

Phytochemical Screening and Antioxidant Activity of *Dyera polyphylla* (Miq) Steenis Extract Using the DPPH Method

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Manuscript received: 07 July, 2025. Revision accepted: 15 August, 2025. Published: 26 November, 2025.

Abstract

This research aimed to determine the chemical profile and antioxidant activity of the jelutung rawa plant (*Dyera polyphylla*). Phytochemical screening tests were conducted using specific reagents, thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC). Determination of antioxidant activity was performed the DPPH method. The research results showed the presence of secondary metabolite compounds, namely alkaloids, flavonoids, phenolics, tannins, and terpenoids in the leaves, while the contain alkaloids, phenolics, tannins, and terpenoids. The antioxidant activity in this plant has been identified as a strong natural antioxidant.

Keywords: Antioxidant; DPPH; *Dyera polyphylla*.

INTRODUCTION

Jelutung (*Dyera spp.*) is a type of plant from the Apocynaceae family. Based on its habitat, jelutung plants are divided into two types: terrestrial *jelutung* (*Dyera costulata*) and swamp *jelutung* (*Dyera polyphylla*) (Tanjung Sari et al., 2016). In society, the *D. costulata* plant has become one of the sources of income because it has a high market value. The *D. costulata* plant has many benefits, including in the rubber industry where *jelutung* sap is used as the main ingredient in the production of chewing gum, paint, and electrical cable insulators, while *D. costulata* wood is used for making pencils, wood carvings, ceilings, wooden sandals, partition boards, and plywood (Sofiyuddin & Janudianto, 2018). Moreover, the use of *D. costulata* in the health field as a traditional medicine has been known for a long time. In Indonesia, the Anak Dalam tribe utilizes the sap of *D. costulata* as an external medicine for animal bites, for swollen toothaches, and for boils (Aminah et al., 2016). The barks and leaves of *D. costulata* are used as medicine for fever, inflammation, and pain (Subhadhirasakul et al., 2003). However, there is limited information on the use of *D. polyphylla*. Meanwhile, this plant is commonly found in the peat swamp forests of Sumatra and Kalimantan, including West Kalimantan (Tata et al., 2015).

Pharmacological studies of *D. costulata* extract showed that this plant has antioxidant, analgesic, and

antidiabetic activities. Meanwhile, chemical studies of the *D. costulata* plant reported such as β -amyrin, rhamnazin, quercetin-3-O- α -rhamnopyranoside, benzoic acid, lupeol, okrolifuanin A, okrolifuanin E, okrolifuanin F, dehydrochrolifuanin A, dehydrochrolifuanin E, and dehydrochrolifuanin F from this plant (Subhadhirasakul et al., 2003; Reanmongkol et al., 2002; Arjinal et al., 2020; Mirand et al., 1983).

Dyera polyphylla is a plant that naturally grows in peat swamp areas. This plant has good adaptations to waterlogged environments because it has breathing roots (pneumatophores) (Tata et al., 2015). The ability of a plant to adapt to its environment can affect the phytochemical content within it. In addition to internal factors, plant adaptation is also influenced by several external factors such as temperature, light, pH, humidity, altitude, and nutrient content in the soil, which can affect the development and growth of a plant (Katuuk et al., 2018). Extreme environmental conditions can increase the production of reactive oxygen species (ROS) free radicals in plant tissues, which can trigger cell damage. As a result, plants will protect themselves by producing antioxidant compounds (Utomo et al., 2020). The difference in habitat between *D. polyphylla* and *D. costulata* suggests that these two plants have different compound contents and biological activities. Therefore, screening the chemical content and antioxidant activity of *D. polyphylla* is necessary, so that it can provide

information regarding the potential utilisation of *D. polyphylla* in society and related fields.

MATERIALS AND METHODS

Materials and equipment

The sample used in this study was *D. polyphylla*, which had been identified by the National Research and Innovation Agency (BRIN) Cibinong, Bogor with the code B-3284/II.6.2/IR.01.02/9/2024. The solvents used were distilled solvents including methanol, *n*-hexane, and ethyl acetate. The reagents used in the phytochemical screening included Dragendorff, Mayer, Wagner, Shinoda, Liberman-Burchard, FeCl₃ 5% and 10%. F254 silica gel plates (Merck KGaA, Germany) were used in phytochemical screening. The materials used in determining antioxidant activity were DPPH (Aldrich Chemistry) and methanol p.a (Smart-lab, Indonesia). The equipment used in this research included an oven, blender (Philips 600W), a digital analytical balance (Advank Innotech), a rotary vacuum evaporator (IKA® RV8), hot plate (IKA® C-MAG HS 7), an UV lamp 254 nm, UV-Vis spectrophotometer (Shimadzu UV-19900I), and cuvette (Shimadzu).

