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Phytochemical screening and antioxidant activity of methanol extract of *Dillenia excelsa* leaf

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Abstract. *Lisdiani, Susanto D, Manurung H. 2022. Phytochemical screening and antioxidant activity of methanol extract of* Dillenia excelsa *leaf. Biodiversitas 23: 3827-3835.* The aim of this study was to determine the phytochemical compounds, total flavonoid, and phenolic content, and antioxidant activity of the methanol extract of *Dillenia excelsa* (Jack) Martelli ex Gilg (or *tanikkara*) leaves. Sample *D. excelsa* leaves were obtained from swamp forests in Melintang Village, Muara Wis sub-district, Kutai Kartanegara district, East Kalimantan province, Indonesia. This research includes sample preparation, extraction and maceration, extract concentration using a rotary evaporator, phytochemical test, total phenolic and flavonoid content test, and antioxidant activity test using 2,2-diphenyl-1-picrylhydrazil (DPPH) and Ferric reducing antioxidant power methods (FRAP). The results of this study indicate that the methanolic extract of *D. excelsa* leaves contains secondary metabolites, including alkaloids, flavonoids, phenolics, triterpenoids, and tannins. The total phenolic content was 181.25 g GAE/g extract, while the total flavonoid content was 36 g QE/g extract. The antioxidant activity of the methanol extract of *D. excelsa* leaf DPPH method had moderate activity strength with a value of IC_{50} is 145 ppm, while the antioxidant activity of the methanol extract of *D. excelsa* leaf using the FRAP method has the highest antioxidant activity value at a concentration of 100 ppm with a value of 70.833%. The result indicated that the methanolic extract *D. excelsa* leaves had many metabolites and potentially an antioxidant.

Keywords: Antioxidant activity, *Dillenia excelsa*, phytochemicals, total flavonoids, total phenolics

INTRODUCTION

Some people traditionally use plants of the genus Dilleniaceae for various medical purposes (Lima et al. 2014; Yazan and Armania 2014; Sabandar et al. 2017; Goh et al. 2017; Ray and Sajwan 2020). In Indonesia, *Dillenia excelsa* (Jack) Martelli ex Gilg) or *tanikkara* has several different regional names such as *D. excelsa* (Kutai), ki seal (West Java), simpor water (Pontianak), simpor male (Belitung). In addition to having regional names, this plant also has different names in several countries, including simpor (Brunei, Sabah), zinbyun or maisaman (Burma), san or masan (Thailand), san (Cambodia), katmon (Philippines), and others (Yazan and Armania 2014; Barua et al. 2018). Sofiah et al. 2018 reported that the genus Dilleniaceae is the tenth most important plant family in the Besiq Bermai Forest, East Kalimantan, for the sapling category. *D. excelsa* is constituent vegetation in the Wetland Central Zone in the Mesangat wetland, East Kutai-district, East Kalimantan Province, Indonesia (Lariman et al. 2021)

This plant is often used for its leaves, especially the leaf buds, which are generally used by local people as a diarrhea medicine by soaking in hot water, then drinking it (Mukhlisi et al. 2018). The bark of *D. excelsa* is used as wound medicine by the Dayak community in Kalimantan (Pasaribu et al. 2019; Santoso et al. 2019; Az-Zahra et al. 2021), sap, leave, and bark as a remedy for Malaria, toothache in Lampung province, Indonesia (Denny et al. 2021). In addition, this plant can be used as a spice ingredient, namely spices that usually come from plants, either used in fresh or dry form, which can later be mixed into dishes as flavoring ingredients and vegetables (Sunariyati 2018).

Dillenia excelsa even though it is known as a medicinal plant, its phytochemical content is not yet known. And information from various references, both digitally and in print, is generally minimal to be used as material to support research materials because this type of plant is a rare plant species in Indonesia. In previous studies, many researchers have used several samples of medicinal plants by conducting phytochemical screening and testing their antioxidant activity using different solvents. Study using *Dillenia indica* samples with positive phytochemical test results containing alkaloids, phenols, tannins, flavonoids, steroids, and triterpenoids, as well as saponins (Shipra et al. 2018; Bordoloi et al. 2019; Prananda et al. 2019; Nkop et al. 2020), antimicrobial, cytotoxicity, and sedative activity (Akter et al. 2021; Lashin et al. 2022), the fruit powder potential antidiabetic (Kamboj et al. 2019), also make them pharmaceutically useful (Bose et al. 2010; Kumar et al. 2018). *Dillenia suffruticosa* ethyl acetate extract showed that the positive sample contained secondary metabolites of tannins, polyphenols, and triterpenoids (Putra et al. 2018;

