

Free Radical Scavenging Activity of 2,2-diphenyl-1-picrylhydrazil from Ethanolic Extract of Salam Leaves (*Syzygium polyanthum*)

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Syzygium polyanthum (Salam) leaves are widely utilized as both a culinary spice and a traditional medicinal plant in Indonesia. They are rich in phenolic compounds, which are known to exhibit potent antioxidant properties. This study aimed to quantitatively evaluate the in vitro antioxidant activity of the ethanolic extract of *S. polyanthum* leaves. The antioxidant capacity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay via visible spectrophotometry. The extract was tested at concentrations ranging from 12.5 to 200 ppm, with ascorbic acid serving as the positive control. The median inhibitory concentration (IC₅₀) was calculated using linear regression analysis. The DPPH radical exhibited a maximum absorption wavelength (λ_{\max}) at 517.8 nm. The ethanolic extract of *S. polyanthum* leaves demonstrated a concentration-dependent radical scavenging activity, achieving 95.27 ± 0.17 % inhibition at 200 ppm. The calculated IC₅₀ value for the extract was 36.37 ± 6.02 ppm, whereas the ascorbic acid standard exhibited an IC₅₀ of 1.36 ± 0.08 ppm. According to the standard antioxidant potency classification, the ethanolic extract of *S. polyanthum* leaves falls into the "very strong" antioxidant category (IC₅₀ < 50 ppm). These findings suggest that *S. polyanthum* leaves represent a highly promising natural source for the development of functional antioxidant products.



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In recent years, oxidative stress has garnered significant attention in biomedical research due to its critical role in the pathogenesis of various chronic and degenerative conditions, including cardiovascular diseases, diabetes, neurodegenerative disorders, and cancer. Oxidative stress occurs when there is a severe imbalance between the systemic production of reactive oxygen species (ROS) or free radicals and the biological system's ability to readily detoxify

these reactive intermediates [1]. Free radicals are highly unstable atoms or molecules possessing one or more unpaired electrons, making them fiercely reactive as they seek to capture electrons from cellular components such as lipids, proteins, and DNA. To counteract this cellular damage, the deployment of antioxidants—compounds capable of neutralizing free radicals—is imperative [2]. Although synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are widely

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available, concerns regarding their potential long-term toxicity and carcinogenic risks have driven a global shift toward identifying safe, effective, and sustainable natural antioxidants derived from botanical sources [3].

Salam (*Syzygium polyanthum*) is a well-known plant that is predominantly utilized as a culinary spice to enhance flavor in traditional Indonesian dishes. Beyond its widespread culinary applications, *S. polyanthum* leaves have historically been integrated into traditional medicine to manage various ailments. Empirical evidence and pharmacological studies have validated that *S. polyanthum* leaves possess a diverse spectrum of biological activities, including anti-inflammatory, antifungal, antimicrobial, and anti-cancer properties. These multi-faceted therapeutic benefits are intimately linked to the plant's rich phytochemistry. Previous phytochemical screenings [4] have revealed that the ethanolic extract of *S. polyanthum* leaves contains a high concentration of secondary metabolites belonging to the phenolic group. The use of Salam leaves as medicinal plants includes being used as anti-inflammatory [5], antioxidants [6] and antifungal [7].

Phenolic compounds, including simple phenols and polyphenols, constitute one of the largest and most ubiquitous groups of natural antioxidants in the plant kingdom. Structurally, these molecules are characterized by the presence of at least one aromatic ring bearing one or more hydroxyl (-OH) functional groups. This specific configuration enables them to act as highly efficient hydrogen donors. When a phenolic compound encounters a free radical, it readily donates a hydrogen atom to neutralize the radical's unpaired electron. The resulting phenoxyl radical intermediate is remarkably stable because its unpaired electron can be effectively delocalized across the conjugated π -electron system of the aromatic ring, thereby successfully halting the chain reaction of oxidative damage [8]. Given this biochemical background, exploring the precise radical scavenging capacity of *S.*

polyanthum leaf extracts is crucial for establishing its empirical value in functional food and pharmaceutical industries

To quantitatively evaluate this antioxidant potential, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was employed in this study. The DPPH method is universally preferred for primary antioxidant screenings because it offers a simple, rapid, cost-effective, and highly sensitive platform to measure the radical-scavenging efficiency of plant extracts *in vitro*. It operates based on a distinct spectrophotometric shift, allowing researchers to evaluate the precise concentration required to inhibit 50% of the radical population (IC_{50} value). Therefore, this research was designed to systematically assess the *in vitro* antioxidant activity of the ethanolic extract of *S. polyanthum* leaves through the DPPH radical scavenging assay. By determining its exact IC_{50} value and comparing it with a standard antioxidant reference, this study aims to provide robust scientific justification for the development of *S. polyanthum* leaves as a premium, natural product-based antioxidant agent.

