay leaf (*Syzygium polyanthum*) were obtained at a maceration time of 9 days, a mass of crude plant material of 50 grams, and an ethanol solvent ratio of 2:1. Longer maceration periods lead to a decrease in antioxidant activity due to the degradation of bioactive compounds during the extraction process. In addition, the 2:1 ethanol solvent ratio shows the optimal ability to extract phenolic compounds and flavonoids because it is able to attract polar and semipolar compounds simultaneously through the balance of solvent polarity. Thus, optimization of extraction parameters is an important factor in obtaining a high and stable antioxidant content in bay leaves. The results of this research are expected to be the basis for the development and standardization of natural ingredient extraction processes, especially for applications in the functional food industry, herbal medicines, and antioxidant-based cosmetics.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper. There is no conflict of interest in this study.

AUTHOR CONTRIBUTION

BS: Review and Editing, Supervision, Methodology, Conceptualization; JYBT: Writing – Original Draft, Visualization, Validation, Investigation, Formal analysis; AS: Writing – Original Draft, Visualization, Validation, Investigation, Formal analysis; ATR: Writing – Original Draft, Visualization, Validation, Investigation, Formal analysis.

DECLARATION OF GENERATIVE AI

During the preparation of this work, the authors used ChatGPT in order to assist with data analysis and structuring the preliminary findings. The authors have used this tool/service. Then, the authors have reviewed, verified, and edited all generated material as needed to ensure scientific accuracy, academic rigor, and proper contextual flow. The authors bear full and sole responsibility for the integrity, validity, and overall content of the published article.

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