Rahayu et al. 2019; Yacop et al. 2020; Syafriana et al. 2021). *Dillenia serrata* has the potential as an antioxidant prospect to be used in herbal and drug development from nature (Sabandar et al. 2020). Liana and Murningsih 2019 reported that *Dillenia auriculata* bark extract has total phenolic content of 19.70±0.05 mg GAE/g extract and a total flavonoid of 19.53±0.08 mg RE/g extract, and potential as an antioxidant. *Dillenia philippinensis* contains flavonoids, phenolic compounds, glycosides, coumarins, and quinones, and potential as antioxidants (Yu et al. 2020; Ansari et al. 2021). While, the ethanolic extract of *Dillenia pentagyna* contains phenolics, flavonoids, tannins, saponins, alkaloids, and terpenoids (Patle et al. 2020), and potentially as anticancer, antidiarrheal, antimicrobial, antioxidant (Ghandi and Mehta 2013). On the other hand, extract ethanolic *Dillenia bracteata* contains glycosides, carbohydrates, triterpenoids, proteins, amino acids, gums, mucilages, and flavonoids, antioxidants potentially (Shama et al. 2019).

Based on the description above, this study examined the phytochemical compounds and antioxidant activity of *D. excelsa* leaves.

MATERIALS AND METHODS

Plant collection and study location

This research was conducted from November 2021 to January 2022. The samples of *Dillenia excelsa* leaves were obtained from the swamp forest in Melintang Village, Muara Wis sub-district, Kutai Kartanegara district, East Kalimantan. Sample preparation, extraction, and maceration were carried out at the Laboratory of Plant Physiology and Development, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, East Kalimantan, Indonesia.

Preparation of extracts

Dillenia excelsa leaf samples were cleaned, put in a plastic bag, and then taken to the laboratory, then air-dried for ±7 days at 27ºC, and mashed using a blender. Samples were macerated with 98% methanol for 3 days, shaken for 3 days while stirring once a day, then macerated 3 times and filtered using filter paper. The filtrate of the *D. excelsa* leaf methanol extract was evaporated using a rotary evaporator at 50-65ºC and placed in a vacuum oven to detect the dryness of the extract. The % yield of the extract was calculated using the following equation:

$$\%\text{Rendemen} = \frac{\text{Extract Weight}}{\text{Simplicity Weight}} \text{ x } 100\ \%$$

The methanol extract of *D. excelsa* leaves was used to test phytochemicals, total phenolic content, total flavonoid content, and antioxidant activity tests (Masyudi et al. 2022).

Phytochemical analysis

Weighed 1 g of *D. excelsa* leaf methanol extract, then dissolved in 100 mL of methanol and called the stock solution for qualitative phytochemical tests (Manurung et al. 2017; Herawati et al. 2021; Armansyah et al. 2022).

Alkaloid-Dragendroff test

Take 5 mL of stock solution, then add 3-4 drops of Dragendorff's reagent, positive for alkaloids is indicated by the formation of orange to red-brown precipitate.

Flavonoid test

A total of 2 mL of stock solution of *D. excelsa* leaf methanol extract, heated for ±5 minutes, added with 0.1 g of Mg metal (Mg powder) and 5 drops of concentrated HCl, positive flavonoids will be marked with yellow, orange to red colors.

Phenolic test

A total of 5 mL of stock solution of *D. excelsa* leaf methanol extract was added with $FeCl_3$ (3-4 drops), phenolic positive was indicated by producing a green, red, purple, blue, or black solution (Herawati et al. 2021).

Saponin test

A total of 5 mL of stock solution of *D. excelsa* leaf methanol extract, added to 5 mL of hot water, shaken vigorously for 10 minutes, positive for saponins is indicated by the formation of foam for approximately 10 minutes with a foam height of 1-10 cm and does not disappear (Herawati et al. 2021).

Triterpenoid and steroid test - Liebermann Burchard

Two (2) mL of the stock solution of *D. excelsa* leaf methanol extract was taken. Chloroform and a few drops of anhydrous CH_3COOH (Anhydrous Acetic Acid) were added. Sulfuric acid 1 drops add through the sidewall of the test tube, positive for steroids will form blue or green, while positive for triterpenoids will form red or purple (Herawati et al. 2021).

Tannin test

Take 2 mL of the stock solution of *D. excelsa* leaf methanol extract, add 2 mL of chloroform and a few drops of $(CH_3COOH)_2$ Pb 1% lead acetate, positive for tannins will form a yellow color (Manurung et al. 2017).

