Antioxidant capacity of Dillenia sp. leaf extract against DPPH (1,1-Diphenyl-2picryl Hidrazil) radical

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Abstract. Some plants have capacity as exogenous antioxidant. Simpor (Dillenia sp.) is plant of the Dilleniaceae family which may have the property of antioxidant. The purpose of this study was to measure the antioxidant capacity of the 96% ethanolic simpor leaf extract. Factorial design was applied with 2x3 factors of maceration time (24 and 48 hours) and concentration series of (10, 15, 20%). Normality was done by Kolmogorov-Smirnov and homogeneity by Levine. Data was analysed by ANOVA (α= 0.05). Post Hoc was analysed by Duncan. Antioxidant property was analysed qualitatively and quantitatively on flavonoid and also leaves water content. The antioxidant capacity assay was measured using DPPH (1,1-Diphenyl-2picryl Hidrazil) scavenging ability at 517 nm with ascorbic acid as control. The result showed that water content of the plant was 73.65%. Simpor contain flavonoids with highest flavonoid content (2.953 mg QE / mg) in 24 hours maceration time at 15% concentration. Furthermore, it also has ability to scavenge DPPH (1,1-Diphenyl-2picryl Hidrazil) radical with the highest value (97.10 ± 0.01%) in 24 and 48 hours maceration at 20% and 10% extract concentration. Statistical analysis on flavonoid content showed that time and concentration gave significant effect on flavonoid content (α=0.003). Duncan result revealed that almost all groups didn’t significantly different only the 24 hours x 15% concentration was. DPPH inhibition based on statistic proved that both factors didn’t affect scavenging ability of Dillenia (α=0.079). It can be concluded that simpor leaves has antioxidant capacity against DPPH radical due to its flavonoid content. Furthermore, concentration and maceration time significantly affect flavonoid content and didn’t affect DPPH radical scavenging ability of Dillenia plant.

1. Introduction
Antioxidants are compounds that can fight free radicals. Free radicals are obtained from the rest of the results of body metabolism, cigarette smoke, chemicals in food, air pollution, sunlight, and so forth [1]. Antioxidants collect in balancing free radical molecules in the metabolic process [2]. The mechanism of antioxidants is to make redox and absorb free radicals quickly inside the membrane [3]. The need for sources of antioxidants from plants needs to be optimized because they have no side effects and prices are relatively affordable. Naturally the body is able to produce antioxidants, but the amount of free radicals is excessive so the antioxidants are no longer able to overcome them. Another alternative to fight free radicals is consuming antioxidants from outside the body to help endogenous antioxidant to cope with it [4]. One source of antioxidants can come from plants. These sources of antioxidants can be...
obtained from stems, leaves, fruits and seeds. Many plants have been widely known contain antioxidant [5]. One of the plants which has an antioxidant source is the simpor.

Simpor (Dillenia sp.) is a plant species from the Dilleniacea tribe. These plants are spread in various Asian regions including Bhutan, India, Laos, Nepal, Philippines, Sri Lanka, Thailand, Vietnam, Myanmar, Malaysia and Indonesia [6]. In India there are only two types of symposiums that pose as medicinal plants, namely Dillenia indica Linn. and Dillenia penagyana Roxb [7]. In the Belitung area the community uses simpor leaves as a fish wrap, rice cake wrappers and vegetable wrappers in traditional markets.

The content of secondary metabolites in D.indica leaves is flavonoids, triterpenoids, steroids, tannins [8], while those in fruits contain 34% phenolics [9]. In D. indica seeds, there are fixed oil, color matter, sterols, glycosides, saponins, proteins, free amino acids, sugars, free acids and tannins [10]. Different types of plants contain different secondary metabolites. D. penagyana contains 6% tannins. The stem contains naringenin-41-O-b-D-xylopyranoside, flavonoid glycosides, 7-galactosyl glucoside naringenin and dihydroquercetin 5-galactoside along with rhamnetin-3-glucoside, saponins. Both plants have betalin, betulinic acid, and β-sitosterol [6,10,11].

