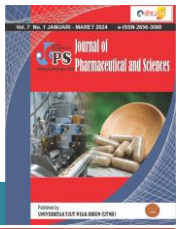
Journal of Pharmaceutical and Sciences
Electronic ISSN: 2656-3088 DOI: https://doi.org/10.36490/journal-jps.com
Homepage: https://journal-jps.com
ORIGINAL ARTICLE JPS. 2026, 9(1), 60-67



Identification of Active Compounds in Dadap Serep Leaves (*Erythrina subumbrans* (Hassk.) Merr) and Their Potential as Antioxidants

Identifikasi Senyawa Aktif dalam Daun Dadap Serep (*Erythrina subumbrans* (Hassk.) Merr) dan Potensinya sebagai Antioksidan

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Abstract

Background: *Erythrina subumbrans* (Hassk.) Merr, locally known as dadap serep, is a medicinal plant traditionally used to treat various ailments, suggesting the presence of bioactive compounds. Scientific exploration of its phytochemical profile and antioxidant potential, however, remains limited compared to other species in the genus. **Objective:** This study aimed to (1) identify the bioactive compound groups in the 70% ethanol extract of *Erythrina subumbrans* leaves through phytochemical screening and Thin Layer Chromatography (TLC), and (2) evaluate its antioxidant activity quantitatively using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. **Methods:** The leaves were extracted via maceration using 70% ethanol. The extract was subjected to standard phytochemical screening tests for flavonoids, alkaloids, saponins, and tannins. Flavonoid profiling was performed using TLC with a quercetin standard. Antioxidant activity was assessed by the DPPH method, with vitamin C as a reference standard, and the IC_{50} value was determined. **Results:** Phytochemical screening confirmed the presence of flavonoids, alkaloids, saponins, and tannins. TLC analysis indicated the presence of flavonoid compounds, with one major spot showing an Rf value similar to the quercetin standard. The DPPH assay revealed that the extract possesses strong antioxidant activity, with an IC_{50} value of 49.94 ppm. According to established antioxidant strength criteria, this IC_{50} value is categorized as very strong.**Conclusion:** The 70% ethanol extract of *Erythrina subumbrans* leaves contains diverse bioactive compounds, particularly flavonoids, and exhibits very strong antioxidant activity. These findings scientifically support its traditional use and highlight its potential as a promising source of natural antioxidants for further pharmaceutical or nutraceutical development.

Keywords*: *Erythrina subumbrans, TLC, DPPH, antioxidant

Abstrak

Latar Belakang: *Erythrina subumbrans* (Hassk.) Merr yang dikenal sebagai dadap serep merupakan tanaman obat yang secara tradisional digunakan untuk mengobati berbagai penyakit, mengindikasikan adanya senyawa bioaktif. Namun, eksplorasi ilmiah mengenai profil fitokimia dan potensi antioksidannya masih sangat terbatas dibandingkan spesies lain dalam genus yang sama. **Tujuan:** Penelitian ini bertujuan untuk (1) mengidentifikasi golongan senyawa bioaktif dalam ekstrak etanol 70% daun *Erythrina subumbrans* melalui skrining fitokimia dan Kromatografi Lapis Tipis (KLT), serta (2) mengevaluasi aktivitas antioksidannya secara kuantitatif menggunakan metode peredaman radikal DPPH (2,2-difenil-1-pikrilhidrazil). **Metode:** Daun diekstraksi menggunakan metode maserasi dengan pelarut etanol 70%. Ekstrak yang diperoleh diuji dengan skrining fitokimia standar untuk mendeteksi flavonoid, alkaloid, saponin, dan tanin. Profil flavonoid dianalisis dengan KLT menggunakan standar kuersetin. Aktivitas antioksidan diuji dengan metode DPPH, menggunakan vitamin C sebagai pembanding, dan nilai IC_{50} ditentukan. **Hasil:** Skrining fitokimia mengonfirmasi keberadaan flavonoid, alkaloid, saponin, dan tanin. Analisis KLT menunjukkan adanya senyawa flavonoid, dengan satu bercak utama yang memiliki nilai Rf mendekati standar kuersetin. Uji DPPH menunjukkan bahwa ekstrak memiliki aktivitas antioksidan yang kuat dengan nilai IC_{50} sebesar 49,94 ppm.

