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Evaluation of Antioxidant Activity in Lemon Juice (*Citrus limon*) Marketed in Makassar City Using the DPPH (2,2 diphenyl-1-picrylhydrazyl) Method

Uji Aktivitas Antioksidan pada Minuman Sari Buah Lemon (*Citrus limon*) yang Dipasarkan di Kota Makassar dengan Menggunakan Metode DPPH (*2,2 diphenyl-1-picrylhydrazyl*)

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ABSTRACT

Human lifestyle is currently experiencing many changes that lead to unhealthy lifestyles. An instant lifestyle can trigger degenerative diseases, which are initiated by excessive oxidation reactions in the body, which can cause the formation of free radicals. Lemon juice, derived from the *Citrus limon* plant, is considered a functional beverage due to its antioxidant properties, which have the potential to counteract the harmful effects of free radicals. This study aimed to measure the antioxidant activity of lemon juice beverage based on the IC_{50} value. The present study adopted a descriptive laboratory approach, utilizing lemon juice beverages from three distinct brands as representative samples. The antioxidant activity test was carried out by adding DPPH and measured using UV-Vis Spectrophotometry. The antioxidant levels obtained were 1,091.17 µg/mL for sample A, 1,114.50 µg/mL for sample B, and 527,261 µg/mL for sample C. All samples exhibited antioxidant activity values that fell within the "very weak" classification, as shown by IC_{50} values above 200 µg/mL.

Keywords: Lemon juice beverage, antioxidant, DPPH,

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1. INTRODUCTION

The current human lifestyle has undergone numerous transformations throughout time. In addition to lifestyle factors, alterations in dietary habits have also occurred, characterized by the adoption of unhealthy eating patterns such as fast food consumption, imbalanced nutrition, insufficient physical activity and rest, and the deterioration of environmental conditions, which will cause people to be frequently exposed to dangerous substances. Thereby increasing the risk of developing degenerative or hereditary diseases and conditions (Yuslianti, 2018).

Most degenerative diseases are initiated by excessive oxidation reactions in the cells of the human body. Oxidation reactions occur at any time, including during respiratory and metabolic processes in the body, which can cause the formation of free radicals. Unstable molecules known as free radicals possess unpaired electrons, making them susceptible to inducing chronic and degenerative diseases (Sine & Pardosi, 2021). Free radicals in normal amounts are beneficial for health, for example, to stop inflammation, kill bacteria, and control the smooth muscle tone of blood vessels and organs. In excess amounts, free radicals can cause oxidative stress, an imbalance between free radical production and the body's antioxidant defense system. This situation can cause oxidative damage starting from

the cell, tissue, and organ levels, which accelerates the aging process and the emergence of disease (Puspitasari *et al*., 2016).

The body requires an intake of antioxidant compounds that can capture and neutralize these free radicals, preventing future oxidative stress reactions and cell damage that can lead to disease. Antioxidants can be obtained from vegetables, fruit, and flowers (Parwata, 2016). Indonesia is rich in biodiversity, such as plants, animals, and minerals, which can be used empirically for health (Zakiyah *et al*., 2021). One potential resource that can be utilized is fruit, as it can fulfill the dietary requirements for fiber and essential vitamins. Lemon (Citrus lemon) is a well-favored and readily accessible fruit in Indonesia. Lemon is a plant whose main composition is sugar and citric acid. According to Krisnawan *et al*. (2017), lemons contain several vitamins (C, A, B1, and P), flavonoids, limonene, tannins, and minerals. The study further suggests that the peel extraction from *Citrus limon* and *Citrus sinensis* has oxidant activity.

Along with the increasing busyness of society, especially in urban areas, and the number of instant products that are fast and practical, various food products have emerged. One of them is a lemon juice beverage. Fruit juice is a clear or slightly clear, non-fermented liquid derived by pressing ripe, fresh fruit, which can be consumed to meet daily vitamin and fiber intake. According to food consumption statistics for 2021, during 2016, apple cider consumption data was at 5.22 and increased in 2021 to 11.47 per capita. This indicates a significant increase in the consumption of fruit beverages in Indonesia between 2016 and 2022 (Ministry of Agriculture, 2021). Nurviana *et al*. (2021) analyzed the beverage composition of limus (*Mangifera feotida* Lour) juice. They found that the antioxidant activity of limus juice in instant powder had increased significantly, in contrast to fresh juice. The study is similar to the research by Darwis *et al*. (2018).

