Antioxidant activity of Ethanol Extract, \textit{n}-Hexane fraction, Ethyl Acetate fraction and Water fraction of Garut Orange Leaves (\textit{Citrus reticulata} Blanco)

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**Abstract.** Antioxidants are compounds that are able to eliminate, cleanse, and resist radical effects. Antioxidants can stabilize free radicals by complementing the lack of electrons possessed by free radicals. Several studies have shown that citrus leaves of other species have been shown to have antioxidant activity. This study aims to determine the antioxidant activity of ethanol extract, \textit{n}-hexane fraction, ethyl acetate fraction and water fraction of Garut orange leaves (\textit{Citrus reticulata} Blanco) using DPPH (2,2-Diphenyl-1-picrylhydrazyl) method. Garut orange leaves were extracted with ethanol 96\% by macerator apparatus. Concentrated extracts were fractionated by liquid extraction method with \textit{n}-hexane, ethyl acetate and water solvent. Furthermore, the ethanol extract, \textit{n}-hexane fraction, ethyl acetate fraction and water fraction of Garut orange leaves were tested for antioxidant activity using the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) method. The antioxidant activity test results showed that ethanol extract of Garut orange leaves had better antioxidant activity with IC\textsubscript{50} value of 42.925 ppm, followed by \textit{n}-hexane fraction with IC\textsubscript{50} value 46.156 ppm, ethyl acetate fraction with IC\textsubscript{50} value 49.371 ppm and water fraction with IC\textsubscript{50} 55.662 ppm.

1. Introduction
Free radicals are reactive and play a role in the occurrence of various diseases. This is because free radicals can bind to carbohydrates, proteins, lipids and even DNA [1]. Therefore, the body needs antioxidants to counteract the presence of free radicals [2]. Several studies have shown that citrus leaves of other species have been shown to have antioxidant activity. Garut orange from fruits, roots and leaves contain flavonoids and terpenoids [3]. The efficacy of Garut oranges has been believed as medicine for nausea, increasing endurance, diarrhea, rheumatoid arthritis, helping the digestive process, increasing endurance, fever and flu and losing weight [4]. However, the efficacy of Garut orange leaves has not been scientifically proven. Therefore, this study aims to determine the antioxidant activity of ethanol extract, \textit{n}-hexane fraction, ethyl acetate fraction and water fraction of Garut orange leaves (\textit{Citrus reticulata} Blanco) using the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) method. This study is expected to be a reference for the use of Garut orange leaves which are efficacious as antioxidants.
2. Method

2.1. Plant material
500 gram of dry leaves of Garut orange (Citrus reticulata Blanco) were collected at Eptilu’s Farm, Cikajang, Garut, West Java in February 2017. Confirmatory identification of the plant was done at Herbarium Bandungense, School of Life Science and Technology, Bandung Institute of Technology, West Java, Indonesia. Dry leaves of Garut orange were characteristic check and qualitatively tested for the presence of secondary metabolite by using phytochemical screening. Garut orange leaves were extracted with ethanol 96% by macerator apparatus, then kept 3x24 hours by changing ethanol every 24 hours. Liquid extract was then separated by using flannel cloth and the filtrate was concentrated by evaporator. The concentrated extract is then carried out by liquid extraction with solvents n-hexane, ethyl acetate and water. The concentrated extract used was weighed in a ratio 1:1 with the solvent. The first solvent used was water, then n-hexane was added. Shake and replace n-hexane solvent until n-hexane fraction was cleared. Furthermore, the water fraction is mixed with ethyl acetate solvent. Shake and replace ethyl acetate solvent until ethyl acetate fraction was cleared. Then from each fraction remove the solvent using rotary evaporator and water bath until the fraction was concentrated [5].

2.2. Experimental design
Testing of antioxidant activity using the DPPH method included preparing samples, preparing vitamin C solution, making DPPH solution, determining the maximum wavelength, and determining IC50 for each sample. The samples tested were ethanol extract, n-hexane fraction, ethyl acetate fraction and water fraction from Garut orange leaves [5-7].

3. Result and discussion
Check characteristics to see the quality of dry leaves of Garut orange (Citrus reticulata Blanco). The result can be seen in Table 1.