Berdasarkan kriteria kekuatan antioksidan yang berlaku, nilai IC_{50} ini tergolong dalam kategori sangat kuat. **Kesimpulan:** Ekstrak etanol 70% daun *Erythrina subumbrans* mengandung berbagai senyawa bioaktif, khususnya flavonoid, dan menunjukkan aktivitas antioksidan yang sangat kuat. Temuan ini mendukung penggunaan tradisionalnya secara ilmiah dan menguatkan potensinya sebagai sumber antioksidan alami yang menjanjikan untuk pengembangan lebih lanjut dalam bidang farmasi atau nutrasetikal.

Kata Kunci: *Erythrina subumbrans, TLC, DPPH, antioksidan*

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Article History:
Received: 02/11/2025,
Revised: 24/12/2025,
Accepted: 25/12/2025,
Available Online: 17/01/2025.
QR access this Article

https://doi.org/10.36490/journal-jps.com.v9i1.1164

Introduction

Free radicals are unstable molecules generated naturally by metabolic processes (endogenous) or derived from external factors (exogenous) such as air pollution, ultraviolet radiation, cigarette smoke, and exposure to chemicals. Excessive free radicals lead to oxidative stress, defined as an imbalance between the production of free radicals and the capacity of the body's endogenous antioxidant defense system. Prolonged oxidative stress can cause oxidative damage to essential biomolecules such as DNA, proteins, and cell membrane lipids. The accumulation of this damage is a major trigger in the pathogenesis of various chronic degenerative diseases, including cancer, cardiovascular disease, diabetes mellitus, neurodegenerative disorders (such as Alzheimer's and Parkinson's), and the aging process [1, 2].

To counteract the adverse effects of free radicals, the body requires antioxidants, which can originate from within the body (endogenous) or from external sources (exogenous). Exogenous antioxidants are primarily obtained from food, supplements, and natural materials. Antioxidant compounds work by donating electrons or hydrogen atoms to neutralize free radicals, thereby halting the chain reaction of cellular damage. Currently, there is a global trend towards utilizing natural materials as safer antioxidant sources with minimal side effects compared to synthetic antioxidants such as BHA (Butylated Hydroxyanisole) and BHT (Butylated Hydroxytoluene), which are suspected of having carcinogenic potential with long-term use [3, 4]. Indonesia, as a mega-biodiversity country, possesses a vast wealth of medicinal plants that have been used traditionally, making them promising candidates for development as natural antioxidant sources.

One plant genus reported to contain various bioactive compounds is *Erythrina* (Fabaceae family). This genus is known to contain alkaloids, flavonoids, terpenoids, saponins, and tannins, which exhibit pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties [5, 6]. Several of its species, such as *E. variegata*, *E. crista-galli*, and *E. indica*, have been extensively studied. For instance, leaf extracts of *E. variegata* have been reported to possess strong DPPH radical scavenging activity, attributed to their high total flavonoid and phenolic content [7].

Erythrina subumbrans (Hassk.) Merr, locally known in Java as *dadap serep*, is one species also used in traditional medicine to treat fever, postpartum disorders, internal bleeding, and respiratory issues [8, 9]. This traditional use suggests the presence of bioactive compounds with specific physiological activities. However, compared to other *Erythrina* species, scientific research on *E. subumbrans* remains very limited, particularly concerning the comprehensive characterization of its active compounds and antioxidant potential. Some initial reports may mention its traditional use or basic phytochemical screening, but significant research gaps persist. These gaps include: (1) The lack of in-depth identification of specific active compounds (such as particular

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types of flavonoids or alkaloids) from the leaves of *E. subumbrans* using more advanced analytical methods beyond preliminary screening; (2) The antioxidant potential of *E. subumbrans* leaf extracts has not been extensively evaluated quantitatively using standard methods such as DPPH to determine a precise IC_{50} value; and (3) The relationship between the identified compound profile and the resulting antioxidant activity has not been elucidated.