Research on secondary metabolites in D. indica and D. penagyana has been studied extensively, ranging from isolation, separation and quantification. Nevertheless, no report dealing with the Dillenia sp. leaves from Belitung has been informed. This study has been carried out to analyze the ability of Dillenia sp. leaf extract as antioxidant in reducing DPPH (1,1-Diphenyl-2-picryl Hidrazil) free radicals. DPPH was applied in the study as free radicals model to be scavenged by antioxidant from simpor.

2. Material and method

2.1. Place and time
The research was conducted at the Biochemical Laboratory Biology Faculty of Mathematics and Natural Sciences, Universitas Negeri Jakarta on February-April 2019.

2.2. Plant material
Leafy Dillenia sp. were collected from Belitung. Dillenia sp. leaves are cut into small pieces, weighed as much as 100 grams and dried in direct sunlight for 2 days to reduce water content. Calculation of water content [12].

\[ \text{Water content} \% = \frac{(w1 - w2)}{w1} \times 100 \]

W1: initial weight; W2: final weight.

2.3. Extraction
The leaves powdered was weighed and extracted with ethanol 96% with variations in maceration time 24 and 48 hours and concentration series of (10%, 15%, and 20%). The solvent was removed by using rotary evaporator at temperature 45°C [13,14].

2.4. Qualitative Flavonoid Assay
30 mg of extraction was put into a test tube. Magnesium powder and a few drops of concentrated HCl was added. Positive flavonoids were seen as yellow orange color formation [15].

2.5. Flavonoid

2.5.1. Configuring standard quercetin solution: A total of 2.5 mg of quercetin was weighed and dissolved in 25 ml of ethanol as standard, 100 ppm. A series of standard concentrations of 20, 30, 40, 50 and 60 ppm is made. 0.5 mL of standard liquid solution added 0.1 mL aluminum (III) chloride 10%, 0.1 mL sodium acetate 1 M and 2.8 mL distilled water. Taken one of the concentrations of a standard solution, the absorbance is measured at a wavelength of 400-800 nm.
2.5.2. Setting a standard quercetin curve: The standard curve is made by connecting the concentration of the standard quercetin solution with the absorbed results obtained by measurement using a UV-Vis spectrophotometer at a wavelength of 437.55 nm [15].

2.6. Determination of Total Flavonoid Levels in Extracts

A total of 20 mg samples were weighed and dissolved in 10 mL pa ethanol and then centrifuged to obtain a concentration of 2000 ppm. 0.5 mL of the test sample was added with 0.1 mL aluminum (III) chloride 10%, 0.1 mL sodium acetate 1 M and 2.8 mL distilled water. After incubation for 30 minutes, the absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength of quercetin 437.55 nm. The total flavonoids from the ethanol extract of the leaves of *Dillenia* sp. were calculated using a linear regression equation from the quercetin calibration curve that had been previously measured. Total flavonoid content is expressed as the number of mg quercetin equivalent to each mg of extract [15].

2.7. DPPH Radical Scavenging Assay

Referring to the study of Kanta et al., the modified antioxidant capacity of the extract was measured DPPH (1,1-Diphenyl-2-picryl Hidrazil), free radical 1ml 0.1mM of DPPH solution in ethanol was mixed with 1ml of leaf solution extract of varying concentration (10, 50, 100, 150 and 200) ppm [16]. Corresponding blank samples were prepared and ascorbic acid was used as a standard reference. As control as used 1ml ethanol and 1ml DPPH solution. The DPPH measured absorption at 517 nm after 30 minutes in dark using UV-Vis spectrophotometer. The% inhibition was measured using the following formula.

\[
\text{Inhibition \%} = \frac{\text{Ac} - \text{As}}{\text{Ac}} \times 100
\]

Where Ac is the absorbance of the control and As is the absorbance of the sample.

