Al Ulum: Jurnal Sains dan Teknologi (e-ISSN 2477-4731) Vol. 11, No. 1, 2025
DOI: http://dx.doi.org/10.31602/jst.v11i1.18488

AL ULUM: JOURNAL OF SCIENCE AND TECHNOLOGY

ORIGINAL ARTICLE

EFFECT OF DPPH CONCENTRATION VARIATION ON ANTIOXIDANT ACTIVITY OF EXTRACT OF RASAM FERN LEAF (*Dicranopteris linearis*)

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Received: 03 March 2025 | Revised: 18 March 2025 |Accepted: 20 March 2025 | Published: 30 April 2025
UPT Publication and Journal Management Uniska-JST 2025

Abstract *Dicranopteris linearis* (rasam fern) is known to contain flavonoid compounds that exhibit significant antioxidant activity. In this study, the antioxidant potential of rasam fern leaf extract was assessed using the DPPH assay at three different concentrations: 30 ppm (P1), 40 ppm (P2), and 50 ppm (P3). Phytochemical screening confirmed the presence of flavonoids, tannins, and saponins in the extract. The IC50 values obtained for each treatment were as follows: P1 = 8.37 ppm, P2 = 12.31 ppm, and P3 = 13.57 ppm, all of which fall within the category of very strong antioxidant activity. Statistical analysis using one-way ANOVA revealed a significant difference in IC50 values among the three DPPH concentrations. These results indicate that variations in DPPH concentration significantly influence the IC50 values, thereby affecting the accuracy and interpretation of antioxidant activity measurements.

Keywords. *Dicranopteris linearis* · DPPH · Antioxidant ·

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Introduction

Antioxidants are compounds capable of inhibiting or slowing down oxidation processes induced by free radicals (Irmawati, 2015). Free radicals have been associated with the onset of various degenerative diseases, including hypertension, osteoporosis, diabetes, and cancer (Amia et al., 2021). In recent years, the exploration of medicinal plants as natural sources of antioxidants has gained considerable attention, with ferns emerging as one of the promising candidates.

Ferns represent a diverse group of vascular plants, comprising an estimated 10,000 species globally. Indonesia alone is home to approximately 1,300 fern species, each possessing unique morphological and phytochemical characteristics (Syukur, 2019). Several fern species have been identified to exhibit notable antioxidant activities, including *Gleichenia linearis* (commonly known as rasam fern), *Acrostichum aureum* (sea fern), and *Stenochlaena palustris* (kalakai) (Rizal, 2017; Hanin et al., 2017; Hakim et al., 2021). The antioxidant properties of these ferns are attributed to their diverse phytochemical constituents, notably alkaloids, flavonoids, saponins, and terpenoids-compounds known for their ability to scavenge free radicals and inhibit oxidative stress (Pardede et al., 2018; Wirdayanti & Sofiyanti, 2019; Pardede & Wardhani, 2024).

The antioxidant activity of plant extracts can be evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. DPPH is a stable free radical that readily reacts with antioxidant compounds by accepting hydrogen atoms,

resulting in its reduction to a non-radical form (Sayuti & Yenrina, 2015). This method is based on the principle that antioxidant compounds donate hydrogen atoms to the DPPH radical, thereby neutralizing its free radical character. The reduction of DPPH is typically accompanied by a color change from deep violet to yellow, which can be quantitatively measured using a spectrophotometer. The degree of discoloration reflects the scavenging ability of the antioxidant compounds present in the extract.

The concentration of antioxidant activity tested using DPPH is expressed as IC50 value. This number states the concentration of antioxidants needed to reduce DPPH by 50%. The smaller the IC 50 value, the stronger the antioxidant activity (Maryam. 2015).

Previous studies on antioxidant activity commonly utilize DPPH solutions at varying concentrations, such as 40 ppm (Sundu et al., 2018), 50 ppm (Oktavia & Hidayah, 2022), 60 ppm (Nurhasnawati et al., 2019), and 200 ppm (Islamiati et al., 2022). However, the influence of DPPH concentration variations on the IC50 value-an indicator of antioxidant potency-specifically in the measurement of antioxidant activity from *Dicranopteris linearis* (rasam fern) leaf extract, has not yet been reported in the literature. Therefore, this study aims to evaluate the antioxidant activity of rasam fern extract by employing different concentrations of DPPH solution, to determine their effect on the resulting IC50 values.