Glycoside test

A total of 3 mL of stock solution of *D. excelsa* leaf methanol extract, added 0.5 mL of DMSO, shaken and added 1 mL of anhydrous acetate, and added 6 drops of sulfuric acid, positive for glycosides, a purple ring will be formed (Sudrajat et al. 2016a; Manurung et al. 2017).

Coumarin test

Take 2 mL of stock solution of methanol extract, add 3-4 drops of NaOH and 3-4 drops of alcohol, positive coumarin will form a yellow color and produce a lighter color solution (Manurung et al. 2017).

Carotenoid test

Two (2) mL of the stock solution of *D. excelsa* leaf methanol extract was taken, added 2 mL of chloroform and

3-4 drops of 85% sulfuric acid, positive for carotenoids will form a blue color on the surface (Sudrajat et al. 2016b; Manurung et al. 2017).

Test for Total Phenolic Content (TPC)

The total phenolic content test was carried out according to the modified Manurung et al. (2019) method as follows.

Preparation of standard solutions of gallic acid

As a comparison, gallic acid was used; 1 mg of gallic acid was weighed and dissolved with 10 mL of DMSO so that it became a concentration of 100 ug/mL, then diluted into several variations in concentration, namely 0, 2, 4, 6, 8, 10, 12 and 14 ppm. Then a Folin-Carbonate solution was made, which weighed as much as 7.5 mg of Na_2CO_3 and dissolved with distilled water to a volume of 100 mL. A Folin-Carbonate (FC) solution was made with a ratio of 1:9. Standard solution of gallic acid 100 µg/mL added 250 µL of FC solution and added 7.5% Na_2CO_3 solution of 1250 µL each; each concentration was repeated 3 times, incubated for 1 hour in the darkroom and measured absorbance using a UV-Vis spectrophotometer with a wavelength of 760 nm.

Determination of total phenolic extract of D. excelsa leaf methanol

Calculation of the total phenolic content using the following formula:

$$T = \frac{(C \times V)}{M}$$

Where:

T: Total phenolic content (µg GAE/mg extract)

C: Gallic acid concentration formed from the calibration curve (µg/mL)

V: Extract volume (mL)

M: Weight of methanol plant extract (mg)

In determining the total phenolic content of the *D. excelsa* leaf methanol extract, 2 mg was weighed and dissolved in 10 mL of DMSO, then 100 µL of the *D. excelsa* leaf methanol extract solution was taken, and 400 µL of distilled water was added, 250 µL of FC solution was added, and 1250 µL of Na_2CO solution was added, 7.5%, was replicated 5 times, incubated for 1 hour in a dark room and measured the absorbance using a UV-Vis spectrophotometer with a wavelength of 760 nm (Ieamkheng et al. 2022).

Total Flavonoid Content (TFC) Test

The total flavonoid content test was carried out according to Manurung et al. (2017) method as follows.

Determination of quercetin standard solution

As a comparison, quercetin was used, weighed 1 mg of quercetin, and dissolved in 10 mL of DMSO so that it became a concentration of 100 mg/mL, diluted into several variations in concentration, namely 0, 2, 4, 6, 8, 10, 12, 14 ppm. Then made a solution of AlCl: 10%, weighed 10 mg AlCl, and dissolved in methanol to a volume of 100 mL, then made a solution of $NaNO_2$ 5% by weighing 5 mg of $NaNO_2$ and dissolving in 100 mL of distilled water. Then 1 M NaOH solution was made by weighing 4 mg of NaOH and dissolved in 100 mL of distilled water. The standard solution of quercetin 100 g/mL was added with 5% $NaNO_2$ solution of 100 µL each, 10% AlCl solution: 100 µL each, and 500 µL of 1 M NaOH solution. Each concentration was replicated 3 times and incubated for 10 minutes in a dark room, and the absorbance was measured using a UV-Vis spectrophotometer with a wavelength of 510 nm.

Determination of total flavonoids of Dillenia excelsa *leaf methanol extract*

D. excelsa leaf methanol extract solution was prepared by weighing 2 mg and dissolved in 10 mL of DMSO, then pipette 100 µL of *D. excelsa* leaf methanol extract solution, add 700 µL of distilled water, add 100 µL of 5% $NaNO_2$ solution, 100 µL of AlCl solution: 10% and 500 µL of NaOH 1 M solution were replicated 5 times and then incubated for 10 minutes in a dark room, and the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 510 nm.

Calculation of total flavonoid content uses the following formula:

$$T = \frac{(C \times V)}{M}$$

Where:

T: Total flavonoid content (µg QE/mg extract)

C: Concentration of quercetin formed from the calibration curve (µg/mL)

V: Extract volume (mL)

M: Weight of methanol plant extract (mg)

Antioxidant activity

According to research from Molyneux (2004) and Manurung et al. (2017) in testing the antioxidant activity of the DPPH method are as follows.

