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Comparison of Antioxidant Activity of Extracts and Fractions of Bay Leaves (*Syzygium polyanthum* (Wight.) Walp.) Using the DPPH (2,2-diphenyl-1-picrylhydrazyl) Method

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Abstract

Free radicals contribute to oxidative stress and chronic diseases, driving interest in natural antioxidants. This study evaluated the antioxidant activity of bay leaf (*Syzygium polyanthum*) ethanol extract and its dichloromethane and ethyl acetate fractions. Extraction used 70% ethanol maceration, followed by liquid-liquid fractionation. Antioxidant capacity was assessed via the DPPH radical scavenging assay at various concentrations (50–200 ppm), with results expressed as half-maximal inhibitory concentration (IC_{50}) values. Phytochemical screening was also performed. The ethanol extract exhibited the strongest activity (IC_{50} = 47.89 ppm, classified as very strong), followed by the dichloromethane fraction (IC_{50} = 73.88 ppm, strong), while the ethyl acetate fraction showed weak activity (IC_{50} = 326.60 ppm). Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, and steroids, with distribution varying across fractions. Statistical analysis of inhibition percentages showed no significant difference ($p > 0.05$), likely due to limited replicates, but the IC_{50} trend clearly indicates superior radical scavenging by the polar constituents of the crude ethanol extract.

Keywords: Antioxidant, DPPH, Fractionation, *Syzygium polyanthum,* IC_{50}

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1 Introduction

Oxidative stress, resulting from an imbalance between free radicals and the body's antioxidant defense system, is implicated in the development of numerous degenerative diseases, including diabetes, cardiovascular disorders, and cancer [1]. Synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commonly used but raise safety concerns regarding potential toxicity and carcinogenicity with long-term use [2]. Consequently, the search for effective and safe natural antioxidants from medicinal plants has gained significant momentum [14] [16].

Indonesia's rich biodiversity harbors numerous plants with therapeutic potential. Bay leaf (*Syzygium polyanthum* (Wight.) Walp.), locally known as "daun salam," is extensively used as a culinary spice and in traditional medicine for treating hyperglycemia, hypertension, gastritis, and hyperuricemia [3]. Its pharmacological properties are attributed to a diverse array of secondary metabolites, including flavonoids, alkaloids, tannins, saponins, and terpenoids, which are known for their antioxidant capacities [4].

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay is a widely adopted, rapid, and economical method for preliminary evaluation of antioxidant activity due to its stability and simple protocol [5]. The activity is often expressed as the IC_{50} value, representing the concentration required to scavenge 50% of DPPH radicals.

Solvent-solvent partitioning (fractionation) is a crucial step in bioassay-guided isolation. It separates crude extracts based on compound polarity, helping to pinpoint fractions with the highest bioactivity and providing clues about the nature of the active constituents [6]. Polar solvents like ethanol are generally effective in extracting a broad spectrum of phenolic antioxidants.

Previous studies have reported the antioxidant activity of *S. polyanthum* extracts [7], [8]. However, a comparative evaluation of the antioxidant potency of its fractions obtained through sequential partitioning with solvents of varying polarity (dichloromethane, ethyl acetate) remains limited. This study, therefore, aimed to compare the DPPH radical scavenging activity of the ethanol extract and its derived fractions from *S. polyanthum* leaves and to identify the most active fraction for potential future application.

2 Method

2.1 Plant Material and Extraction

Fresh leaves of *Syzygium polyanthum* were collected from Bekasi, West Java, in January 2025. The plant was identified and authenticated at the UPT Herbal Materia Medica Laboratory, Batu, East Java (voucher specimen no: 01/SP/II/2025). The leaves were washed, air-dried in the shade, and ground into a fine powder (40 mesh). One kilogram of the powder was macerated with 10 L of 70% ethanol for 24 hours with occasional stirring. The filtrate was concentrated using a rotary evaporator (Heidolph, Germany) at 50°C to obtain a crude ethanol extract (CEE).

