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Article

Antioxidant Activities Extracts N-Hexane, Ethyl Acetate and Methanol of Limau Sundai *(Citrus nobilis* Lour*)* Peels

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Abstract The Limau Sundai plant (*Citrus nobilis* Lour) is widely known in West Sumatra. The fruit of this plant is commonly consumed as a cooking spice. This antioxidant activity test research aims to find out the potential of Lime Sundai peel as a source of antioxidant compounds. The peel of Limau Sundai fruit is ensnared with methanol solvent and concentrated using a rotary evaporator to obtain a coarse extract. The crude extract is then partitioned successively in n-hexane and ethyl acetate so that n-hexane, ethyl acetate, and methanol extracts are obtained. Sundai lime peel has good antioxidant activity against DPPH, which is shown in the ethyl fraction of acetate, methanol, and liquid methanol. The antioxidant activity of the three fractions is smaller than vitamin C and more significant than the hexane fraction. In the ethyl acetate, methanol and methanol/aqueous fractions with concentrations of 1076, 1822, and 1372 ppm in a row can reduce 50% of DPPH radical activity.

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1. Introduction

Antioxidants are compounds with low molecular weight in plasma, such as Vitamin A, bilirubin, and uric acid [1-4]. Antioxidants can reduce oxidative damage caused by reaction oxygen species (ROS) [5-6]. Some of the functions of antioxidants are new radical inhibitors, Captured free radicals to avoid chain reactions, and restoring disturbances caused by free radicals. Among the antioxidant compounds commonly found in fruits or vegetables are carotenoids, Vitamin C, and Vitamin E [7-10].

Citrus plants such as sweet oranges, limes, mandarin oranges, and lemons belong to the family rutaceae, which is one of the largest and most popular fruit crops in the world. In general, oranges are used as orange juice and a large amount of waste of underutilized orange peel [11-12]. Many studies mentioned that orange peel contains vitamin C, dietary fiber, and phytochemical compounds such as flavonoids, carotenoid alkaloids, triterpenes, amino acids, and phenolic acids [13-15]. Based on phytochemical tests on the peel of fruit *Citrus nobilis* Lour it can be found that the peel of *Citrus nobilis* Lour lime fruit contains many phytochemical compounds, namely flavonoids, phenolics, and alkaloids (ethyl acetate extract and methanol extract) while n-hexane extract peel of *Citrus nobilis* Lour contains only steroid compounds [16-17].

Several of the studies that have been conducted are tests of antioxidant activity in lemon cultivars [18-19], citrus peels of *Citrus sinensis* [20-21], and citrus flesh and peel *Citrus auranthium* [22-25]. The study used a variety of solvents in extracting samples, such as research conducted by Park et al. (2014) using acetone, ethanol and methanol solvents in extracting *Citrus auranthium* peels and meat. Using various solvents aims to determine the antioxidant activity in each solvent. In addition, Hegazy & Ibrahium (2012) also used a variety of solvents (methanol, ethanol, dicloromethane, acetone, hexane, and ethyl acetate) in testing antioxidant activity in citrus peels citrus sinensis obtained the highest antioxidant activity owned by ethanol solvents with a percentage of 78% [24-27].

Based on the chemotaxonomic approach, there is a possibility of the same compound in the fruit peel of *Citrus nobilis* Lour plant that has the potential as an antioxidant [28-29]. The fruit is commonly consumed in the form of juice and used as a cooking spice, while the peel is rarely consumed but can be used as a complement to certain dishes [30-31]. Therefore, phytochemical screening testing is carried out to determine the phytochemical content of n-hexane extract, ethyl acetate, and methanol of *Citrus nobilis* Lour peels which has the potential as an antioxidant and testing antioxidant activity in n-hexane extract, ethyl acetate and methanol fruit peel of *Citrus nobilis* Lour which is reviewed from IC_{50} value.