DPPH method (2,2-diphenyl-1-picrylhydrazyl)

Ascorbic acid standard solution determination

The ascorbic acid standard was prepared by weighing 1 mg dissolved in 10 mL of ethanol to form a 100 µg/mL concentration. Concentration variations were made, namely 1, 1.5, 2, 2.5, and 3 ppm, then a solution of 27% DPPH was made by weighing 27 mg of DPPH and dissolving it in 100 mL of methanol. Ascorbic acid standard solution of 100 µg/mL with 66 µL of each concentration was pipetted, added 934 µL of ethanol and 1000 L of 27% DPPH solution, incubated for 20 minutes in a dark room, and the absorbance was measured using a UV-Vis spectrophotometer with a wavelength of 517 nm.

Determination of the antioxidant activity of Dillenia excelsa *leaf methanol extract*

The solution of *D. excelsa* leaf methanol extract was prepared by weighing 5 mg and diluting with 10 mL of ethanol so that a concentration of 500 µg/mL was formed; variations in concentration were made, namely 200, 100,

50, 25, 12.5, and 6.25 ppm, each concentration was pipetted. 33 µL, added 467 µL of ethanol and 500 mL of 27% DPPH solution, incubated for 20 minutes in a dark room, and measured absorbance using a UV-Vis spectrophotometer with a wavelength of 517 nm (Rahayu et al. 2019)

The percentage of antioxidant activity was calculated using the following equation:

$$\%Antioxidant\ activity = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \text{ x } 100\ \%$$

Where:
Abs control: Absorbance control
Abs sample: Absorbance sample

The IC_{50} value was calculated using a linear regression analysis plot where the abscissa represents the concentration of the extract solution and the ordinate is the percent of antioxidant activity. A lower IC_{50} value indicates a higher antioxidant activity (Alfarabi et al. 2022; Sujana et al. 2022).

FRAP (Ferric Reducing Antioxidant Power) method

Ascorbic acid standard solution determination

As a comparison, ascorbic acid was used, weighed 1 mg, and dissolved in 10 mL of distilled water so that the concentration was 100 µg/mL. Several concentration variations were made, namely 1, 1.5, 2, 2.5, and 3 ppm. Each concentration was taken 250 µL, added with 500 µL phosphate buffer solution (0.2 M, pH 6.6), and added 500 µL potassium ferricyanide 1%. Then the sample solution was put in the oven for 20 minutes at 50°C; after that, the sample solution was added with 500 µL of 10% trichloroacetic acid (TCA), and the sample solution was centrifuged at a speed of 1000 Rcf (Relative Centrifugal Force) for 10 minutes. The sample solution will be divided into two layers; 750 µL of the top layer is taken and added with 750 µL of distilled water and 50 µL of 0.1% ferric chloride. All sample solutions were incubated for 10 minutes in a dark room, and the absorbance was measured using a spectrophotometer at a wavelength of 700 nm.

Determination of the antioxidant activity of Dillenia excelsa *leaf methanol extract*

In determining the antioxidant activity of the methanol extract of *D. excelsa* leaves, 5 mg was weighed and dissolved in 10 mL of distilled water to become a concentration of 500 ppm. Several concentration variations were made, namely 6.25, 12.5, 25, 50, and 100 ppm. Each concentration was taken 250 µL, added with 500 µL phosphate buffer solution (0.2 M, pH 6.6), and added 500 µL potassium ferricyanide 1%. Then the sample solution was put in the oven for 20 minutes at a temperature of 50°C; after that, the sample solution was added with 500 µL trichloroacetic acid 10% (TCA), and the sample solution was centrifuged at a speed of 1000 Ref (Relative Centrifugal Force) for 10 minutes. The sample solution will be divided into two layers; 750 µL of the top layer is taken and added with 750 µL of distilled water and 50 µL of 0.1% ferric chloride. All sample solutions were incubated for 10 minutes in a dark room, and the absorbance was measured using a spectrophotometer at a wavelength of 700 nm (Munteanu and Apetrei 2021).

The percentage of antioxidant activity was calculated using the following equation:

$$\%\text{Antioxidant activity} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \text{ x } 100\ \%$$

Where:
Abs control: Absorbance control
Abs sample: Absorbance sample

Data analysis

Phytochemical data were qualitatively analyzed descriptively, and total phenolic and flavonoid content and antioxidant activity were expressed as standard deviation using excel. The antioxidant activity value was calculated using linear regression analysis based on the IC50 value.