2.2 Solvent-Solvent Fractionation

The liquid-liquid fractionation was performed on the CEE. Briefly, 10 g of CEE was dissolved in 10 mL of warm water and successively partitioned three times with an equal volume of dichloromethane (DCM) in a separatory funnel. The combined DCM layers were evaporated to yield the DCM fraction (FD). The remaining aqueous layer was then similarly partitioned three times with ethyl acetate (EtOAc) to obtain the ethyl acetate fraction (FE). The residual aqueous layer was discarded. All fractions were stored at 4°C until use.

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2.3 Phytochemical Screening

Standard qualitative chemical tests were performed on the CEE, FD, and FE to detect the presence of major phytochemical groups: alkaloids (Meyer's, Wagner's, Dragendorff's, and Bouchardat's reagents), flavonoids (Mg-HCl test), steroids/triterpenoids (Liebermann-Burchard test), tannins (FeCl3 test), and saponins (foam test) [9] [15].

2.4 DPPH Radical Scavenging Assay

The antioxidant activity was determined using the stable DPPH radical method with slight modifications [10]. A 0.1 mM stock solution of DPPH in ethanol was prepared. Each sample (CEE, FD, FE) and ascorbic acid (as a standard) was dissolved in methanol to prepare stock solutions of 500 ppm. Serial dilutions were made to obtain final test concentrations of 50, 100, 150, and 200 ppm for samples, and 2, 4, 6, 8, 10 ppm for ascorbic acid. In a test tube, 1 mL of sample/standard solution was mixed with 4 mL of DPPH solution. The mixture was vortexed and incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Thermo Scientific, USA). A control was prepared using 1 mL of methanol plus 4 mL of DPPH solution. The percentage of DPPH radical scavenging activity (% Inhibition) was calculated using the formula:

$$\%\text{Inhibition}=(A\text{control}/A\text{control}-A\text{sample})\times 100\%$$

where Acontrol is the absorbance of the control and Asample*A*sample is the absorbance of the sample/standard. The IC_{50} value (concentration providing 50% inhibition) was calculated from the linear regression equation of the plot of % inhibition against sample concentration.

2.5 Statistical Analysis

Data are presented as mean ± standard deviation (SD) of % inhibition. Normality and homogeneity of variances were assessed using Shapiro-Wilk and Levene's tests, respectively. Since each concentration per fraction was tested without replication for ANOVA, the % inhibition values from all fractions across all concentrations were combined into one dataset for a One-Way ANOVA to examine the overall effect of concentration on antioxidant activity, followed by a post-hoc test if applicable. Statistical significance was set at $p < 0.05$. All analyses were performed using IBM SPSS Statistics version 25.

3 Result and Discussion

3.1 Extraction Yield and Phytochemical Profile

The maceration of *S. polyanthum* leaf powder with 70% ethanol yielded 111.23 g of a viscous, dark brown extract, representing an 11.12% extraction yield. The phytochemical screening results (Table 1) confirmed the presence of alkaloids, steroids, flavonoids, tannins, and saponins in the CEE. Upon fractionation, the metabolite distribution shifted: the FD tested positive for alkaloids, steroids, flavonoids, and tannins; the FE was positive only for alkaloids and steroids. The absence of saponins in both FD and FE aligns with their highly polar nature, making them less soluble in semi-polar (EtOAc) and non-polar (DCM) solvents [11] [17]. The selective distribution validates the effectiveness of the fractionation process in segregating compounds based on polarity [18].

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Table 1 Results of Phytochemical Screening of Crude Ethanol Extract (CEE), Dichloromethane Fraction (FD), and Ethyl Acetate Fraction (FE) of *S. polyanthum* Leaves.

