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# Bioactive And Bioactivity Test Of Moringa (Moringa sp.) Starfruit Wuluh (Averrhoa bilimbi), And Keres (Muntingia calabura)

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**ABSTRAK**

Penelitian ini mengevaluasi bioaktivitas ekstrak tanaman Kelor (Moringa oleifera), Belimbing Wuluh (Averrhoa bilimbi), dan Keres (Muntingia calabura) terhadap aktivitas antioksidan dan antibakteri. Ekstraksi dilakukan menggunakan metode maserasi dengan pelarut etanol 96%. Uji antioksidan dilakukan menggunakan metode DPPH (2,2-difenil-1-pikrilhidrazil), sedangkan uji antibakteri dilakukan terhadap bakteri Staphylococcus aureus menggunakan metode difusi sumuran. Hasil penelitian menunjukkan bahwa ekstrak Kelor memiliki hubungan linier yang kuat antara konsentrasi dan aktivitas antioksidan, sedangkan ekstrak Belimbing dan Keres menunjukkan aktivitas antioksidan yang rendah dengan nilai IC50 di atas 100 ppm yang menunjukkan aktivitas yang lemah. Pada uji antibakteri, ekstrak Belimbing menghasilkan zona hambat yang signifikan, meskipun tergolong respon lemah. Temuan ini menunjukkan bahwa ketiga tanaman tersebut berpotensi sebagai sumber senyawa bioaktif, meskipun efikasi yang optimal memerlukan optimasi metode ekstraksi dan konsentrasi. Penelitian lebih lanjut diperlukan untuk mengidentifikasi senyawa aktif utama dan mengeksplorasi mekanisme kerjanya.

**Kata kunci:** Flavonoid, Antioksidan, Antibakteri, Moringa oleifera, Averrhoa bilimbi, Muntingia calabura

**ABSTRACT**

This study evaluated the bioactivity of Moringa plant extracts (Moringa oleifera), Star Fruit Wuluh (Averrhoa bilimbi), and Keres (Muntingia calabura) on antioxidant and antibacterial activities. Extraction was carried out using the maceration method with a 96% ethanol solvent. The antioxidant test was carried out using the DPPH method (2,2-diphenyl-1- picrylhydrazyl), while the antibacterial test was carried out on Staphylococcus aureus bacteria using the well diffusion method. The results showed that Moringa extract had a strong linear relationship between concentration and antioxidant activity, while Star Fruit and Keres extracts showed low antioxidant activity with IC50 values above 100 ppm, indicating weak activity. In the antibacterial test, Star Fruit extract produced a significant inhibition zone, although it was classified as a weak response. These findings suggest that all three plants have potential as sources of bioactive compounds, although optimal efficacy requires optimization of extraction and concentration methods. Further research is needed to identify the main active compounds and explore their mechanisms of action.

**Keywords**: Flavonoid, Antioxidant, Antibacterial, Moringa oleifera, Averrhoa bilimbi, Muntingia calabura

## I. INTRODUCTION

### 1. Background

Bioactivity is the ability of a compound or material to exert specific biological effects on living organisms, either at the cellular, tissue, or body system levels. Bioactivity is often used to evaluate the therapeutic potential or toxicity of a compound, such as in drug or nutrition research. For example, secondary metabolites such as flavonoids, alkaloids, and terpenoids have significant bioactivity in various aspects, such as antioxidants, antibacterial, and anticancer (Dhanani et al., 2017). Flavonoids are secondary metabolite compounds that are widely found in various plants. These compounds are known to have a variety of important biological activities, including as antibacterial and antioxidant agents. In the field of phytochemistry, studies on the bioactivity of flavonoids continue to grow due to their potential in pharmaceutical and medical applications (Panche et al., 2016). Antibacterial tests are carried out to evaluate the ability of flavonoids to inhibit or kill bacterial growth. These compounds are known to interfere with the function of bacterial cell membranes, inhibit important enzymes, or damage bacterial DNA (Cushnie & Lamb, 2011). The effectiveness of flavonoids as antibacterial agents is often evaluated through disc diffusion or microdilution methods. In addition, flavonoids have significant antioxidant activity, which allows them to ward off free radicals and protect cells from oxidative damage. Oxidative damage is often associated with various chronic diseases such as cancer, diabetes, and cardiovascular disease (Pietta, 2000). Antioxidant tests usually involve measurement methods such as DPPH (2,2-diphenyl-1-picrylhydrazyl). The DPPH method is a measurement of antioxidants with the ability to capture free radicals with the free radicals used being DPPH. Thus, testing the bioactivity of flavonoid extracts against antibacterial and antioxidant activities is essential to explore their therapeutic potential as well as their development as an effective pharmaceutical agen.