RESULTS AND DISCUSSION

Phytochemical test

The results of the methanol extraction of *D. excelsa* leaves that were evaporated using a rotary evaporator obtained 65.9406 g of methanol extract with a yield of 13.2%. Based on the results obtained, the extract's yield is large. Bordoloi et al. 2019 reported that ethanolic extract of *D. indica* has a 4.7% yield, 10.07% of methanol extract of fruit (Gogoi et al. 2012), and 21.8% in methanolic extract of *D. suffruticosa* leaves (Yakop et al. 2020).

Based on a qualitative phytochemical test, the methanol extract of *D. excelsa* leaves contains alkaloids, flavonoids, phenolic, triterpenoids, and tannins compounds (Table 1). The results of phytochemical tests on the plant of the other *Dillenia* showed that the leaf and stem extract *D. indica* contained alkaloid, flavonoid, tannin, saponin, terpenoid, and steroid compounds, and the root extract contained alkaloids, flavonoids and tannins (Gandhi and Mehta 2013; Abdel-Kader et al. 2015; Shipra et al. 2018; Utami and Anjani 2020; Alam et al. 2020; Gupta et al. 2020). While, the ethanolic extract of *D. indica* leaves was positive for saponins, glycosides, phenols, alkaloids, flavonoids, tannins, carbohydrates, and resins (Parvin et al. 2009; Barua et al. 2018; Prananda et al. 2019; Bordoloi and Das 2019; Nkop et al. 2020). *D. pentagyna* (Roxb) showed that the ethanol extract is enriched with phenolics, flavonoids, tannin, saponin, alkaloid, and terpenoids (Gandhi and Mehta 2013; Patle et al. 2020).

Table 1. Phytochemical test results of methanol extract of *Dillenia excelsa* leaves

Phytochemical test	Result	Description
Alkaloids	+	It is dark green, and an orange precipitate is formed
Flavonoids	+	Orange
Phenolic	+	Solid black color
Saponins	-	No foam is formed for ± 10 min.
Triterpenoids	+	Red or orange
Steroids	-	Dark green color
Tannins	+	A yellow solution is formed
Glycoside	-	Brick red solution

Coumarin	-	Dark green solution
Carotenoids	-	Light green solution

Note: (+): detected to contain secondary metabolites, (-): not detected to contain secondary metabolites

The secondary metabolite compounds of *Dillenia serrata* were researched by Illing et al. 2017 showed that ethanol extract of fruits contained alkaloids, flavonoids, saponin, phenolics, and terpenoids, while methanol extract of its stem bark contained flavonoids, tannins, terpenoids, steroids, and saponins (Sabandar et al. 2020). Thooptianrat et al. (2017); Putra et al. (2019); Yakop et al. (2020) showed that ethyl acetate extract of *D. suffruticosa* leaves contains tannins, polyphenols, and triterpenoids and triterpenoids. On the other hand, Shama et al. (2019) reported that the ethanolic extract of *D. bracteata* contains glycosides, carbohydrates, triterpenoids, proteins, amino acids, gums, mucilages, and flavonoids.

Test of the total phenolic content of *Dillenia excelsa* leaf methanol extract

Before the total phenolic test, the absorbance value of the standard solution of gallic acid was first measured. The results of measuring the absorbance value of the standard solution of gallic acid were used to determine the total phenolic content. The regression curve (linear) of the absorbance value of the standard solution of gallic acid is presented in Figure 1.

Figure 2 shows the absorbance value of gallic acid is increasing. This is because the higher the concentration of gallic acid given, the higher the color intensity, so the higher the absorbance value. The gallic acid calibration curve results obtained a regression equation Y=0.008X +0.106 with a correlation coefficient (R) of 0.98. The results of the total phenolic calculation of *D. excelsa* leaves are presented in Table 2.

The linear regression equation for gallic acid was used to calculate the total phenolic content of the methanolic extract of *D. excelsa* leaves. The total phenolic content of the *D. excelsa* leaf methanol extract was 181.25 g GAE/g extract, meaning that 1 g of the *D. excelsa* leaf methanol extract contained 181,25 g of total phenolic. Gogoi et al. (2012); Vedika (2020) reported that the fruit of *D. indica* has phenolic content (59.99±2.21 mg/g) recorded in 50% aqueous methanolic extract. The total phenolic content of the bark extract of *D. auriculata* has total phenolics equivalent to 19.70±0.05 GAE/g extract (Liana and Murningsih 2019). Ethanolic extract of *D. bracteata* leaves has 68.67 mg GAE/g extract (Shama et al. 2019), while the total phenolic concentration of stem bark *D. serrata* was 59.2 mg GAE/g (Sabandar et al. 2020). Methanol extract *D. suffruticosa* leaves has total phenolic content of 309.91 ±3.42 mgGAE/g dry weight extract (Yakop et al. 2020).