Procedures

Sample Preparation and Extraction

The *D. polyphylla* samples studied were parts of the leaves, bark, and stems. The extraction process began with processing the identified plant into simplicia through several stages, namely sorting, washing, cutting, drying, and grinding.

The powdered simplicia of leaves (3.53 g), bark (68.8 g), and wood (313 g) were macerated using the maceration method. In total maceration, one-third of the total weight of the simplicia was macerated using methanol solvent for 3x24 hours with solvent replacement every 24 hours. Meanwhile, in stepwise maceration, two-thirds of the total weight of the simplicia was macerated with *n*-hexane for 3x24 hours with solvent replacement every 24 hours. The residue obtained was re-macerated using ethyl acetate and methanol solvents with the same treatment. The obtained macerate was concentrated using a rotary vacuum evaporator. The yield calculation was obtained from the comparison of the thick extract obtained with the initial weight of the sample before the extraction treatment (Putri et al., 2024a). A yield is considered good if the value obtained is more than 10% (Lutfiah et al., 2024).

Phytochemical Screening

Qualitative testing of the extract from the leaves, stems, and bark of jelutung rawa was performed using phytochemical screening methods, thin-layer chromatography (TLC), and chromatogram profile analysis on HPLC. Dragendorff, Mayer, and Wagner reagents for alkaloid tests, Shinoda reagent for flavonoid

tests, Liebermann-Burchard reagent for terpenoid tests, 5% FeCl₃ reagent for phenolic tests, and 10% FeCl₃ reagent for tannin tests (Khafid et al., 2023; Oktavia & Sutoyo, 2021; Tunny et al., 2020). The complexity and polarity properties of the compounds contained in the *D. polyphylla* extract were analysed using thin-layer chromatography (TLC) technique. The eluent systems used were *n*-hexane:ethyl acetate (7:3), *n*-hexane:ethyl acetate (1:9), and CHCl₃:acetone (1:1). The extract that has been dissolved in methanol is spotted on the TLC plate and eluted with each eluent. Observation of the spots was conducted under UV light at 254 nm. The plate was then sprayed with 2% CeSO₄ and heated. The results of the TLC chromatogram were analysed based on the number of spots. Next, the determination of the chromatogram profile was analysed using High-Performance Liquid Chromatography (HPLC) instrumentation in reverse phase using a C8 column with an isocratic eluent system of methanol: H₂O (9:1) at a flow rate of 1.5 mL/min and an operation time of 25 minutes at a UV wavelength of 254 nm. The HPLC chromatogram results were analysed based on the retention times obtained for each *D. polyphylla* extract.

Determination of Antioxidant Activity

The determination of antioxidant activity qualitatively was carried out using the procedure described by Putri et al., (2024) through the TLC technique. Each extract with a concentration of 1000 ppm and ascorbic acid 1000 ppm as a positive control was spotted on the TLC plate with the same number of spots. The plate was then sprayed with 50 ppm DPPH and incubated for 30 minutes in the dark. Antioxidant activity is indicated by the presence of yellow spots on the TLC plate. Quantitative analysis of antioxidant activity was conducted to determine the concentration of an extract that can neutralise free radicals based on the IC₅₀ value. In this test, the positive control used was trolox. The 100 ppm trolox solution was diluted into seven series of concentrations: 20 ppm; 10 ppm; 5 ppm; 2.5 ppm; 1.25 ppm; 0.625 ppm; and 0.3125 ppm. Meanwhile, the 1000 ppm *D. polyphylla* extract was diluted into seven series of concentrations: 500 ppm; 250 ppm; 125 ppm; 62.5 ppm; 31.25 ppm; 15.625 ppm; and 7.8125 ppm. Each extract (1000 ppm – 7.8125 ppm) was taken in an amount of 2.5 mL and added to 4 mL of 40 ppm DPPH. Then it was covered and incubated for 30 minutes at room temperature in the dark. After incubation, the samples were measured using a UV-Vis spectrophotometer at a wavelength of 516 nm in triplicate. The same treatment was also applied to the control solution.