2. Experimental Section

2.1. Materials

The tools used in this study were glassware, rotary evaporators, reagent bottles, analytical balance sheets, micropipettes, notch pipettes, and Uv-Vis spectrophotometers. The ingredients used in this study were samples of lime sundai (*Citrus nobilis* Lour) fruit peels, ascorbic acid, sulfuric acid, hydrochloric acid, iron (III) chloride, Mayer reagents, Lieberman-Buchard reagents, sodium hydroxide, magnesium bands, distilled technical solvents namely hexane, ethyl acetate, methanol, aquades, DPPH (1,1-diphenyl-2-picrilhydrazil). Limau sundai fruit (*Citrus nobilis* Lour) was obtained from Kuranji Subdistrict Area of Padang City, West Sumatra and the plant was identified in the Bogorinse Herbarium, the Botanical field of the Bogor Biological Research Center-LIPI.

2.2. Procedure

The absorption of antioxidant activity of n-hexane extract, ethyl acetate and methanol of sundai lime peel (*Citrus nobilis* Lour) includes several stages [11][32].

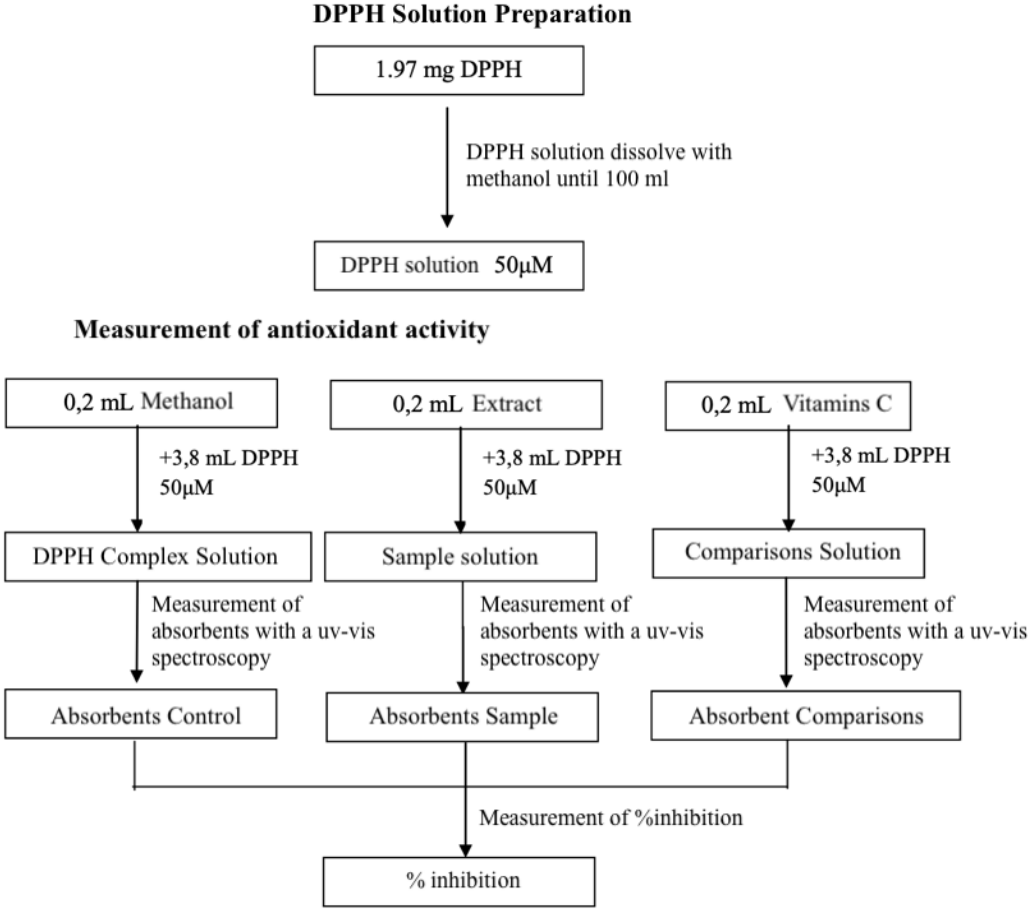

Figure 1. Experimental section

Then the antioxidant test is done. Percent of inhibition is calculated by the formula:

$$\% \text{ inhibition} = \frac{\text{Control A} - \text{Sampel A}}{\text{Control A}} \; x \; 100\% \qquad [38\text{-}39]$$

where A is the absorbance.