Based on this background and the identified research gaps, this study aims to: (1) perform phytochemical screening and Thin Layer Chromatography (TLC) analysis on the 70% ethanol extract of *dadap serep* leaves to detect groups of bioactive compounds, with a focus on flavonoid identification; (2) evaluate the antioxidant activity potential of the extract using the DPPH radical scavenging method and determine its IC_{50} value; and (3) provide an initial scientific basis regarding the potential of *E. subumbrans* leaves as a natural antioxidant source for further development.

Experimental Section

Materials and Apparatus

The instruments used included an analytical balance, test tubes, micropipettes, blue tips, beakers, measuring cylinders, chromatography chamber, UV-Vis spectrophotometer, oven, water bath, separating funnel, glass jars, filter paper, aluminum foil, grinder, silica gel, and UV lamp.

The materials used included ethanol extract of dadap serep leaves (*Erythrina subumbrans* (Hassk.) Merr), 70% ethanol, quercetin, acetone, distilled water, magnesium powder (Mg), ascorbic acid, HCl, $FeCl_3$, Mayer's reagent, and DPPH.

Preparation of Extract

Dadap serep leaf simplicia powder was extracted using maceration. A total of 200 g of powder was soaked in 1500 mL of 70% ethanol in a glass container, covered, and left for 5 days in the dark with occasional stirring. The mixture was filtered, stored in a closed vessel for 1 day in a cool, dark place, and then filtered again. The filtrate was evaporated using a water bath until a thick extract was obtained [3].

Phytochemical Screening

Phytochemical screening was conducted using standard colorimetric assays. For flavonoid detection, 0.5 g of extract was dissolved in 10 mL of 70% ethanol and stirred. The solution was filtered, and 2 mL of the filtrate was transferred to a test tube. Subsequently, 0.5 g of magnesium (Mg) powder and a few drops of concentrated HCl were added. The formation of a dark red to purplish color indicated the presence of flavonoids [4]. For alkaloid detection, 0.5 g of extract was mixed with 2 mL of 70% ethanol, followed by the addition of 5 mL of 2 N HCl and heating in a water bath. After cooling, the mixture was filtered, and a few drops of Mayer's reagent were added to the filtrate. The appearance of turbidity or precipitate confirmed the presence of alkaloids [5]. Saponins were tested by dissolving 0.5 g of extract in 2 mL of 70% ethanol, adding 20 mL of distilled water, and shaking vigorously. The formation of stable foam persisting for less than 10 minutes indicated saponin content [5]. For tannin detection, 0.5 g of extract was mixed with 2 mL of 70% ethanol, and 3 drops of $FeCl_3$ solution were added. A blackish-green color change confirmed the presence of tannins [5].

Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) was carried out to separate and identify the active compounds in the leaves extract of *Erythrina subumbrans*. First, the prepared leaf extract was dissolved in 70% ethanol at a concentration of 100 mg/mL. Then, 10 µL of the extract solution and 10 µL of the quercetin standard solution with the same concentration were spotted onto a 10 cm × 5 cm GF254 silica gel plate using a micropipette, ensuring sufficient distance between the spots to prevent mixing during separation.

The plate was then placed in a chamber saturated with the mobile phase, which consisted of a mixture of chloroform, acetone, and formic acid in a ratio of 10:2:1, and left until the solvent reached the predetermined limit, approximately 8 cm from the base of the plate. After the separation process was complete, the plate was air-dried and observed under ultraviolet light at a wavelength of 366 nm. To enhance the visualization of the spots, the plate was sprayed with AlCl3 reagent, and any color changes detected indicated the presence of flavonoid compounds.