3. Results and discussion

3.1. Water content

Moisture content of sample used was the leaves of *Dillenia* sp. which had been dried in the sun for two days. The results of the analysis of *Dillenia* sp. leaves obtained water content of 73.65%. Decrease in water content occurs due to the evaporation process. Water and compounds found in leaves easily evaporate so that the levels decrease and affect the weight of the leaves. The reduction in water content in the leaves aims to be easily stored and inhibit the enzymatic process. Large water levels allow the simplicia to become a growth medium for microorganisms [17,18].

3.2. Qualitative flavonoid assay

| Treatment | Inferences |
|-----------|------------|
| 10%       | +          |
| 15%       | ++         |
| 20%       | +++        |
| 10%       | +++        |
| 15%       | ++         |
| 20%       | +          |

Table 1. Preliminary qualitative flavonoid analysis of ethanolic leaf extract of *Dillenia* sp.

(+) present.
Qualitative test results of flavonoids on leaf extract of *Dillenia* sp. positively contains flavonoids. Indications that the extract is positive for flavonoids will change colour after adding magnesium and HCl reagents to yellow. The addition of magnesium and HCl reagent in the extract aims to reduce the nucleus of benzopirone in flavonoid compounds. Addition of HCl causes a reduction between magnesium and flavonoid compounds [15].

3.3. **Quantitative flavonoid assay**

Determination of flavonoid content aims to determine the flavonoid compounds contained in the leaf of *Dillenia* sp. Levels of flavonoid compounds were expressed in mg QE / mg. Determination of flavonoid levels uses the AlCl$_3$ colony method with the principle of complex formation. This principle forms a shift in the wavelength of the visible direction so that the color of the solution turns yellow. AlCl$_3$ will form complex and stable compounds after reacting with C4 ketone groups and hydroxyl groups on C3 or C5 from flavone and flavonol compounds [19]. The formation reaction of the Flavonoid-AlCl$_3$ complex can be show in Figure 1 and 2.

![Figure 1](image1.png)  ![Figure 2](image2.png)

**Figure 1.** The formation reaction of the Flavonoid-AlCl$_3$ complex.  
**Figure 2.** Complex formation reaction between AlCl$_3$ and flavone [20].

Quesertin was used in designing flavonoid standard curves since it belongs to the flavonoid group [21], which have ketone groups on C4 atoms and hydroxyl groups on C3 or C5 atoms. In addition, quesertin easily reacts with AlCl$_3$ [22]. The flavonoids have chelating properties so they can bind to metals and prevent increased oxidation and gives positive effect [23].

The absorbance of the questionnaire was plotted against the concentration so that it gets the calibration curve equation. From the graph obtained the regression equation $y = 0.0007x - 0.002$; with a correlation coefficient ($R^2$) = 0.9689. The correlation coefficient ($R^2$) value close to 1 indicates regression analysis can be trusted (Figure 3).

![Figure 3](image3.png)  
**Figure 3.** Standard quecertin curve.
The results of the research in Table 2 shows that the highest concentration of flavonoid compounds were at 24 hours maceration time with a concentration of 15% (2.953 mg QE / mg). The lowest was at the maceration time of 48 hours with a concentration of 20% (2.875 mg QE / mg). Statistical analysis showed that data was homogenous and normally distributed ($\alpha> 0.005$). ANOVA results obtained maceration time and concentration significantly affect flavonoid content ($\alpha = 0.003$). Duncan Pos Hoc test showed that almost all group were not different unless the 24 hours x 15% one.

### Table 2. Flavonoid content of ethanolic leaf extract of *Dillenia* sp.

| Time maceration (hours) | Concentration (%) | Flavonoid content (mg QE/mg) |
|-------------------------|-------------------|-----------------------------|
|                         | 10                | 2.904                       |
| 24                      | 15                | 2.953                       |
|                         | 20                | 2.877                       |
| 48                      | 10                | 2.893                       |
|                         | 15                | 2.887                       |
|                         | 20                | 2.875                       |

3.4. **DPPH Radical Scavenging Assay**

Free radicals are relatively unstable because they have unpaired electrons in the outer orbit. These electrons are very reactive if they are compounded with other molecules. Substances that are able to prevent and protect from the side effects of free radicals are antioxidants. Antioxidants can to subvert one molecule and stabilize it.