3. Results and Discussion

Antioxidant Activities Test

DPPH (2,2-diphenyl-2-picrylhyrazyl) is a measurement method for determining antioxidant activity in testing the activity of natural compounds in plants and biological systems. The advantages of the DPPH method are easy, fast, and affordable for researchers [33-34]. Tested of antioxidant activity is carried out by measuring the absorbance of DPPH reagent solution at the maximum wavelength reacted with the test solution (sample and comparison) marked purple decay in DPPH [33][35]. The color change can be measured by a spectrophotometer expressed by % damping and then plotted

against the concentration. Then the EC_{50} value is calculated from the linear regression obtained [36-37].

In this study, measurements were taken on a sample solution of *Citrus nobilis* Lour fruit peel and an ascorbic acid comparison solution with a concentration of 10 µg/mL to find out its ability to capture free radicals [33][38]. The methanol extract was chosen because it has greater antioxidant activity than other extracts [39-40]. The 3.8 mL solution of DPPH 50 µM added in 0.2 mL of methanol is used as a control obtained absorbent of 0.463 [41-42]. The results of absorbent measurements of hexane fraction samples, ethyl acetate, methanol, and methanol/aqueous and vitamin C obtained absorbents from each sample with several concentrations [43-44], namely 1000 ppm, 500 ppm, 250 ppm, 125 ppm, 62.5 ppm, 31.25 ppm [45-46] can be seen in Table 1.

Table 1. Results of absorbent measurement and percent inhibition of ethyl acetate fraction, methanol, methanol/aqueous fruit peel extract *Citrus nobilis* Lour fruit at some concentrations

No	Fraction	Concentration (ppm)	Absorbance	% Inhibition
1.	Ethyl Acetate	1000.00	0.253	45.35
		500.00	0.336	27. 42
		250.00	0.399	13.82
		125.00	0.424	8.42
		62.50	0.438	5.39
		31.25	0.451	2.59
2.	Methanol	1000.00	0.335	27.64
		500.00	0.389	15.98
		250.00	0.425	8.20
		125.00	0.443	4.31
		62.50	0.448	3.23
		31.25	0.453	2.15
3.	Methanol/watery	1000.00	0.292	36.93
		500.00	0.369	20.30
		250.00	0.405	12.52
		125.00	0.428	7.55
		62.50	0.439	5.18
		31.25	0.445	3.88
4.	N-Hexane	1000.00	0.395	14.68

Based on Table 2. It is known that the hexane fraction with a concentration of 1000 ppm has a very small percent inhibition [8]. This indicates that the sample does not have good antioxidant activity. While the ethyl acetate, methanol and methanol/watery fractions showed quite good antioxidant activity [47-48]. From the percent of inhibitions obtained calculated IC_{50} from these three factions [49-50]. The IC_{50} values of the three fractions can be seen in Table 2.

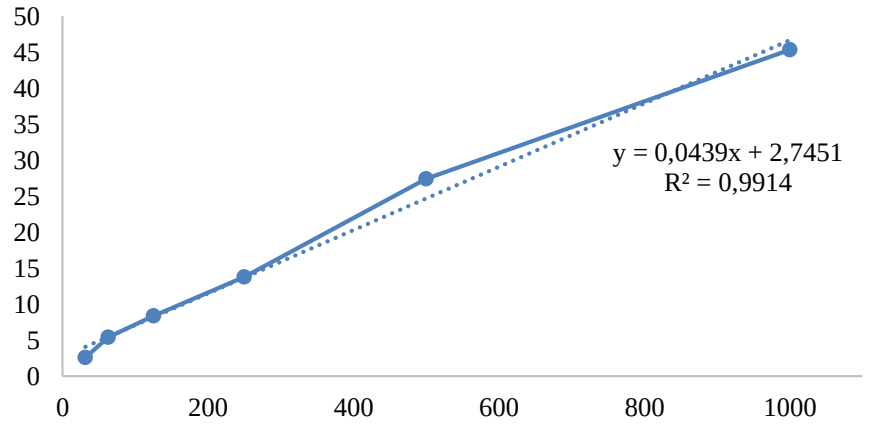

Figure 2. Graph of relation % inhibition with concentration ethyl acetate fraction