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For the calculation of the Retention Factor (Rf), the distance traveled by the compounds and the distance traveled by the solvent were recorded, where the Rf value was calculated using the established formula, namely the distance traveled by the compound divided by the distance traveled by the solvent [6].

Antioxidant Activity Test Using DPPH

The antioxidant activity test was conducted to evaluate the ability of *Erythrina subumbrans* leaf extract to neutralize DPPH free radicals. The DPPH solution was prepared by dissolving 0.04 g of DPPH powder in 25 mL of 70% ethanol, resulting in a solution with a concentration of approximately 1.6 mM. From this DPPH solution, 1 mL was taken and diluted with 4 mL of 70% ethanol, making a total volume of 5 mL, which was then left for 30 minutes in the dark to avoid light interference.

Afterward, 1 mL of the diluted DPPH solution was mixed with 5 mL of the ethanol extract of *Erythrina subumbrans* leaves at various concentrations 5 ppm, 10 ppm, 25 ppm, and 50 ppm [7], and then incubated for 30 minutes in the dark. The absorbance of the resulting mixture was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. The same measurements were also performed for vitamin C as the reference solution at the same concentrations. To clarify the final concentrations, the 1:1 mixing ratio between DPPH and the sample solution results in a final sample concentration equal to half of its initial stock concentration. Likewise, the DPPH concentration used for absorbance measurement becomes 0.8 mM after dilution from the 1.6 mM stock.

The percentage of DPPH free radical inhibition was calculated using a formula that measures the difference between the absorbance of the blank and that of the sample solution. With the steps described, this study is expected to produce valid data regarding the active compounds and the potential antioxidant activity of *Erythrina subumbrans* leaves. The antioxidant activity test was carried out by calculating the inhibition percentage obtained from the absorbance data using the following formula:

$$\%h = \frac{Ab - As}{Ab} \; x \; 100\%$$

Description:
%h= inhibition (free radical scavenging activity)
Ab= Absorbance of blank
As= Absorbance of sample

Results and Discussion

A total of 200 g of simplicia powder was macerated in 1.5 L of 70% ethanol for five days with occasional stirring. Ethanol 70% was selected as the extraction solvent due to its intermediate polarity, which effectively extracts both polar and non-polar secondary metabolites [8]. Maceration was chosen for its simplicity, cost-effectiveness, and ability to promote the release of intracellular compounds through osmotic pressure differences between the inside and outside of plant cells [9]. The obtained macerate was filtered and evaporated using a rotary evaporator to remove the solvent, yielding a concentrated extract with a specific rendemen value (Table 1).

Table 1. Weight and Yield of Dadap Serep Leaf Extract

Sample	Weight of Simple Substance (grams)	Extract Weight (grams)	Yield (%)	Thick Extract Color	Odor
Dadap Serep Leaves	200	24,83	12,41%	Deep dark green	The distinctive aroma of dadap serep leaves

Phytochemical screening revealed that the ethanolic extract of *Erythrina subumbrans* leaves contained flavonoids, alkaloids, saponins, and tannins (Table 2) and (Figure 1). The alkaloid test using Mayer's reagent produced a turbid precipitate, confirming the presence of alkaloids known for their ability to terminate free radical chain reactions [10]. The flavonoid test with magnesium powder and concentrated HCl yielded a dark red coloration, consistent with the reduction reaction typical of flavonoid compounds [4]. The saponin test produced stable foam that persisted for approximately 10 minutes, indicating the presence of amphipathic saponins that reduce surface tension [11]. Meanwhile, the tannin test with $FeCl_3$ resulted in a bluish-black coloration due to the formation of a complex between polyphenolic tannins and Fe^{3+} ions [12]. These results