Test of the total flavonoid content of *Dillenia excelsa* leaf methanol extract

Before the total flavonoid test, measurements were made of the absorbance value of the gallic acid solution. The results of measuring the absorbance value of the standard solution of gallic acid were used to determine the total flavonoid content. The regression curve (linear) of the absorbance value of the standard solution of gallic acid is presented in Figure 2.

The total flavonoid content in the methanolic extract of *D. excelsa* leaves was measured three times. The linear regression equation on quercetin was used to calculate the total flavonoid content of the methanolic extract of *D. excelsa* leaves. The results of the total flavonoid calculation in *D. excelsa* leaves are presented in Table 3.

Calculating the total flavonoid content of methanol and *D. excelsa* extracts obtained 36 g QE/g extract, meaning that 1 g of *D. excelsa* leaf methanol extract contains 36 g of total flavonoids. The total flavonoid content is influenced by several factors, namely the drying process and room temperature. The *D. excelsa* leaf sample was dried at room temperature of around 27°C; the higher the temperature used to dry, the higher the flavonoid content in the sample.

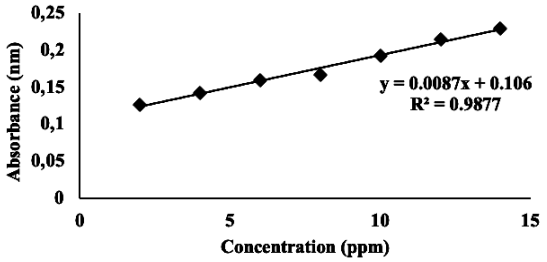

Figure 1. Gallic acid calibration curve at a wavelength of 760 nm

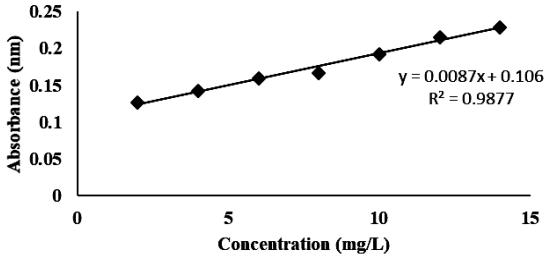

Figure 2. The regression curve (linear) of the absorbance value of the standard solution of gallic acid

Table 2. The results of the total phenolic calculation of *Dillenia excelsa* leaves

Sample	Replicate	Absorbance	Mean	Total phenolic (µgGAE/g extract)
Methanol extract	1	0.134	0.135 ± 0.001	181.25
	2	0.137		
	3	0.133		

Table 3. The results of the total flavonoid calculation in *Dillenia excelsa* leaves

Sample	Replicate	Absorbance	Mean	Total flavonoid (µgQE/g extract)

Methanol extract	1	0.172	0.170 ± 0.001	36
	2	0.170		
	3	0.168		

From the test results, the total phenolic and flavonoid content of the methanol extract of *D. excelsa* leaves has different values, several factors influence this, namely leaf age. The leaves at an older age can synthesize more secondary metabolites. Light, temperature, drought, and salinity affect plants' secondary metabolite content. Rahayu et al. (2019) reported that ethanol extract of *D. indica* leaves contains a flavonoid content of 2.953 mg (QE/mg) in macerated 24 hours at 15% concentration. Ethanolic extract of *D. bracteata* leaves has 61.67 mg quercetin equivalents per g of dry extract (Shama et al. 2019). While total flavonoid content in the stem bark extract *D. serrata* was 23.4 mg QE/g (Sabandar et al. 2020). Methanol extract *D. suffruticosa* leaves have total flavonoid content of 36.24±1.36 mgQE/g dry weight extract (Yakop et al. 2020). In addition, research from Liana and Murningsih (2019) on antioxidant activity, total phenolic and flavonoid content of the bark extract of *D. auriculata* contain total flavonoids are equivalent to 19.53±0.08 RE/g extract so that it has differences in the total phenolic and flavonoid content of the methanol extract of *D. excelsa* leaves, this is influenced by plant varieties, climate, geographical conditions so that it affects the chemical content in plant extracts and their bioactivity.