Data analysis

Measurement of antioxidant activity using a UV-Vis spectrophotometer conducted in triplicate. The obtained data was expressed in the form of IC₅₀.

RESULTS AND DISCUSSION

The *D. polyphylla* sample was obtained from the Sungai Sepeti district, Kayong Utara Regency, West Kalimantan. The samples were sorted to separate them from unwanted plant parts such as grass, soil, or damaged samples (Widodo & Subositi, 2021). The sample was then washed using running water, cut into small pieces to reduce the sample size, dried using an oven at 50°C, and grinded to increase the sample surface area so that during the maceration process, the secondary metabolites contained in the sample can be maximally extracted (Gamah et al., 2023). The obtained simplicia

was extracted using the total maceration and fractional maceration methods. In total maceration, the extract obtained is a total extract that can be extracted in the solvent used, while fractional maceration produces specific compounds according to the solvent used (Hamka et al., 2022). The obtained macerate was concentrated using a rotary vacuum evaporator to obtain a thick extract. The yield calculation is obtained from the comparison of the thick extract obtained with the initial weight of the sample before the extraction treatment (Putri et al., 2024).

Table 1. Yield and antioxidant data of *D. polyphylla* extract.

Extraction method	Solvent	Sample	Yield (%)	IC ₅₀ Antioxidant *(ppm)
Total maceration	Methanol	Leaf	13.63	51.95 ± 0.087
		Bark	10.55	72.21 ± 0.462
		Stem	13.36	421.03 ± 0.598
Tiered maceration	n-Hexane	Leaf	2.85	> 500
		Bark	2.20	> 500
		Stem	10.73	> 500
	Ethyl acetate	Leaf	5.38	77.63 ± 0.061
		Bark	0.91	> 500
		Stem	10.93	> 500
	Methanol	Leaf	22.68	55.26 ± 0.063
		Bark	17.00	10.28 ± 0.059
		Stem	5.69	> 500
Trolox				2.14 ± 0.002

*The value is the average ± SD, n = 3.

The highest yield value in total maceration was found in the leaf extract at 13.63%, followed by the stem extract at 13.36%, and the bark extract at 10.55%. The selection of methanol as a solvent is based on its ability to dissolve both polar and nonpolar compounds because its small molecular structure can penetrate plant cells, thereby maximising the dissolution of secondary metabolites (Muaja et al., 2017; Mutmainnah et al., 2017). Meanwhile, in the stepwise maceration using *n*-hexane solvent, the highest yield value is found in the stem extract compared to the leaf and bark extracts. In the ethyl acetate solvent, the highest yield value is found in the stem extract compared to the leaf and bark extracts. Whereas in the stepwise methanol solvent, the highest yield value was found in the leaf and bark extracts compared to the stem extract. This indicated that the compounds dissolved in the stem extract were predominantly semi-polar and polar compounds. Meanwhile, in the leaf and bark extracts, the dissolved compounds are predominantly polar compounds. This is in line with the principle of like dissolves like, where compounds will dissolve in solvents that have similar properties (Alif et al., 2023). The higher the yield value of an extract, the greater the concentration of dissolved substances in the extract (Senduk et al., 2020). The differences in yield values obtained are influenced by

several factors, namely the type of solvent polarity, the ratio or concentration of the solvent used, the particle size of the simplicia, and the duration of the extraction performed (Handoyo, 2020).

Phytochemical screening is an initial stage in research to provide an overview of the groups of compounds contained in the extract of *D. Polyphylla* (Minarno, 2015). Phytochemical screening using specific reagents such as Dragendorff, Mayer, and Wagner reagents were used to detect alkaloid compounds, Shinoda reagent was used to detect flavonoid compounds, FeCl₃ 5% reagent was used to detect phenolic compounds, FeCl₃ 10% reagent was used to detect tannin compounds, and Liberman-Burchard reagent was used to detect terpenoid compounds.