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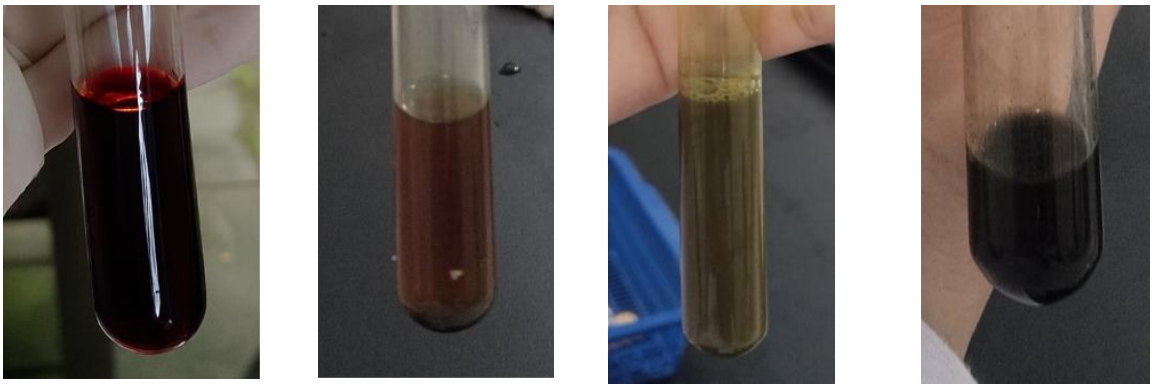
confirm that *Erythrina subumbrans* leaves are rich in secondary metabolites with potential antioxidant properties.

Thin Layer Chromatography (TLC) was performed for qualitative identification of compounds in the ethanolic extract. Silica gel 60 GF254 plates served as the stationary phase, while a mixture of chloroform:acetone:formic acid (10:2:1) was used as the mobile phase, suitable for flavonoid separation [13]. Spots were visualized under UV light at 366 nm, and retention factor (Rf) values were calculated to compare sample components with known standards. The TLC analysis successfully separated several compounds in the extract, producing distinct spots with visible fluorescence under UV 366 nm, suggesting the presence of flavonoid constituents. The Rf values obtained supported the polarity characteristics of the detected compounds [14] the (Figure 2).



a b c d

Figure 1. Phytochemical screening results of Dadap serep leaves, showing the presence of (a) flavonoids, (b) alkaloids, (c) saponins, and (d) tannins.

Table 2. Identification of Secondary Metabolites in the 70% Ethanol Extract of Dadap Serep Leaves

Phytochemical Compound Test	Description	Result
Flavonoids	The solution turned deep red after addition of Mg and concentrated HCl, indicating a typical flavonoid reduction reaction	(+)
Alkaloids	A yellowish-white precipitate formed and the solution became turbid, confirming alkaloid presence	(+)
Saponins	After shaking, a stable foam layer of approximately 1–2 cm persisted for more than 10 minutes, indicating saponins	(+)
Tannins	The solution changed to bluish-black or greenish-black, consistent with Fe^{3+} tannin complex formation	(+)

The thin-layer chromatography (TLC) analysis revealed distinct differences in the chromatographic profiles of the quercetin standard and the ethanolic extract, allowing detailed comparison of their Rf values and fluorescence characteristics. In lane **P** (quercetin standard), a single major spot was observed with an Rf value of approximately 0.75, exhibiting bright yellow fluorescence after $AlCl_3$ treatment, which served as the primary reference for flavonoid identification. In lane **S** (extract), several spots were detected with distinct chromatographic characteristics.

The major spot showed an Rf of approximately 0.74, with intense yellow fluorescence after $AlCl_3$ spraying, closely matching both the Rf value and fluorescence pattern of the quercetin standard. This strong similarity indicates the presence of quercetin-type flavonoids in the extract. Additionally, an intermediate spot with an Rf of about 0.42 appeared violet-blue before and after $AlCl_3$ treatment, suggesting the presence of aromatic non-flavonoid constituents. Another more polar spot, with an Rf of around 0.26, exhibited a light green coloration that shifted to yellow-green following $AlCl_3$ application, indicating the presence of other flavonoids with higher polarity, such as flavonols or flavanones. Overall, the close resemblance between the major spot in the extract and the quercetin standard confirms that the extract contains flavonoid compounds with chromatographic characteristics comparable to quercetin.