### 2. Problem formulation

The formulation of the problem in this study is how to conduct research on the bioactive test and biological activity of Bioactive And Bioactivity Test Of Moringa (Moringa sp.) Starfruit Wuluh (Averrhoa bilimbi), And Keres (Muntingia calabura)

### 3. Research purpose

This research aims to obtain research results from the title Bioactive And Bioactivity Test Of Moringa (Moringa sp.) Starfruit Wuluh (Averrhoa bilimbi), And Keres (Muntingia calabura).

### 4. Benefit of Research

Benefits of research is to obtain results and implications and implementation of the research title Bioactive And Bioactivity Test Of Moringa (Moringa sp.) Starfruit Wuluh (Averrhoa bilimbi), And Keres (Muntingia calabura).

## II. METHOD

### Time and location of research

This research was conducted in October - November 2024 at the University of Muhammadiyah Lamongan. The bioactivity test of flavonoids, antioxidant activity, and antibacterial activity were carried out at the Integrated Laboratory of the University of Muhammadiyah Lamongan.Materials and tools.

### Materials :

Starfruit, Keres leaves, Moringa leaves, simplicia powder, 96% ethanol, methanol, ice cubes, refill water/distilled water, 75% ethanol, 2 N hydrochloric acid, distilled water, Mayer’s reagent, Bouchardat’s reagent, Dragendorff’s reagent, hot water, magnesium

powder, concentrated HCl, amyl alcohol, ferric (III) chloride, 2 N HCl, chloroform, anhydrous acetic acid, concentrated sulfuric acid.

Tools :

Knife, cutting board, bucket, tampah/tray, oven, black cloth, blender, analytical balance, digital scale, plastic spoon, beaker glass, maceration jar, stirrer, sterile flannel cloth, filter paper, bottle/sealed container, rinsing container, knife, heating container, Erlenmeyer

**Preparation of Simplicia**

Prepare the materials to be used. Perform wet sorting on the materials, then wash them several times with running water. Drain until the water is completely removed. Slice the materials thinly. Dry the materials using the following methods: oven-dry at a specified temperature and duration or sun-dry them while covering with black cloth.

**Maceration**

Weigh 250 grams of simplicia powder. Prepare 1 liter of solvent (96% ethanol or analytical-grade methanol). Place the weighed simplicia powder into a maceration jar. Add the measured solvent into the jar and stir until homogeneous. Close the jar and store it in a dark place. After 24 hours, filter the solution. The filtrate (liquid) is stored in a bottle, and the residue is returned to the jar. Add the measured solvent again to the jar, stir until homogeneous, and store for another 24 hours (48 hours total). Filter the solution again, store the filtrate, and return the residue to the jar. Add the measured solvent once more, stir until homogeneous, and let it sit for another 24 hours (72 hours total). Filter the solution again. Combine all the filtrates and concentrate them using a water bath.