The presence of alkaloids is marked by the formation of orange precipitates with Dragendorff's reagent, brown precipitates with Wagner's reagent, and white precipitates with Mayer's reagent. The precipitate formed occurs due to the formation of a potassium-alkaloid complex between the K⁺ metal ion, which forms a coordinate covalent bond with the free electrons on the nitrogen atom present in the alkaloid (Sangkal et al., 2020). Flavonoids are indicated by a colour change to orange to red due to the reduction of concentrated hydrochloric acid with magnesium forming the complex

[Mg(OAr)₆]⁴⁺. Phenolic compounds show a colour change to green or blue due to the formation of iron (II) hesasianoferat as phenolic compounds reduce Fe³⁺ to Fe²⁺. Tannin compounds are indicated by a colour change to dark blue or greenish-black. Meanwhile, terpenoid compounds are indicated by a bluish-green colour for steroid-type terpenoids and an orange-brown colour for triterpenoid-type terpenoids (Pratama et al., 2022). The extract of *D. polyphylla* leaves contains groups of

alkaloid, flavonoid, phenolic, tannin, and terpenoid compounds. Meanwhile, the extract of *D. polyphylla* stems and bark contains groups of alkaloid, phenolic, tannin, and terpenoid compounds. The presence of flavonoid compounds was not detected in the stems and bark of *D. polyphylla*. This is because not all groups of flavonoid compounds can be detected with the Shinoda reagent.

Table 2. Results of phytochemical screening of *D. polyphylla* extract

	Sample	Alcaloide			Flavonoide	Phenolic	Tannin	Terpenoide
		1	2	3				
Methanol	Leaf	+	-	-	+	+	+	+
	Bark	+	+	-	-	+	+	+
	Stem	+	-	-	-	-	+	+
n-Hexane	Leaf	-	+	+	-	+	-	+
	Bark	+	-	+	-	-	-	+
	Stem	-	-	-	-	-	-	+
Ethyl acetate	Leaf	+	+	+	+	+	+	+
	Bark	+	-	+	-	+	+	-
	Stem	+	-	+	-	+	+	-
Methanol	Leaf	+	-	-	+	+	+	+
	Bark	+	-	-	-	+	+	-
	Stem	+	-	-	-	-	+	-

Note: 1) Dragendorff's reagent, 2) Wagner's reagent, 3) Mayer's reagent. (+) Contains secondary metabolite compounds (-) Does not contain secondary metabolite compounds.

The analysis of the complexity and polarity properties of the compounds contained in the *D. polyphylla* extract was carried out using TLC. The eluent systems used were n-hexane:ethyl acetate (7:3), n-hexane:ethyl acetate (1:9), and CHCl₃:acetone (1:1). The extract that has been spotted on the TLC plate was eluted with each eluent. Among the various eluents used, n-hexane:ethyl acetate (7:3) provided clear and well-separated spots (Ridwanuloh & Syarif, 2019). Observation was conducted under UV light at 254 nm. During the observation under UV light at 254 nm, the TLC plate fluoresced and the sample appeared dark in colour (Izzah et al., 2019). Observation under UV light at 254 nm showed that the spots on the leaf and stem bark extracts exhibit dark spots, especially in the ethyl acetate leaf and stem bark extracts. Meanwhile, the stem extract did not show any fluorescent spots. Therefore, the TLC

plate was sprayed with cerium sulphate (CeSO₄), which is a universal stain developer (Arnida & Sutomo, 2008). After the plate was sprayed with CeSO₄ 2% and heated, purple spots appeared, indicating the presence of phenylpropanoid derivatives, and brown spots indicated the presence of flavonoid compounds (Mutmainnah et al., 2017; Nuari et al., 2019). In the leaf and stem extracts, the spots that appear tend to be purple and brown, while in the bark extract, the spots that appear are predominantly brown. The abundance of spots in the leaf and stem extracts indicates that both extracts have more complex components compared to the bark extract. The TLC chromatogram results indicated that the compound components contained in the *D. polyphylla* extract have a polar polarity level. This was shown by the spots that appear with an R_f value >0.7 in the nonpolar eluent system (n-hexane-ethyl acetate (7:3)).



Figure 1. Chromatogram of the extract observed under UV light at 254 nm (a), after spraying with 2% CeSO₄ and heating (b)

Note : Leaf extracts from total methanol (1), methanol (2), n-hexane (3), ethyl acetate (4). Stem extracts from total methanol (5), methanol (6), n-hexane (7), ethyl acetate (8). Bark extracts from total methanol (9), methanol (10), n-hexane (11), ethyl acetate (12).