The antioxidant activity of the ethanolic extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The assay was conducted at a wavelength of 517 nm, where

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antioxidant activity was expressed as the percentage inhibition of DPPH radicals [7The IC_{50} value, defined as the extract concentration required to inhibit 50% of DPPH radicals, was used as an indicator of antioxidant activity. Based on the percentage inhibition data obtained at various concentrations, the IC_{50} value was determined using linear regression analysis. The IC_{50} was calculated by substituting a 50% inhibition value into the regression model to estimate the corresponding extract concentration.

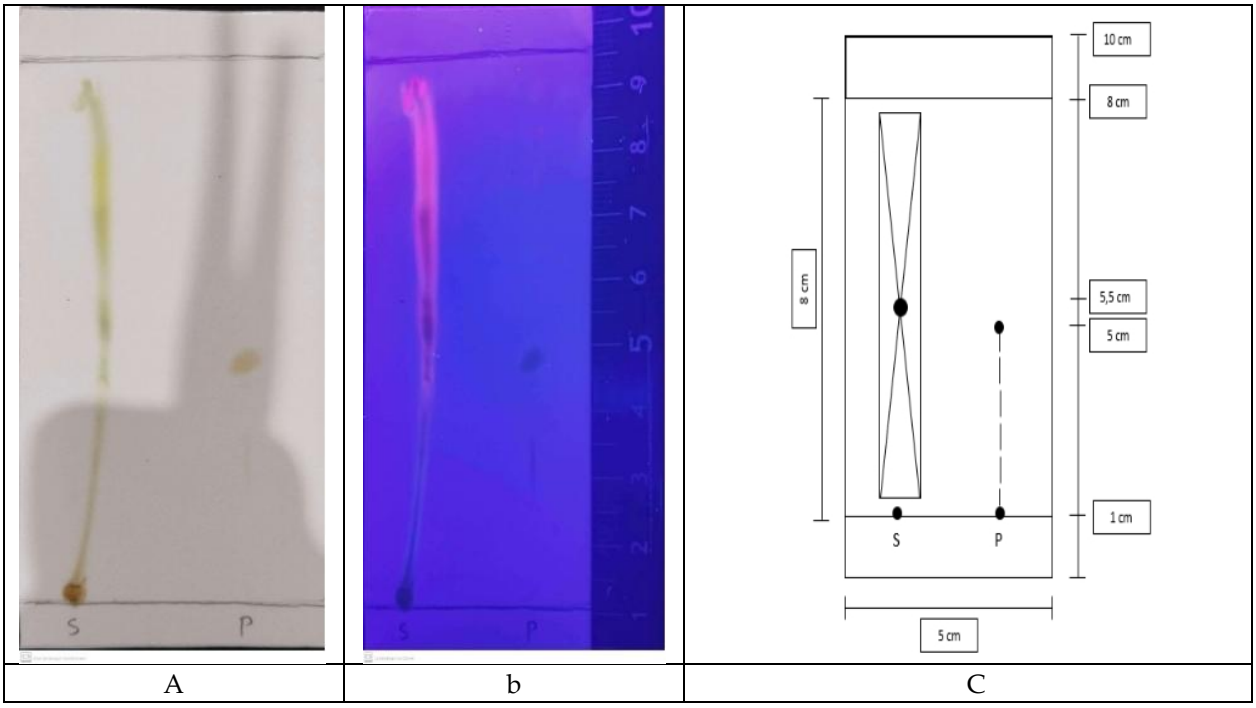

Figure 2. Thin Layer Chromatography Test Results. The chromatogram labeled a shows the results observed under UV light at 366 nm after spraying with $AlCl_3$ reagent, while b represents the chromatogram observed under UV light at 366 nm before any treatment. The chromatogram labeled c was observed under visible light. In this analysis, lane S corresponds to the Dadap serep leaf extract, whereas lane P represents the quercetin standard.