**Bioactivity of Antioxidant**

a. Preparation of 100 PPM Concentration Master Solution

The extract was weighed as much as 2 mg diluted using methanol as much as 20 ml in a measuring flask until the limit mark was shaken until homogeneous.

b. Preparation of 20, 40, 60, 80 PPM Concentration Test Solution

Each solution with a concentration of 100 ppm was pipetted as much as 0.5; 1; 1,5; And 2 ml is then put into a measuring flask, 25 ml is added methanol until the limit mark, then beaten until homogeneous.

c. Preparation of DPPH Solution Concentration 40 PPM

The manufacture of this raw solution is made by weighing 1 mg of DPPH powder and then dissolved in hetanol with a 25 ml measuring flask until the limit mark, shaken until homogeneous.

d. DPPH Absorbance Measurement

The 40 ppm DPPH solution was pipetted as much as 2 ml was put into the test tube, 2 ml of methanol, divoertex was added and left for 30 minutes after 30 minutes were put into the cuvette, observed using a UV-Vis spectrophotometer for its absorption using a wavelength of 480 nm.

e. Extract Absorbance Measurement

The 40 ppm DPPH solution was pipetted as much as 2 ml, put in a test tube, and 2 ml of test solution from each concentration (20, 40, 60, 80, and 100 ppm) was added. Divortex and let stand for 30 minutes. Then its absorption is observed at maximum wavelength with the UV-Vis spectrophotometer alternately at all five concentrations. The absorbance of each solution is recorded. Then from the absorbance, the percentage of immersion is calculated with a formula.

$$\%\ \text{immersion} = \frac{absorbansi\ DPPH - absorbansi\ sampel}{absorbansi\ DPPH} x\ 100\%$$

From the value of the immersion percentage in each concentration, a regression curve is made, so that the equation y = bx - a is obtained and the IC50 value will be obtained by linear regression where the sample concentration (ppm) is the absis (x- axis) and the immersion percentage value is the coordinate (y-axis). The IC50 value is obtained from the calculation of the percentage of attenuation of 50%.

## III. RESULT AND DISCUSSION

### Bioactif of Flavonoid

The chromatogram above shows the results of the analysis of DPPH (2,2- diphenyl-1-pyrrylhydrazile) blank solution using HPLC with a detector at a wavelength of 360 nm, which is the maximum absorption wavelength of DPPH. The main peak appears at a time retention of about 2,263 minutes, which indicates the presence of pure DPPH in the environment. The height and area of these peaks reflect the initial DPPH concentration in the absence of interaction with other compounds. In addition to the main peaks, there were minor peaks at retention times of about 1,891 minutes, 2,878 minutes, and 4,656 minutes, which were most likely derived from solvents such as methanol or ethanol, or minor impurities (Brand-Williams et al., 1995).

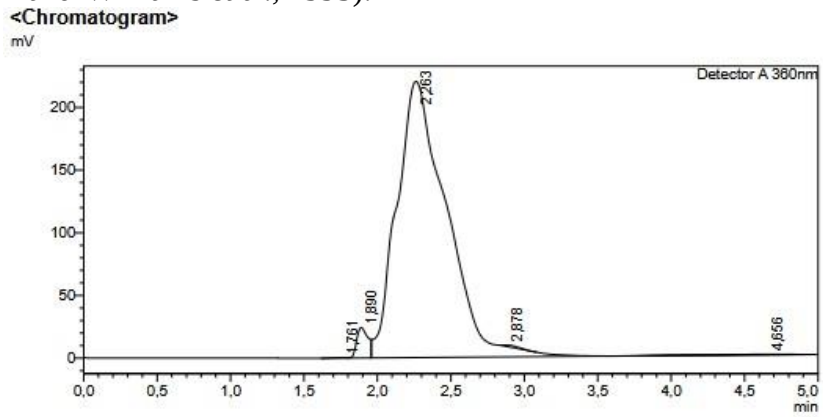


**Figure 1**. DPPH blank solution chromatogram

**Table 1**. Radical Reduction Activity Extract

| Sampel | Konsentrasi | Peredaman Radikal |
|---|---|---|
| Belimbing | 0,5 | 0,98 |
| Kelor | 25 | 0,98 |
| Kelor | 20 | 0,96 |
| Keres | 0,5 | 0,31 |
| Keres | 2,5 | 0,98 |
| Keres | 25 | 0,97 |