The complexity analysis and polarity properties of the extracts from the leaves, stems, and bark of *D. polyphylla* were further analysed using reverse phase HPLC (RP-HPLC). HPLC has a high sensitivity level, quick analysis time, and requires only a small amount of sample (Rosydiati & Saleh, 2019). The principle of reverse phase HPLC is based on polarity, where more polar compounds will elute first and have a shorter retention time compared to semi-polar or nonpolar compounds (Aulia et al., 2016). The compounds that elute will be detected by the detector and recorded in the form of a chromatogram. The number of peaks in the chromatogram indicates the number of components, and

the area of the chromatogram peaks indicates the concentration of the components in the *D. polyphylla* extract (Khairun et al., 2021). The number of peaks that appear at the initial retention time indicated that the *D. polyphylla* extract contains more polar compounds than nonpolar ones (Rudiana et al., 2022). The number of peaks obtained showed that the total extracts of leaves and stems have a more varied component of compounds than the bark extract. Additionally, the similarity in retention time between the leaf and stem extracts of *D. polyphylla* indicated that both extracts have almost similar compound components.

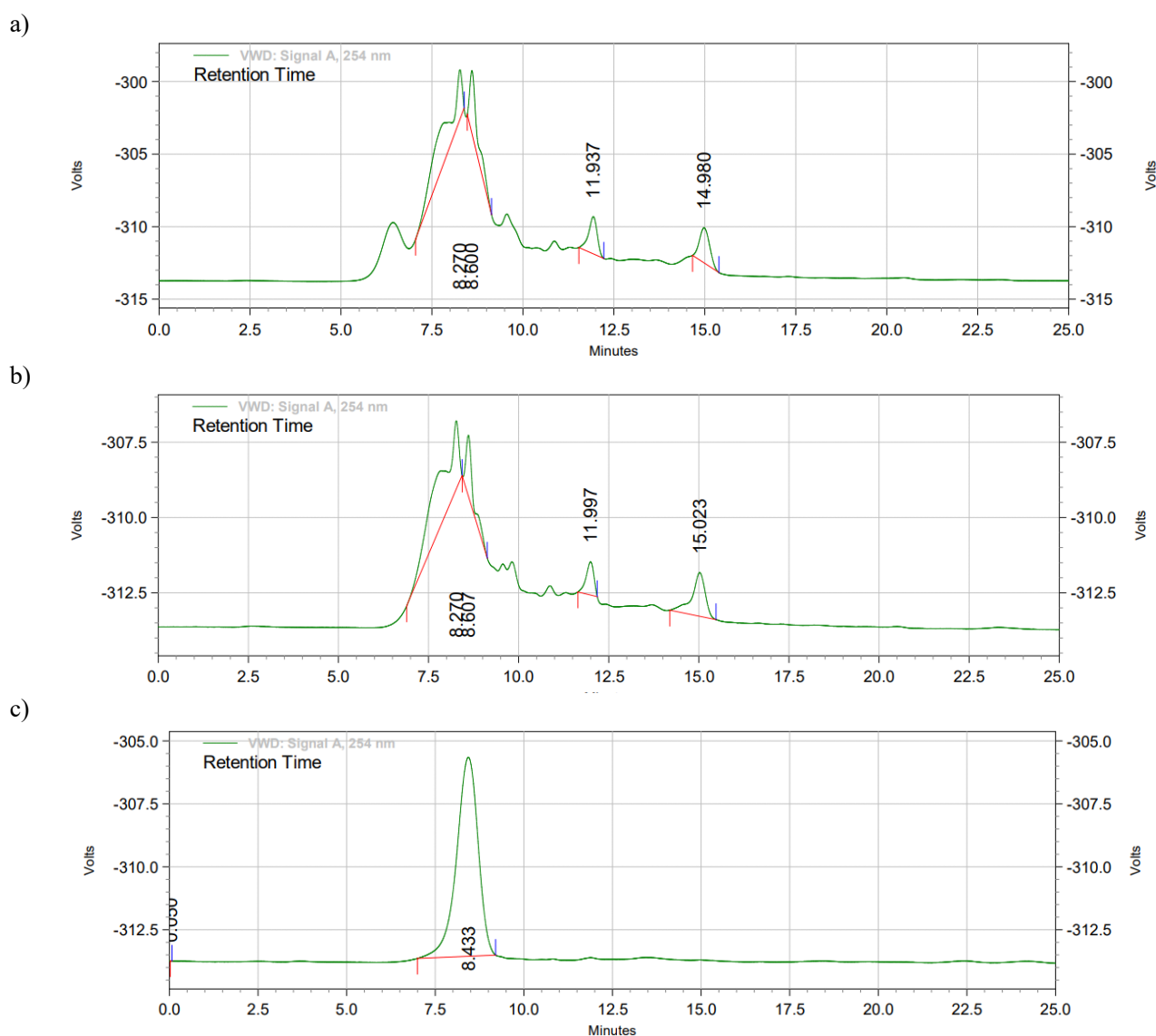


Figure 2. HPLC chromatogram of total extracts (a) Leaves, (b) Stem, and (c) Bark of *D. polyphylla*.

Qualitative analysis of antioxidant activity was conducted to identify *D. polyphylla* extracts with antioxidant activity using the TLC method. *D. polyphylla* extracts with antioxidant potential are marked by a colour change of DPPH from purple to yellowish-white with a purple background (Putri et al., 2024). The colour