Antioxidant activity

DPPH method (2,2-diphenyl-1-picrylhydrazyl)

Antioxidant activity was analyzed using the DPPH method at a wavelength of 517 m. Based on the data from the absorbance value, the percentage of free radical inhibition (% inhibition) of ascorbate and methanolic extract of *D. excelsa* leaves is shown in Tables 4 and 5.

Table 4 percentage of inhibition of the sample concentration of methanol extract of *D. excelsa* leaf with DPPH method based on Table 4, it can be seen that the antioxidant activity of the *D. excelsa* leaf methanol extract at the highest concentration of 100 ppm obtained a percent inhibition of 121.737%. In comparison, at the lowest concentration of 6.25 ppm, the percentage inhibition was 71.461%, radical inhibitory activity was characterized by increasing sample concentration, and the high concentration of *D. excelsa* leaf methanol extract, which is 100 ppm, indicates that the content of active antioxidant compounds is more so that the ability to inhibit free radicals will increase. Ethanol extract of *D. indica* has an inhibition percentage of 97.10±0.01% (Saha et al. 2009; Rahayu et al. 2019). Ethanolic extract of *D. bracteata* leaves inhibited 74% with DPPH (Shama et al. 2019). Patel et al. (2020) reported that the ethanolic extract of *D. pentagyna* leaves has an inhibition percentage of 76.46± 1.58% with the DPPH method. Methanol extract of stem bark of *D. serrata* scavenged DPPH radicals (48.2-59.7%) at 100 µg/mL (Sabandar et al. 2020). Methanol extract *D. suffruticosa* leaves has IC_{50} 305.09±4.53 µg/mL DPPH radical scavenging (Yakop et al. 2020; Sinala et al. 2020). This study used ascorbic acid as a comparison (Table 5).

Based on the data from Table 5, it can be seen that the antioxidant activity of ascorbic acid at the highest concentration has an inhibition percentage of 105.991%, while at the lowest concentration obtained % inhibition of 69.527%, it can be seen that the greater the concentration, the higher the inhibition value, meaning the content of secondary metabolites contained in ascorbic acid has a higher antioxidant value and changes the color of the cuvette solution from purple DPPH to yellow. In addition, ascorbic acid functions as a very strong antioxidant source because it can donate hydrogen atoms and form relatively stable ascorbyl free radicals (Yoga 2015).

Based on the value of % inhibition on the concentration of ascorbic acid and methanol extract of *D. excelsa* leaves, a linear regression curve was presented so that the standard curve equation was used in determining the IC_{50} value obtained. The following is a comparison of IC_{50} values between ascorbic acid and methanol extract of *D. excelsa* leaves presented in Figure 3.

Based on Figure 3, it can be seen that the IC_{50} value of ascorbic acid is 2.62 ppm which means that the strength of the antioxidant activity of ascorbic acid is very strong, ascorbic acid is a pure compound that has a very strong antioxidant activity value because it has an IC_{50} value of less than 50 ppm and ascorbic acid is used as control positive. In comparison, the IC_{50} value of the methanol extract of *D. excelsa* leaves was 145 ppm, which means that the strength of the antioxidant activity was moderate.

Table 4. Percent inhibition of methanol extract of *Dillenia excelsa* leaves with the DPPH method

Concentration (ppm)	Inhibition
0	-
100	121.737
50	105.162
25	89.417
12.5	80.853
6.25	71.461

Tabel 1. Percent inhibition of ascorbic acid with the DPPH method

Concentration (µg/mL)	% Inhibition
1	69.527
1.5	77.262
2	97.428
2.5	102.400
3	105.991

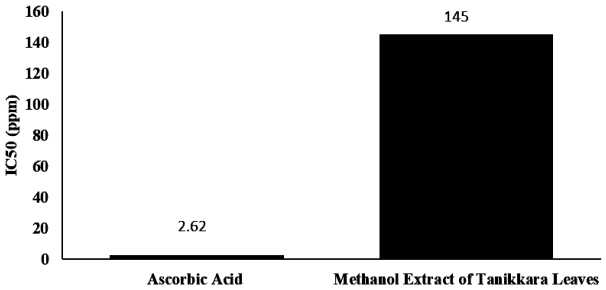

Figure 3. Comparison of IC_{50} values of ascorbic acid and methanol extract of *excelsa* leaves

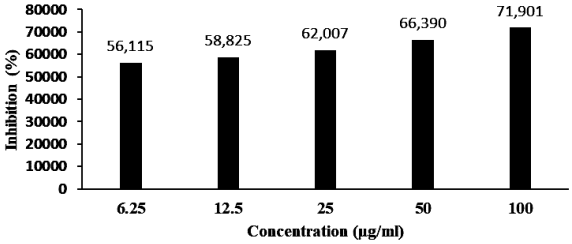

Figure 4. Measurement inhibition percentage of methanol extract of *Dillenia excelsa* leaf