Results and Discussion

The evaluation of the antioxidant capacity of the *Syzygium polyanthum* leaf ethanolic extract was successfully executed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method via visible spectrophotometry. The DPPH assay is widely regarded as a rapid, reliable, and highly reproducible screening tool for assessing the radical-scavenging efficiency of plant-derived extracts [9]. This test begins with screening the maximum wavelength of DPPH at a concentration of 32 $\mu\text{g/mL}$ at a wavelength of 400-800 nm. The wavelength reading in this range is because the DPPH solution is a dark violet compound that can be analyzed in visible light.

Prior to the sample analysis, a baseline screening was conducted to identify the maximum absorption wavelength (λ_{max}) of the DPPH radical solution (32 $\mu\text{g/mL}$) within the visible spectrum of 400–800 nm. As illustrated in Figure 1, the radical

demonstrated a sharp, distinct absorption peak at 517.8 nm. This deep violet color is a direct consequence of the stable free radical character of DPPH, which features a highly delocalized spare electron across its aromatic chromophore and auxochrome network. The obtained λ_{\max} of 517.8 nm

perfectly aligns with prior literature reports [10, 11], who reported a maximum absorbance around 517 nm. It also closely matches the 515 nm wavelength recorded [12], confirming the structural integrity and stability of the radical reagent used in this experiment.

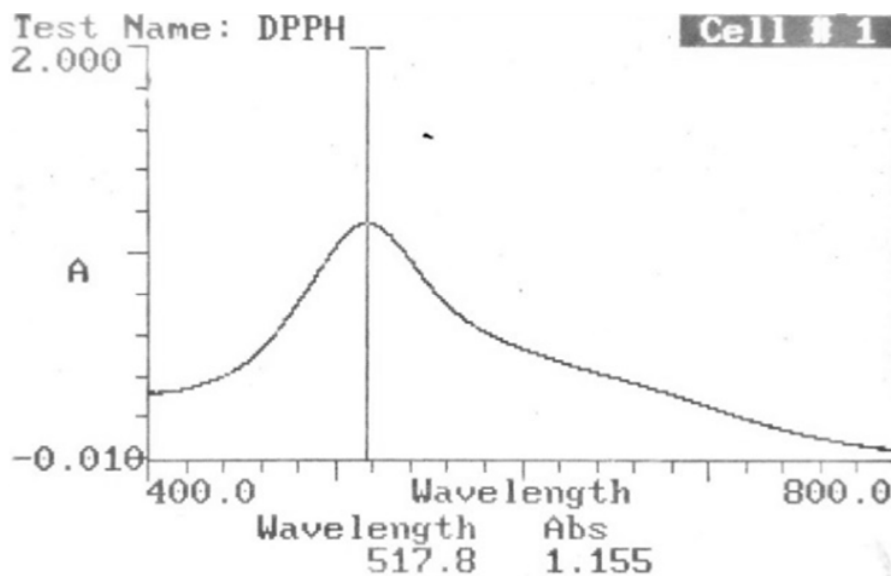


Figure 1. DPPH maximum wavelength screening results at 517.8 nm

DPPH solution can provide absorption because it has chromophore and auxochrome groups in its chemical structure and the delocalization of electrons in DPPH will give a violet color [13]. The fundamental chemical principle underlying this assay revolves around the colorimetric shift of the reaction mixture. When the dark purple DPPH radical accepts an electron or a hydrogen atom from an antioxidant donor, its radical character is neutralized, converting it into the non-radical form (2,2-diphenyl-1-picrylhydrazine). This structural alteration triggers a visual discoloration from dark violet to light yellow, which can be quantitatively monitored via the decreasing absorbance at 517.8 nm [13].

This study used ascorbic acid as a comparison because ascorbic acid is an antioxidant compound that has very high antioxidant activity [14]. The quantitative data summarized in **Table 1** reveals a clear, dose-dependent relationship for both the plant extract and the reference compound. For the *S.*

polyanthum extract, as the concentration escalated from 12.5 $\mu\text{g/mL}$ to 200 $\mu\text{g/mL}$, the percent inhibition systematically climbed from $33.83 \pm 2.87\%$ to a near-complete inhibition of $95.27 \pm 0.17\%$. A similar progressive trend was mirrored by the ascorbic acid standard, which moved from $24.08 \pm 0.77\%$ inhibition at 0.125 $\mu\text{g/mL}$ to $63.97 \pm 0.80\%$ at 2 $\mu\text{g/mL}$.

Table 1. DPPH inhibitory test results

Groups	Concentration ($\mu\text{g/mL}$)	% IC
Salam Leaf	12.5	33.83 ± 2.87
Ethanol	25	42.60 ± 5.05
Extract	50	60.70 ± 5.09
	100	83.20 ± 2.92
	200	95.27 ± 0.17
Ascorbic	0.125	24.08 ± 0.77
Acid	0.25	25.79 ± 0.91
	0.5	32.65 ± 2.56
	1	41.16 ± 4.44
	2	63.97 ± 0.80

IC = Inhibitory Concentration

Antioxidant activity in this study was expressed in IC_{50} values. To precisely evaluate the antioxidant potency, the linear regression equations ($y = bx + a$) mapping the concentration (x) against the percent inhibition (y) were constructed to derive the Inhibitory Concentration 50 (IC_{50}). The IC_{50} value indicates the exact concentration of a substance required to scavenge 50% of the initial DPPH radicals in the reaction environment.