DPPH is a free radical compound that is often used to measure antioxidant activity from various compounds or extracts of natural ingredients. This method is easy to use to measure antioxidant activity in extracts. DPPH which was reacted with ethanolic leaf extract of *Dillenia* sp., caused the intensity of purple to decrease and turn yellow (Figure 4, 5). This color change occurs because of the transfer of electrons from certain compounds in the ethanolic extract of *Dillenia* sp. on DPPH so that it becomes stable.

![Figure 4](image1.png)

**Figure 4.** Results of a qualitative flavonoid analysis of ethanolic leaf extract of *Dillenia* sp. in maceration time 24 and 24 hours and series consentration of 10%, 15%, and 20% (left to right).

![Figure 5](image2.png)

**Figure 5.** DPPH scavenging of ethanolic leaf extract of *Dillenia* sp. the 24 hour in series concentration (10, 50, 100, 150, and 200) ppm shows the change in color of DPPH after being given *Dillenia* sp. ethanolic leaf extract from purple to yellow.

The method used to measure activity is expressed by the value of DPPH reduction. DPPH reduction in ethanolic leaf extract of *Dillenia* sp. in maceration 24 was highest at extract concentrations of 20% (97.097%) while the extracts in maceration 48 hours were highest at extract concentrations of 10% (97.097%) (Table 3).
Table 3. DPPH scavenging of ethanolic leaf extract of *Dillenia* sp. in maceration time 24 and 48 hours (values represent means±SE).

| Concentration (ppm) | Concentration extract | 24 hours |  |  |  | 48 hours |  |  |  |
|---------------------|-----------------------|----------|---|---|---|----------|---|---|---|
|                     |                       | 10%      | 15%| 20%| 10%| 15%      | 20%|  |  |
| 10                  |                       | 88.69 ± 0.01 | 65.47 ± 0.01 | 91.89 ± 0.00 | 94.39 ± 0.00 | 85.09 ± 0.01 | 91.39 ± 0.00 |  |  |
| 50                  |                       | 91.79 ± 0.01 | 89.29 ± 0.01 | 91.89 ± 0.00 | 91.49 ± 0.01 | 78.58 ± 0.01 | 89.89 ± 0.00 |  |  |
| 100                 |                       | 89.79 ± 0.01 | 95.20 ± 0.01 | 96.70 ± 0.00 | 97.10 ± 0.01 | 88.69 ± 0.01 | 91.99 ± 0.01 |  |  |
| 150                 |                       | 95.70 ± 0.01 | 92.59 ± 0.01 | 96.30 ± 0.00 | 91.69 ± 0.01 | 88.59 ± 0.01 | 89.79 ± 0.01 |  |  |
| 200                 |                       | 90.59 ± 0.01 | 84.58 ± 0.01 | 97.10 ± 0.00 | 95.10 ± 0.01 | 94.19 ± 0.00 | 92.49 ± 0.01 |  |  |

Antioxidant properties can be found in extracts with different concentrations. Ethanolic extracts are polar solvents and are very effective for extracting antioxidant compounds in the leaves of *Dillenia* sp. This study provides evidence that the leaf of *Dillenia* sp. from Belitung contains flavonoid compounds and has the potential as a source of antioxidants. Antioxidant activity is influenced by time and concentration during extraction. Statistical analysis showed that concentration and maceration didn’t affect DPPH scavenging ability *Dillenia* (p 0.079).

4. Conclusion
Based on the results of research and data analysis that has been carried out that ethanolic leaf extract of *Dillenia* sp. have flavonoid levels of 2.953 mg QE / mg with a 24 hour maceration time at a concentration of 15%. Ethanol leaf extract *Dillenia* sp. also has antioxidant activity with the DPPH method. The highest ability to scavenge radicals on DPPH was 97.097% at maceration 24 and 48 hours at 20% and 10% extract concentration.

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