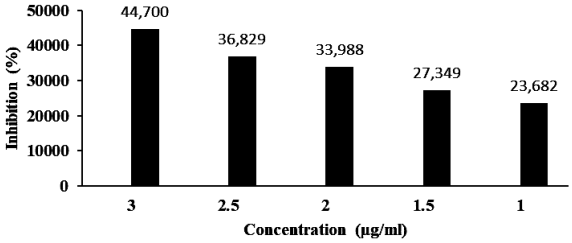

Figure 5. Measurement inhibition percentage of ascorbic acid

A substance has very strong antioxidant properties if the IC_{50} is less than 50 ppm, the strong category is the IC_{50} value of 50-100 ppm, and the medium category is the IC_{50} value is 100-150 ppm. The weak category is that the IC50 value is more than 151 ppm, so the methanol extract *D. excelsa* leaves are categorized as having a moderate level of antioxidant activity. Research from Sabandar et al. (2020); Wibawa et al. (2021), showed that songi bark extract (*D. serrata*) using several organic fractions, with methanol and ethyl acetate fractions from songi bark has the best DPPH radical scavenging activity, namely methanol extract has an IC_{50} value of 59.5 ppm. The ethyl acetate extract had an IC_{50} value of 54.0 ppm. Research from Liana and Murningsih (2019) that the bark extract of *D. auriculata* Mart has an IC_{50} value of 32.28 ppm. Hence, the songi bark has the potential as a natural source of antioxidant agents and has a different IC_{50} value from the methanol extract of *D. excelsa* leaves. On the other hand, the fruit of *D. indica* has antioxidant activity with an IC_{50} value of 56.66±1.55 µg/mL (Gogoi et al. 2012) and root ethanol extract of 30.44±0.003 µg/mL (Bordoloi et al. 2019). At the same time, IC_{50} values of bark and leaves of *D. pentagyna* are 5.64 µg/mL and 6.54 µg/mL (Patle et al. 2020). Some factors also affect the value of antioxidant activity are the extraction method, test method, and conditions during the extraction process, such as solvent volume, leaf powder size, extraction time, temperature, and pressure. In addition, other factors, namely the plant's chemical composition used, can affect the value of the antioxidant activity, the plant's chemical composition is influenced by the conditions of the plant's living habitat, such as light and temperature, which affect the content of chemical compounds in plants. Alfarabi et al. (2022) reported that the differences in metabolite composition in extract result in different characteristics and cause differences in antioxidant activity.

FRAP (Ferric Reducing Antioxidant Power) method

The results of the antioxidant activity test using the FRAP method consist of several concentrations where the higher the concentration, the higher the value of % inhibition in inhibiting free radicals. In Figure 4, it can be seen that the highest antioxidant activity was found at a concentration of 100 ppm at 71.901% and the second-highest antioxidant activity at a concentration of 50 ppm at 66.390%, based on the value of the inhibition % that the concentrations of 100 ppm and 50 ppm could potentially be antioxidant agents.

Sabandar et al. (2020) reported that methanol extract of stem bark of *D. serrata* has FRAP values varied from 0.8-3.4 µg/µg trolox equivalent amount (quercetin and gallic acid were 25.7 dan 32.4 µg/µg), inhibited xanthine oxidase (15.3-50.3%) at 100 µg/mL (allopurinol, 98.2%). A sample can be used as an antioxidant agent if it can reduce Fe^{3+} complex compounds to ferrous Fe^{2+} in an acidic state, indicated by the sample solution in the cuvette forming a turquoise color on absorbance measurement using a spectrophotometer. And on testing, the antioxidant activity of ascorbic acid shows in Figure 5. At a concentration of 1 ppm, it can reduce free radicals by 23.682%, at the highest concentration of 3 ppm, it can reduce free radicals by 44.700%.

The principle of the FRAP method is based on a reduction reaction in an acid environment to a yellow Fe^{3+} (potassium hexacyanoferrate) complex compound into a bluish-green Fe^{2+} complex compound due to electron donors from antioxidant compounds; this method measures the absorption of the Fe^{2+} complex compound formed using a spectrophotometer at a maximum wavelength is 700 nm. Acid conditions in the FRAP method can reduce the ability of antioxidant compounds due to acid protonation. The FRAP method is a very short antioxidant process, so fast results are obtained (Maesaroh et al. 2018). In conclusion, *D. excelsa* leaves extract with methanol solvent had high metabolites and potentially an antioxidant.

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