The DPPH (2,2 diphenyl-1-picrylhydrazyl) method is a simple method that can be used to test antioxidant content. The process is easy and fast. The DPPH method operates on the principle of quantitatively assessing antioxidant activity. It is achieved by employing UV-Vis spectrophotometry to measure the ability of compounds with antioxidant properties to capture DPPH radicals. The resulting IC_{50} (Inhibition Concentration) value represents the degree of suppression of free radicals. The IC_{50} value is defined as the concentration of the test compound that can reduce free radicals by 50% (Yunanto *et al*., 2009). Based on the above information, researchers are interested in studying the antioxidant activity of lemon juice beverages sold in Makassar City using the DPPH method.

2. RESEARCH METHODOLOGY

2.1 Material and Tools

The ingredients used were 3 packaged drinks of lemon juice, DPPH, and ethanol. The tools used were a UV-Vis Spectrophotometer, measuring flask, beaker, dropper pipette, horn spatula, stir bar, and analytical balance.

2.2 Preparation of DPPH Solution

A 0.4 mM DPPH solution was prepared by dissolving 50 mg DPPH with 70% ethanol until it reached 100 mL in a volumetric flask to obtain a concentration of 500 ppm.

2.3 Determination of max wavelength (λ) and blank absorbance

A total of 2 mL of 0.4 M DPPH solution was mixed with 1 mL of 70% ethanol and then left for 30 minutes in a dark place. The absorption of the solution was measured with a UV-Vis spectrophotometer at a wavelength of 400 - 700 nm.

2.4 Determining Operating Time of the Solution

The operating time was determined by mixing 2 mL of 0.4 mM DPPH solution with 1 mL of 100 ppm test solution (test solutions S1, S2, S3). The absorbance of the solution was measured at the maximum wavelength obtained at 5-minute intervals until a stable absorbance was obtained and no visible decrease in absorbance was observed.

2.5 Sample Solution Measurement

A total of 0.5 mL of each lemon juice beverage was dissolved in 70% ethanol in a 100 mL volumetric flask and homogenized so that the concentration was 5000 ppm. The 5000-ppm stock solution was then diluted again by pipetting 2, 4, 6, and 8 mL of solution and increasing the volume with ethanol to 100 mL to obtain 100, 200, 300, and 400 ppm solutions. The test was carried out by mixing 2 mL of sample solutions of various concentrations with 2 mL of 0.4 mM DPPH and 70% ethanol until it reached 2 mL in the vial. The mixture was then homogenized and left for an operating time of minutes. The absorption was measured at the maximum wavelength, and it was calculated using the formula:

$$\% \text{ Inhibition} = \frac{A0-A1}{A0} \times 100\ \%$$

Table 1. Efficient Concentration Value (IC_{50})

IC_{50} Value (µg/mL)	Activity
< 50	Very strong
50 ppm – 100	Strong
101 ppm – 150	Medium
151 ppm – 200	Weak
>200	Very weak

(Handayani, *et al*., 2022)

3 RESULTS AND DISCUSSION

3.1 Wavelength (λ) max and absorbance of DPPH (2,2 diphenyl-1-picrylhydrazyl) standard solution

Maximum wavelength data measured using UV-Vis Spectrophotometry is presented in Figure 1

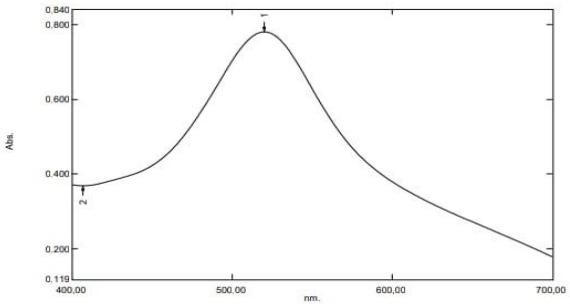

Figure 1. DPPH Maximum Wavelength

Data from determining the absorbance of the blank solution measured at a wavelength of 519.8 nm:

Table 2. Absorbance of Blank Solution

No	P/V	Wavelength	Abs.
1		519.81	0.780
2		406.61	0.369

3.2 Antioxidant Activity of Lemon Juice Beverages (Citrus limon) with DPPH reagent

Table 3 Antioxidant activity of sample A

	Sample Concentration (ppm)	Abs	% Inhibition	Linier Regression Equation	IC_{50}
DPPH		0.780		y = 0.0141x + 34.615	1,091.17 µg/mL
A	100	0.499	36.025	R^2 = 0.918	
	200	0.485	37.820		
	300	0.483	38.076		
	400	0.463	40.641		

Table 4 Antioxidant activity of sample B

	Sample Concentration (ppm)	Abs	% Inhibition	Linier Regression Equation	IC_{50}
DPPH		0.780			1,114.50 µg/mL

B	100	0.577	26.025	y = 0.0241x + 23.141
	200	0.564	27.692	R^2 = 0.9496
	300	0.550	29.487	
	400	0.519	33.461	

Table 5 Antioxidant activity of sample C

	Sample Concentration (ppm)	Abs	% Inhibition	Linier Regression Equation	IC_{50}
DPPH		0.780		y = 0.111x - 8.526	527.261µg/mL
C	100	0.733	6.025	R^2 = 0.9054	
	200	0.725	7.051		
	300	0.564	27.692		
	400	0.498	36.153		

3.3 Discussion

This study conducted antioxidant activity tests on 3 lemon juice (*Citrus limon*) beverages from 3 different brands. The samples were labeled A, B, and C. This study assessed the antioxidant activity and IC_{50} of lemon juice beverages using the DPPH reagent. The mechanism of this process involves the transfer of electrons or hydrogen radicals between antioxidants and free radicals derived from DPPH, leading to their neutralization (Sayuti & Yenrima, 2015). Measurements were carried out using UV-Vis spectrophotometry. UV-Vis spectrophotometry will receive light from a polychromatic light source and convert it into monochromatic light through a dispersion process so that only one type of light with a predetermined wavelength hits the sample. The light is then passed to the detector to be converted into an electric current. The recorder or computer then captures the amount of electric current, converts it into % transmittance or absorbance, and translates it into a spectrum. The spectrum will produce the wavelength and absorbance of the sample by forming a graph (Irawan, 2019).

The results of measuring the maximum wavelength and absorbance of the blank solution are presented in Table 2. The maximum wavelength determines the absorption area produced as an absorbance value. The wavelength of the DPPH solution measured at 400 - 700 nm using UV-Vis spectrophotometry was 519.81 nm, with the absorbance of the blank solution being 0.780 nm.

The IC_{50} value of sample A was 1,091.17 µg/mL, sample B was 1,114.50 µg/mL, and sample C was 527.261 µg/mL. The IC_{50} values of samples A, B, and C are very weak, determined based on the efficient concentration table with IC_{50} values between more than 200 µg/mL. It can also be seen from the color change that occurs, namely from purple to yellow, when the sample solution with a concentration of 400 ppm is added with DPPH. This color change occurs because the DPPH radical is reduced to obtain an electron or hydrogen atom from the antioxidant (Yang *et al*., 25).

All samples exhibit IC_{50} values that fall within the range of the very weak category. Sample C exhibits an IC_{50} value of 527.261 µg/mL, which falls within the closest proximity to the IC_{50} range.

4. CONCLUSION

The antioxidant activity of beverages A, B, and C falls into the very weak category, as shown by their IC_{50} values above 200 µg/mL. Specifically, the IC_{50} values for beverages A, B, and C are 1,091.17 µg/mL, 1,114.50 µg/mL, and 527.261 µg/mL, respectively.

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