Metabolite Class	Test Reagent	CEE	FD	FE
Alkaloids	Mayer's	+	+	+
	Wagner's	-	+	-
	Dragendorff's	-	-	+
	Bouchardat's	+	+	+
Steroids/Triterpenoids	Liebermann-Burchard	+	+	+
Flavonoids	Mg-HCl reduction	+	+	-
Tannins	FeCl3	+	+	-
Saponins	Froth formation	+	-	-
(+) = Present, (-) = Absent			r	

3.2 Antioxidant Activity (DPPH Assay)

The DPPH radical scavenging activity of all samples increased in a concentration-dependent manner. The IC_{50} values, which inversely correlate with antioxidant strength, are summarized in Table 2. Ascorbic acid, the positive control, showed the most potent activity with an IC_{50} of 7.61 ppm. Among the tested samples, the CEE demonstrated the strongest activity (IC_{50} = 47.89 ppm), classified as "very strong" according to standard criteria (IC_{50} < 50 µg/mL) [12]. The FD showed "strong" activity (IC_{50} = 73.88 ppm), while the FE exhibited only "weak" activity (IC_{50} = 326.60 ppm) [20] [22].

Table 2 IC_{50} Values and Antioxidant Strength of Samples.

Sample	IC_{50} (ppm)	Antioxidant Strength Category
Ascorbic Acid (Standard)	7.61 ± 0.15	Very Strong
Crude Ethanol Extract (CEE)	47.89 ± 1.82	Very Strong
Dichloromethane Fraction (FD)	73.88 ± 2.41	Strong

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Sample	IC_{50} (ppm)	Antioxidant Strength Category
Ethyl Acetate Fraction (FE)	326.60 ± 8.75	Weak
Data presented as mean ± SD (n=3).		

The superior activity of the CEE suggests a synergistic effect of the diverse phytochemicals present in the crude extract. Phenolic compounds like flavonoids and tannins are potent hydrogen donors and are typically more soluble in polar solvents like ethanol/water mixtures [13]. Their concentration in the CEE is likely higher than in the partitioned fractions, explaining its low IC_{50} . The FD, containing medium-polarity compounds like some flavonoids and less polar antioxidants, showed moderate activity. The weak activity of the FE suggests that the compounds partitioning into ethyl acetate under these conditions contribute minimally to radical scavenging.

The One-Way ANOVA on the combined % inhibition data from all fractions across concentrations (50-200 ppm) showed no statistically significant difference ($F(3, 8) = 0.93$, $p = 0.460$). This non-significance is likely an artifact of the limited sample size (single measurement per concentration per fraction for the ANOVA model) and the high variance introduced by the weak FE data. However, the clear and consistent trend in the directly calculated IC_{50} values (Table 2) provides a more reliable indicator of the actual bioactivity gradient: CEE > FD > FE [25].

3.3 Implications and Future Perspectives

The findings confirm that *S. polyanthum* is a rich source of natural antioxidants, primarily concentrated in the polar fraction. The ethanol extract, with its very strong activity (IC_{50} - 48 ppm), holds potential for development into standardized herbal preparations or as a source of nutraceuticals. Future work should focus on replicating the bioassay with more robust statistical design, identifying and quantifying the specific phenolic compounds (e.g., via HPLC) responsible for the activity, and evaluating other antioxidant mechanisms (e.g., FRAP, ABTS) and in vivo models to confirm efficacy [25].

4 Conclusion

This study successfully compared the antioxidant potential of *Syzygium polyanthum* leaf extract and its fractions. The crude ethanol extract (70%) displayed the most potent DPPH radical scavenging activity, significantly stronger than its dichloromethane and ethyl acetate fractions. Phytochemical analysis revealed a broad spectrum of metabolites in the crude extract, which may act synergistically. While statistical analysis of the combined dataset showed no significant difference, the IC_{50} values unequivocally demonstrate that the polar antioxidants in the ethanol extract are primarily responsible for the activity. *Syzygium polyanthum* crude ethanol extract is a promising candidate for further investigation as a natural antioxidant agent.

5 Declarations

5.1 Acknowledgements

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5.2 Author contributions

Moh. Firman Irwanto.: Conceptualization, Supervision, Writing – Review & Editing. **Andi Tenri Nurwahidah.:** Methodology, Formal Analysis, Supervision. **Fiki Ita'ul Badiyah.:** Investigation, Data Curation, Writing – Original Draft Preparation. All authors have read and agreed to the published version of the manuscript.

5.3 Ethics

This study did not involve human or animal subjects. The plant material was collected and used in accordance with institutional guidelines.

5.4 Conflict of Interest

The authors declare no conflict of interest

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