Table 1. The result of characteristic check.

| No. | Characteristic Check          | The result (%) |
|-----|------------------------------|----------------|
| 1.  | Total Ash Content            | 10.67          |
| 2.  | Acid Insoluble Ash Content   | 1.80           |
| 3.  | Water Soluble Ash Content    | 3.30           |
| 4.  | Water Soluble Extract Content| 2.30           |
| 5.  | Ethanol Soluble Extract Content| 6.30        |
| 6.  | Loss on Drying              | 7.33           |
| 7.  | Water Content                | 5.00           |

Phytochemical screening to see the secondary metabolite of dry leaves and concentrated extract of Garut orange (Citrus reticulata Blanco). The result can be seen in Table 2.

Table 2. The result of phytocemical screening.

| No. | Secondary Metabolite | Dry Leaves | Concentrated Extract |
|-----|----------------------|------------|----------------------|
| 1.  | Alkaloid             | -          | -                    |
| 2.  | Flavonoid            | +          | +                    |
| 3.  | Saponin              | +          | +                    |
| 4.  | Tannin               | -          | -                    |
| 5.  | Kuinon               | +          | +                    |
| 6.  | Polyphenols          | +          | +                    |
| 7.  | Steroid/Triterpenoid | +          | +                    |

+: Detected  -: Undetected
Testing of antioxidant activity using the DPPH method to determine Inhibition Concentration (IC_{50}). IC_{50} value is defined as the amount of concentration of test compounds that can reduce free radicals by 50%, the smaller IC_{50} value, the greater antioxidant power. This method is used because it is a simple, easy method and uses samples in small amounts with a short amount of time. Testing of antioxidant activity using the DPPH method begins with determining the maximum wavelength to determine the wavelength that has the highest absorption. The results obtained that the maximum wavelength of DPPH solution is 517 nm with an absorption value of 0.769. The principle of measuring quantitative antioxidant activity using the DPPH method is the change in DPPH purple color intensity which is proportional to the concentration of the DPPH solution. DPPH free radicals that have unpaired electrons will give purple. The color will turn yellow when the electrons are in pairs. These color changes affect the DPPH absorbance value, the higher concentration of the sample used, the lower the absorbance value of DPPH solution. This color change occurs because of a compound that gives hydrogen atoms to DPPH radicals so that they are reduced to a more stable DPPH-H [6,7].

![Figure 1. Vitamin C % inhibition curve.](image1)

Positive control is used vitamin C, because vitamin C can dissolve in polar solvents and the compounds contained in vitamin C have the ability to reduce or counteract free radicals very well. The result of Vitamin C % inhibition curve can be seen Figure 1. IC_{50} value is defined as the amount of concentration of test compounds that can reduce free radicals by 50%. The smaller IC_{50} value, the higher free radical reduction activity [8].

![Figure 2. Ethanol extract % inhibition curve.](image2)
Figure 3. \( n \)-Hexane fraction % inhibition curve.

\[
y = 0.6004x + 22.288 \\
R^2 = 0.9837
\]

Figure 4. Ethyl Acetat fraction % inhibition curve.

\[
y = 0.7676x + 12.103 \\
R^2 = 0.9958
\]

Figure 5. Water fraction % inhibition curve.

\[
y = 0.622x + 15.378 \\
R^2 = 0.992
\]
From the results of testing the antioxidant activity using the DPPH method it is known that the ethanol extract of Garut orange leaves (Citrus reticulata Blanco), n-hexane fraction, ethyl acetate fraction has very strong antioxidant activity with a range of <50 [Figure 2,3,4,5].

Identification of compounds suspected of being responsible for antioxidants using monitoring TLC method (Thin Layer Chromatography). The stationary phase used is silica gel GF254 and as its mobile phase using n-hexane: ethyl acetate (7: 3).

![Thin Layer Chromatography](image)

Figure 6. The result of Thin Layer Chromatography (a) Ethanol extract, (b) n-Hexane fraction, (c) Ethyl Acetate fraction and (d) Water fraction.

The results of TLC thought providing antioxidant activity in ethanol extract, n-hexane fraction, and ethyl acetate fraction are flavonoid compounds, because it is seen from the Rf on the plate that has been sprayed with DPPH 0,2% spotting appearance that has Rf 0.86 for which the Rf value is the same as the AlCl3 sprayed plate having Rf 0.86.

4. Conclusion
The ethanol extract, n-hexane fraction, ethyl acetate fraction and water fraction of Garut orange leaves were tested for antioxidant activity using the DPPH (2,2-Diphenyl-l-Picrylhydrazyl) method. The antioxidant activity test results showed that ethanol extract of Garut orange leaves had better antioxidant activity, followed by n-hexane fraction, ethyl acetate fraction and water fraction. The results of TLC thought providing antioxidant activity in ethanol extract, n-hexane fraction, and ethyl acetate fraction are flavonoid compounds.

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