Thus, the IC_{50} value was derived from this computation. The regression and IC_{50} determination can be performed manually or using analytical software such as Microsoft Excel to ensure accuracy and minimize calculation errors. Measurements were performed under dark conditions to prevent photodegradation of DPPH [15] (Table 3).

Table 3. Results of Absorbance Measurement of 70% Ethanol Extract of Dadap Serep Leaves

Concentration (ppm)	DPPH	Absorbance	% Inhibition	Linear Equation
5	2,360	1,729	27,73	y = 1,1466x + 19,615
10		1,650	30,08	
25		1,242	47,38	
50		0,532	77,45	

Vitamin C was used as the reference standard for comparison. Quantitative analysis of antioxidant activity showed that the percentage of DPPH radical inhibition increased proportionally with concentration, ranging from 27.73% at 5 ppm to 77.45% at 50 ppm [15]. The IC_{50} value of the ethanolic extract was determined to be 49.94 ppm, indicating very strong antioxidant activity. This high activity is attributed to the synergistic effects of flavonoids, alkaloids, and saponins, which can donate hydrogen atoms or electrons to neutralize free radicals, thereby changing the DPPH color from purple to pale yellow [16].

For comparison, vitamin C exhibited an IC_{50} value of 1.474 ppm, consistent with its well-known potency as a natural antioxidant [17]. Although the IC_{50} value of Erythrina subumbrans extract was higher than that of vitamin C, its strong radical-scavenging capacity demonstrates promising potential for further development as a natural antioxidant agent. These findings indicate that the ethanolic extract of Erythrina subumbrans (Hassk.) Merr possesses significant antioxidant activity and contains diverse bioactive compounds,

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supporting its potential use as a natural antioxidant source in pharmaceutical and cosmetic formulations (Tabel 4).

Table 4. Results of Vitamin C Absorbance Measure

Concentration (ppm)	DPPH	Absorbance	% Inhibition	Linear Equation
5	2,360	1,856	21,35	y = 1,6127x + 13,789
10		1,624	31,19	
25		1,110	52,97	
50		0,123	94,79	

Conclusions

The 70% ethanol extract of *Erythrina subumbrans* (Hassk.) Merr leaves contains various classes of secondary metabolites, including flavonoids, alkaloids, saponins, and tannins, as confirmed by qualitative phytochemical screening. Thin-layer chromatography (TLC) analysis provided preliminary evidence of flavonoid compounds, with one major constituent displaying chromatographic behavior similar to the quercetin standard. The extract demonstrated significant radical scavenging activity against DPPH, with an IC_{50} value of 49.94 µg/mL. Based on established antioxidant activity classification, this IC_{50} value categorizes the extract as possessing very strong antioxidant capacity. This potent activity is likely attributed to the synergistic action of the identified phytochemicals, particularly the flavonoid constituents. The findings provide scientific validation for the traditional use of dadap serep leaves and highlight their promising potential as a source of natural antioxidants. For further development, subsequent research employing advanced analytical techniques such as HPLC, LC-MS/MS, or GC-MS is recommended to precisely identify, isolate, and quantify the specific active compounds responsible for the observed bioactivity.

Acknowledgment

The authors would like to express their sincere gratitude to the Department of Pharmacy, Faculty of Pharmacy, Muhammadiyah Magelang University, Central Java, Indonesia, for the facilities and support provided during the conduct of this research.

Conflict of Interest

The authors declare that there are no financial, personal, or institutional conflicts of interest that could potentially influence the conduct, analysis, or reporting of this study. All stages of the research, including experimental procedures, data analysis, and interpretation of the results, were carried out independently without any external interference or bias.

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Electronic ISSN : 2656-3088
Homepage: https://www.journal-jps.com