Materials and Methods

Glassware (pyrex®), macerator (Thermo®), Rotary evoporator (Thermo®), micropipette, analytical balance (Ohaus®), oven (Memmert®), water bath (Memmert®),UV-Vis spectrophotometer (Shimadzu ®) , DPPH (Sigma aldrich®), amyl alcohol (Merck®), distilled water, $FeCl_3$ 1%, Ethanol 70% (One Med®), HCl p.a (Merck®), quercetin (Sigma aldrich®), ethyl acetate (Merck®), bouchardat, dragendorf and meyer reagents, Mg powder, rasam fern leaf simplisia.

Extraction Procedure

A total of 150 grams of *Dicranopteris linearis* (rasam fern) leaf simplicia was subjected to maceration using ethanol as the solvent for 24 hours. The resulting macerate was filtered, and the remaining plant residue was remacerated once under the same conditions and using the same volume of solvent. The second macerate was also filtered, and both filtrates were combined. The pooled extract was then concentrated using a rotary evaporator until a viscous (thick) extract was obtained.

Phytochemical screening

The identification of phytochemical constituents in the rasam fern extract included qualitative tests for alkaloids, flavonoids, tannins, saponins, and steroids/triterpenoids. To prepare the test solution, 1 gram of the extract was weighed and dissolved in 50 mL of distilled water by heating. The solution was then filtered, and the resulting filtrate was used for the detection of alkaloid, flavonoid, tannin, and saponin compounds, following the methods described by Supomo et al. (2020); Endarini, (2016); Pardede et al., (2013); Adlhani (2014).

Antioxidant Activity

The antioxidant activity of the extract was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. The prepared extract solution was transferred into a beaker, followed by the addition of DPPH reagent. The mixture was then incubated in a dark room at room temperature for 30 minutes to prevent photodegradation. Absorbance measurements were taken three times at a specific wavelength using a UV-Vis spectrophotometer. The percentage of antioxidant activity was calculated based on the reduction in absorbance, as described by Shalaby and Shanab (2013). In this study, different concentrations of DPPH were used to examine their effect on the antioxidant activity of the extract.

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Preparation of DPPH Solution (30, 40, and 50 ppm)

Respective amounts of 1.5 mg, 2 mg, and 2.5 mg of DPPH were weighed and placed into separate volumetric flasks. Ethanol p.a. was then added to each flask up to the 50 mL mark, and the mixtures were shaken until homogeneous, as described by Susiloningrum and Sari (2021).

Determination of Maximum Absorption Wavelength for DPPH

From the prepared 30, 40, and 50 ppm DPPH solutions, 3 mL of each was transferred into a 10 mL volumetric flask. The volume was adjusted to the mark with 70% ethanol, and absorbance was measured at wavelengths between 450 and 600 nm using a UV-Vis spectrophotometer.

Preparation of Blank Solution for Antioxidant Activity Test of Quercetin

1 mL of 70% ethanol was pipetted into separate test tubes, and 2 mL of each 30, 40, and 50 ppm DPPH solution was added to the respective test tubes.

Preparation of 100 ppm Quercetin Stock Solution

10 mg of quercetin was weighed and dissolved in 70% ethanol to a final volume of 10 mL in a volumetric flask, resulting in a 1000 ppm stock solution. From this stock, 1 mL was pipetted and transferred to a 10 mL volumetric flask, where it was diluted with ethanol to achieve a final concentration of 100 ppm.

Preparation of Quercetin Series Solutions

From the 100 ppm quercetin stock solution, 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, and 0.5 mL were pipetted into separate 10 mL volumetric flasks. Each flask was filled to the mark with 70% ethanol, resulting in series solutions of 1 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm quercetin.