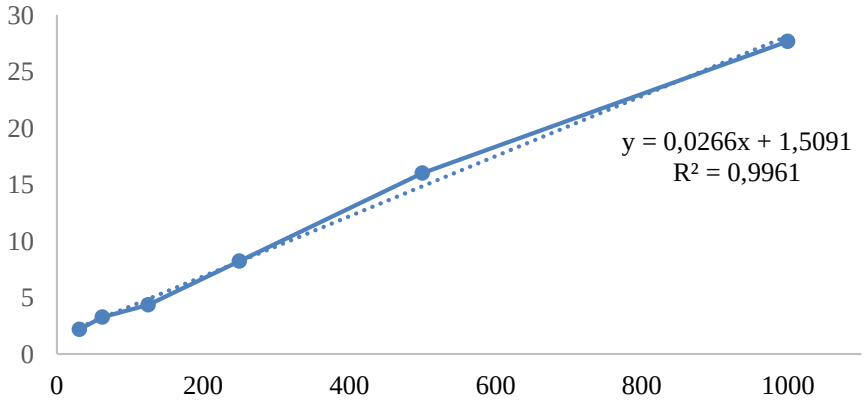

Figure 3. Graph of relation % inhibition with concentration methanol fraction

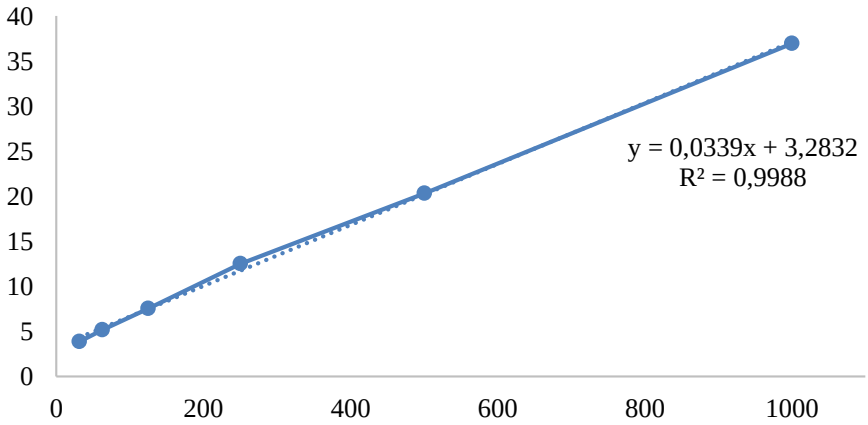

Figure 4. Graph relation % inhibition with concentration watery methanol fraction

Table 2. IC_{50} value of ethyl acetate, methanol and methanol/aqueous fraction of *Citrus nobilis* Lour fruit peel extract

No	Fraction	IC_{50} (ppm)
1	Ethyl acetate	1076
2	Methanol	1822
3	Methanol/watery	1378

Based on Table 1. It can be concluded that the fractions of ethyl acetate, methanol and methanol/aqueous, respectively at concentrations of 1076, 1822, 1372 ppm can reduce 50% of dpph radical activity [38].

4. Conclusion

Sundai lime peel has antioxidant activity against DPPH, which is well indicated in the ethyl acetate fraction, methanol and liquid methanol have less activity than vitamin C and more significant than the hexane fraction. In the ethyl acetate, methanol and methanol/aqueous fractions with concentrations of 1076, 1822, 1372 ppm in a row can reduce 50% of DPPH radical activity. The presence of strong antioxidant activity against methanol extract due to the presence of flavonoid and phenolic compounds.

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