change that occurs was caused by a reaction between compounds that can donate hydrogen atoms from the *D. polyphylla* extract to the DPPH molecule, reducing the DPPH molecule to DPPH-H (Muthia et al., 2019). The leaf and bark extracts of *D. polyphylla* showed colour changes comparable to ascorbic acid as a reference. This

suggests that the leaf and bark extracts of *D. polyphylla* have antioxidant activity.

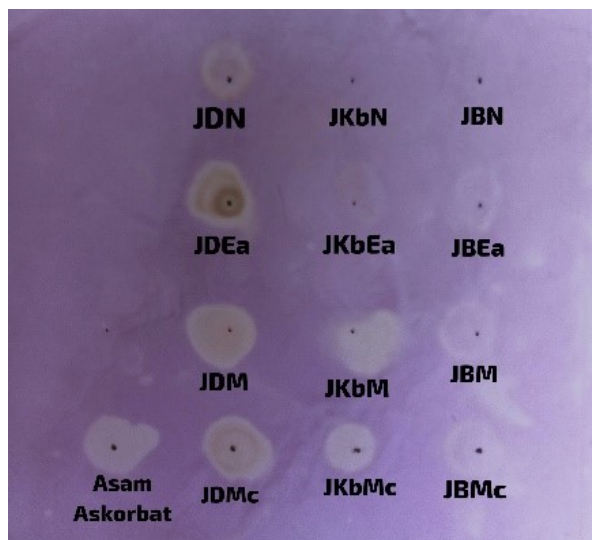


Figure 3. Qualitative antioxidant test using the TLC technique with an extract concentration of 1000 ppm.

The determination of antioxidant activity was conducted using the DPPH method. This method was chosen because it is simple, fast, easy, and requires only a small sample to evaluate the antioxidant activity of natural compound materials (Hasan et al., 2022). The parameter used is the concentration level of *D. polyphylla* extract in inhibiting 50% of DPPH free radicals. Each *D. polyphylla* extract (1000 - 7.8125 ppm) and trolox (20 - 0.3125 ppm) was measured using a UV-Vis spectrophotometer at a wavelength of 516 nm in triplicate. The concentration variations were used to facilitate determining the concentration limit of effective antioxidant activity in *D. polyphylla* extract (Kurnia et al., 2024). Measurements at the maximum wavelength are conducted to increase the sensitivity of the measurements because at this wavelength, the absorption in the test solution will be maximised. Measurements of the control solution show that the concentration is directly proportional to the percentage inhibition. This indicates that the higher the concentration used, the higher the percentage inhibition obtained. (Figure 4).