Antioxidant Activity Assay of Quercetin

1 mL of each quercetin series solution was transferred into separate test tubes. To each test tube, 2 mL of 30, 40, and 50 ppm DPPH solution was added. The mixtures were incubated in the dark for 30 minutes. Absorbance was then measured using a UV-Vis spectrophotometer at the maximum absorption wavelength.

Preparation of 100 ppm Extract Stock Solution

Fifty milligrams of the extract sample were dissolved in ethanol in a 50 mL volumetric flask to obtain a 1000 ppm stock solution. Then, 1 mL of the stock solution was pipetted and diluted with ethanol up to the 10 mL mark, resulting in a final concentration of 100 ppm.

Preparation of 2, 4, 6, 8, and 10 ppm Extract Series Solutions

0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, and 1 mL of the 100 ppm extract stock solution were pipetted into separate 10 mL volumetric flasks. Each flask was then filled to the mark with 70% ethanol, resulting in final concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm extract solutions.

Antioxidant Activity Assay of Extracts Using the DPPH Method

1 mL of each extract series solution was transferred into separate test tubes. To each test tube, 2 mL of 30, 40, and 50 ppm DPPH solution was added. The mixtures were incubated in the dark for 30 minutes. Absorbance was then measured using a UV-Vis spectrophotometer at the maximum absorption wavelength. The percentage of antioxidant activity (percent inhibition) was calculated using the formula described by Shalaby and Shanab (2013). The IC50 value was determined using the regression equation $y=bx+a$ where $y=50$ and x represents the concentration of the test solution that inhibits 50% of DPPH free radicals.

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Results and Discussion

Phytochemical screening is a test performed to identify the classes of compounds present in plants. The results of the phytochemical screening of the ethanol extract from *Dicranopteris linearis* (rasam fern) leaves are presented in Table 1.

Table 1. Screening Phytochemical

Extract	Reagent	Result	Description
Alkaloid	Mayer	Brown sediment	+
	Dragendroff	Red-brown sediment	+
	Bouchardat	No sediment	-
Flavonoid	Amil alcohol	Pink	+
Tannin	$FeCl_3$	Blackish green	+
Saponin	HCl	Foam	+
Steroid/Triterpenoid	$C_2H_4O_2+H_2SO_4$	No blue-green color formed	-

(-) Negative for compounds, (+) Positive for compounds

Based on the results presented in Table 1, it is evident that the *Dicranopteris linearis* (rasam fern) extract contains alkaloids, flavonoids, tannins, and saponins. Alkaloid compounds are known for their potential as antioxidants, as they contain nitrogen atoms in their structure. These atoms possess free electron pairs that can reduce free radical activity in the body (Puspitarsari, 2018). Flavonoids also play a significant role as antioxidants, as they can neutralize free radicals by inhibiting enzymes that produce free radicals, such as xanthine oxidase, lipoxygenase, mitochondrial succinate dehydrogenase, and NADPH oxidase. Additionally, flavonoids can inhibit lipid oxidation (Banjarnahor and Artanti, 2014; Olivia et al., 2015; Pardede et al., 2017). According to Hasim et al. (2019) and Sulandi (2013), tannins exhibit antioxidant activity due to their hydroxyl (OH) groups, whose hydrogen atoms can be donated to free radicals, converting them into non-radical compounds (DPPH-H). Saponins also demonstrate antioxidant activity, as they can reduce superoxide radicals by forming hyperoxide intermediates, thereby preventing biomolecular damage caused by free radicals (Hasan, 2022).

Antioxidant Activity Assay of Rasam Fern Leaf Extract Using the DPPH Method

The free radical scavenging activity of the sample was evaluated by assessing its ability to neutralize DPPH free radicals. In this method, the free radical source is 1,1-diphenyl-2-picrylhydrazyl (DPPH). The principle of the assay relies on the donation of hydrogen atoms from the tested substance to the DPPH radicals, transforming them into non-radical diphenylpicrylhydrazine, as indicated by a change in color (Kurniasih et al., 2015).