Table 2. IC_{50} values for DPPH free radicals

IC_{50} values for DPPH free radicals ($\mu\text{g/mL}$)	
Salam Leaf Ethanol Extract	36.37 ± 6.02
Ascorbic Acid	1.36 ± 0.08

As tabulated in **Table 2**, the IC_{50} of the *S. polyanthum* ethanolic extract was found to be $36.37 \pm 6.02 \mu\text{g/mL}$. This finding strongly corroborates the data published [12], who documented an IC_{50} value of $31.14 \mu\text{g/mL}$ for a similar ethanolic extract of Salam leaves. When juxtaposed with the pure ascorbic acid reference ($IC_{50} = 1.36 \pm 0.08 \mu\text{g/mL}$), the crude plant extract naturally exhibits lower absolute antioxidant activity. This difference is entirely expected from a pharmacological standpoint: ascorbic acid is a pure, highly optimized single compound renowned for its exceptional electron-donating capacity. Conversely, the crude ethanolic extract is a complex matrix

containing a mixture of various non-antioxidant ballast molecules along with active phenolic constituents [14]. Nevertheless, when evaluated against the standard antioxidant intensity index established, any substance that achieves an IC_{50} value below $50 \mu\text{g/mL}$ is classified as a "very strong" antioxidant [8]. Because the IC_{50} of *S. polyanthum* ($36.37 \pm 6.02 \mu\text{g/mL}$) sits comfortably below this $50 \mu\text{g/mL}$ threshold, it officially qualifies as a highly potent natural radical scavenger. This exceptional classification highlights the tremendous potential of *S. polyanthum* leaves as a premium bio-resource for isolating natural antioxidants or formulating therapeutic functional health products.

Conclusion

Salam leaf ethanol extract has antioxidant activity by inhibiting DPPH radicals. The IC_{50} value of the ethanol extract of Salam leaves against DPPH radicals was $36.37 \pm 6.02 \mu\text{g/mL}$ with the intensity category of very strong antioxidant inhibition.

Research Method

Material and Equipment

The materials used in this study included the ethanol extract of Salam leaves obtained from Yunita's research [15], pro-analytical ethanol (Merck), 96% technical ethanol, DPPH (Himada), ascorbic acid (Merck), and distilled water. Antioxidant activity test was carried out using UV-Vis Spectrophotometer (Genesys S10).

Preparation of DPPH Solution

The DPPH solution was prepared at a concentration of $160 \mu\text{g/mL}$ [10] by weighing 0.004 grams of DPPH powder and then dissolving it in 25 mL of ethanol until dissolved.

DPPH Maximum Wavelength Measurement

DPPH solution $160 \mu\text{g/mL}$ was pipetted 0.4 mL, then 1.6 mL of ethanol was added and left for 30 minutes in a dark place. Maximum wavelength

screening was carried out at a wavelength of 400-800 nm. The maximum wavelength obtained is 517.8 nm.

Measurement of Percent Inhibition of DPPH From Ethanol Extract of Salam Leaves

The test solution for the ethanol extract of Salam leaves was made at a concentration of 12.5 µg/mL; 25 µg/mL; 50 µg/mL; 100 µg/mL; and 200 µg/mL. Each solution was then added with DPPH solution, then allowed to stand in a dark place for 30 minutes. The absorbance reading was carried out at a wavelength of 517.8 nm. Ascorbic acid as a comparison was tested for its antioxidant activity at a concentration of 0.125 µg/mL; 0.25 µg/mL; 0.5 µg/mL; 1 µg/mL; and 2 µg/mL. Furthermore, ascorbic acid was prepared in the same way as the ethanol extract of Salam leaves. The antioxidant activity test of Salam leaf extract and ascorbic acid was replicated 3 times each.

Data Analysis

The DPPH radical scavenging activity is expressed in percent inhibition concentration (%IC) which is calculated using formula (1).

$$\%IC = 1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \times 100\% \quad [16].$$

Notes:

A_{control} : Control absorbance

A_{sample} : Sample absorbance

The DPPH radical scavenging activity data were analyzed and the IC_{50} value was calculated using the linear regression equation $y = bx + a$, where x is the concentration of the reference solution or test solution and y is the %IC. The parameter to indicate the antioxidant activity of a compound is the value of Inhibition Concentration 50 (IC_{50}), namely the concentration of an antioxidant substance that can cause 50% of DPPH to lose its radical character [17]. Classification of the level of antioxidant strength by the DPPH method is presented in **Table 3**.

Table 3. Levels of DPPH antioxidant strength [8]

Intensity	IC_{50}
Very strong	< 50 µg/ml
Strong	50-100 µg/ml
Currently	101-150 µg/ml
Weak	>150 µg/ml

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