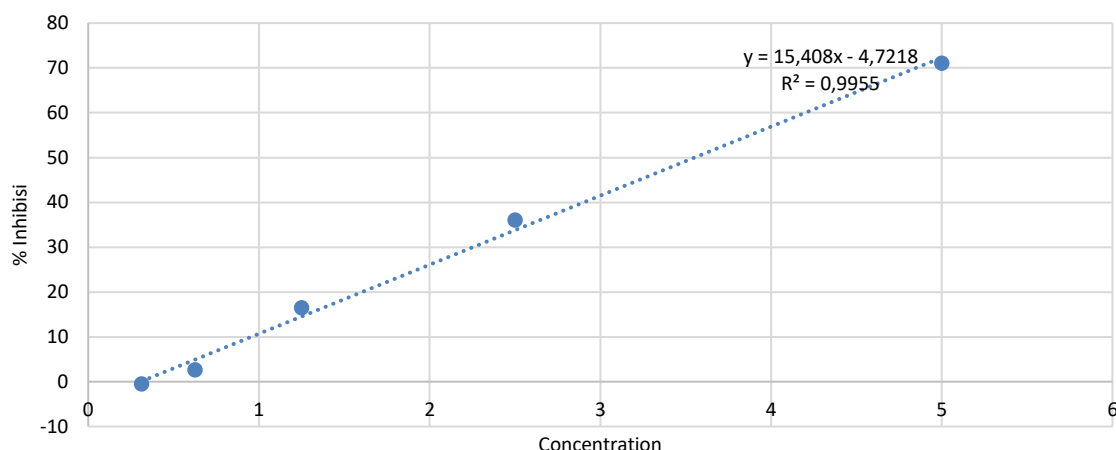


Figure 4. Calibration curve of the control solution (trolox) measured in triplicate.

The IC_{50} value of *D. polyphylla* extract was determined by creating a calibration curve between the concentration of the extract and the percentage of inhibition. The obtained linear equation was used to calculate the concentration of *D. polyphylla* extract that can inhibit DPPH free radicals by 50%. *D. polyphylla* extract was said to have very strong antioxidant activity if the IC_{50} value is < 50 ppm, strong if the IC_{50} 50-100 ppm, moderate if the IC_{50} value is 101-250 ppm, weak if the IC_{50} value is 250-500 ppm, and inactive if the IC_{50} is >500 ppm (Putri et al., 2024). The antioxidant activity in the total leaf extract was classified as very strong compared to the bark extract, while the bark extract did not have antioxidant activity. In addition, the methanol bark extract has very strong antioxidant activity,

followed by the methanol leaf extract and the ethyl acetate leaf extract (Table 1). This indicated that the leaf and bark extracts have potential as strong natural antioxidants, and it is estimated that the compounds actively involved in this activity are polar and semi-polar compounds. Therefore, the isolation of compounds from the *D. polyphylla* plant can be recommended. This finding is consistent with research on another species from the same genus, namely *D. costulata*, which has also been reported to have strong antioxidant activity in total chloroform leaf extracts of $79.8 \pm 0.2 \mu\text{g/ml}$, total *n*-butanol leaf extracts of $12.0 \pm 0.1 \mu\text{g/ml}$, and isolated *n*-butanol leaf extracts of $9.37 \pm 0.02 \mu\text{M}$. The *D. polyphylla* leaf extract has been shown to have greater antioxidant activity than the antioxidant BHT at $80.78 \pm$

0.01 μ M. Compounds isolated from the *D. costulata* leaf extract include rhamnazin, β -amyrin, quercetin-3-O- α -rhamnopyranoside, okrolifuanin A, okrolifuanin E, okrolifuanin F, dehydrochrolifuanin A, dehydrochrolifuanin E, and dehydrochrolifuanin F (Subhadhirasakul et al., 2003; Mirand et al., 1983).

CONCLUSIONS

The leaves of *D. polyphylla* contain compounds from the alkaloid, flavonoid, phenolic, tannin, and terpenoid groups, while the stems and bark of *D. polyphylla* contain compounds from the alkaloid, phenolic, tannin, and terpenoid groups. The extracts of the leaves and bark of *D. polyphylla* have strong natural antioxidant activity. Therefore, the extracts of the leaves and bark of *D. polyphylla* have potential as a source of antioxidant compounds. Additionally, the isolation of active compounds from *D. polyphylla* is highly recommended to broaden the exploration of the *D. polyphylla* plant.

Acknowledgements: Thank you to the MBKM (Merdeka Belajar Kampus Merdeka) Research grant for supporting this research.

Authors' Contributions: Putri Dharma Monika: Writing articles and conducting experiments; Rini Muharini: Supervising research and article, designing and directing projects, processing data and performing analysis; Eni Mayasari: Designing and directing projects.

Competing Interests: The authors declare that there are no competing interests.

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