Determination of DPPH Maximum Absorption Wavelength

In this study, the absorption wavelength range was measured between 450 and 600 nm using a UV-Vis spectrophotometer. The maximum wavelengths obtained for the three different treatments are presented in Table 2.

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Table 2. Maximum Wavelength at Three Variations of DPPH Concentration

Treatment	DPPH Concentration (ppm)	Wavelength (nm)
P1	30	517
P2	40	522
P3	50	520

Table 3. IC50 Value of Antioxidant Activity DPPH Method

Sample	Variation concentration DPPH		
Extract	P1 (30 ppm)	P2 (40ppm)	P3 (50 ppm)
	8,37	12,31	13,57
Quercetin	3,08	4,08	4,34

The results of the maximum wavelength determination across the three DPPH concentrations showed variation in the range of 514–523 nm. In a previous study, Rizal (2017) reported that the methanol extract of rasam fern (*Dicranopteris linearis*) exhibited a maximum absorption wavelength of 517.5 nm. According to Sahri (2019), shifts in the maximum absorption wavelength of DPPH can be influenced by factors such as the type of solvent and the pH of the solution.

Antioxidant Activity Assay of Quercetin

Quercetin was used as a reference antioxidant, as it is a well-known natural compound capable of inhibiting the oxidation of other molecules (Zhao et al., 2018). A series of quercetin solutions at concentrations of 1, 2, 3, 4, and 5 ppm was prepared. At these concentrations, a visible color change from purple to yellow was observed, indicating a reduction in DPPH radicals. The absorbance of each solution was measured at the respective maximum wavelengths of 517, 522, and 520 nm. The percentage of DPPH radical inhibition was then calculated based on the absorbance values, and the IC50 value was subsequently determined. The average IC50 value of quercetin was found to be 3.075 ppm, which, according to Kusumawati (2021), classifies it as a very strong antioxidant (IC50 < 50 ppm).

Antioxidant Activity Assay of Ethanol Extract of Rasam Fern Leaves

For the rasam fern ethanol extract, a series of concentrations (2, 4, 6, 8, and 10 ppm) was used in the antioxidant activity assay. At these concentrations, a gradual color change from purple to yellowish was observed, indicating the reduction of DPPH radicals. The absorbance of each solution was measured at the maximum wavelengths obtained (517, 522, and 520 nm), and the percentage inhibition was calculated accordingly. These values were then used to determine the IC50, which serves as a key parameter in evaluating antioxidant activity. A lower IC50 value corresponds to a higher antioxidant capacity (Syarif et al., 2015). The IC50 values obtained from the three treatments using different DPPH concentrations are presented in Table 3.

The results demonstrated that higher DPPH concentrations led to higher IC50 values, both for the rasam fern extract and for quercetin as a positive control. These findings are consistent with several previous studies that also examined the IC_{50} values of quercetin under varying DPPH concentrations. For example, Rahmawati et al. (2015) reported an IC50 value of 2.62 ppm for quercetin when using 20 ppm DPPH, while Nurhasnawati et al. (2019) observed an IC_{50} of 5.89 ppm with 60 ppm DPPH. These results indicate a trend: as the concentration of DPPH increases, the IC50 value obtained also tends to increase.

Conclusion

The antioxidant activity of *Dicranopteris linearis* (rasam fern) leaf extract was classified as very strong across all three treatments, with IC50 values of 8.37 ppm (P1), 12.31 ppm (P2), and 13.57 ppm (P3). Statistical analysis revealed significant differences among the three treatments, indicating that variations in DPPH concentration had a significant effect on the IC50 values of the rasam fern leaf extract. These findings suggest that the concentration of DPPH plays a critical role in influencing the measured antioxidant activity.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

In vitro antioxidant activity, as measured by IC50 may be influenced by several factors, including the type of free radical used, the reaction time, temperature, and pH of the system. A longer reaction time between the antioxidant and the radical may allow for greater radical scavenging, potentially resulting in a lower IC50 value. Additionally, the temperature and pH conditions of the reaction system can influence antioxidant stability and reactivity, as some antioxidant compounds exhibit higher stability and effectiveness at specific pH levels or temperatures (Irianti et al., 2021).

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