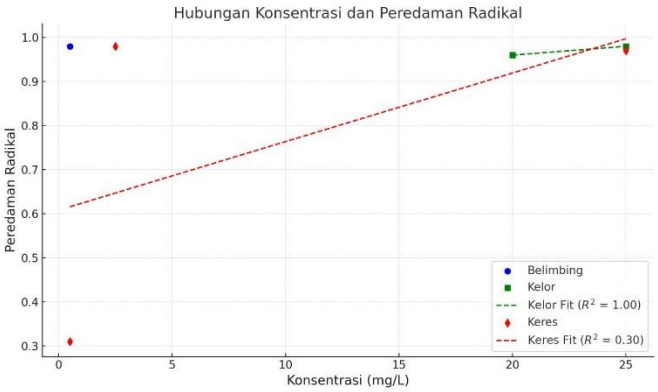


**Figure 2**. Flavonoid Activity Curse Using HPLC

Based on the analysis of the concentration and absorbance relationship data for Belimbing, Kelor, and Keres samples, variations in data quality and linear relationships between parameters were found. In the Belimbing sample, data were only available for one concentration point (0.5 mg/L) with an absorbance of 0.98.

**Bioactivity of Antioxidant**

The low antioxidant activity in these three extracts can be caused by several factors, such as low concentrations of bioactive compounds (flavonoids, phenolics, and ascorbic acid) in the extract, suboptimal extraction methods, and degradation of active compounds during the testing process. The content of bioactive compounds in raw materials such as moringa and star fruit is known to have antioxidant potential, but an inefficient extraction process can reduce the ability of these compounds to inhibit free radicals.

**Table 2.** Sample Absorbance

| Keres | 1 | 2 | 3 | Installment Absorbansi | Value $IC_{50}$ |
|---|---|---|---|---|---|
| 100 | 0,153 | 0,159 | 0,158 | 0,153 | 11,2 |
| 80 | 0,145 | 0,155 | 0,153 | 0,151 | 24,34 |
| 60 | 0,127 | 0,154 | 0,114 | 0,132 | 25,06 |
| 40 | 0,084 | 0,084 | 0,083 | 0,084 | 11,2 |
| 20 | 0,074 | 0,073 | 0,072 | 0,073 | 27,28 |
| Kelor | 1 | 2 | 3 | | |
| 100 | 0,149 | 0,155 | 0,152 | 0,152 | 20,96 |
| 80 | 0,108 | 0,138 | 0,118 | 0,121 | 22,03 |
| 60 | 0,105 | 0,104 | 0,102 | 0,104 | 22,61 |
| 40 | 0,098 | 0,098 | 0,098 | 0,098 | 22,81 |
| 20 | 0,059 | 0,057 | 0,059 | 0,058 | 24,18 |
| Moringa | 1 | 2 | 3 | | |
| 100 | 0,186 | 0,173 | 0,187 | 0,182 | 13,96 |
| 80 | 0,173 | 0,17 | 0,186 | 0,176 | 14,12 |
| 60 | 0,132 | 0,131 | 0,129 | 0,131 | 15,82 |
| 40 | 0,078 | 0,083 | 0,077 | 0,079 | 16,62 |
| 20 | 0,074 | 0,074 | 0,076 | 0,075 | 16,72 |

The $IC_{50}$ value is a parameter that indicates the concentration of the extract needed to inhibit 50% of free radical activity. Based on the test data, the maximum percentage of inhibition for moringa extract was 16.72% (20 ppm), keres extract was 27.28% (20 ppm), and star fruit extract was 24.18% (20 ppm). The three extracts did not reach 50% inhibition values in the tested concentration range, so the $IC_{50}$ values for all three were estimated to be higher than 100 ppm. Based on the classification of Blois (1958), antioxidant activity with an IC50 of more than 150 ppm is classified as weak (Blois, 1958).

## DISCUSSION

### Bioactive of Flavonoids

The use of DPPH blank solution in testing antioxidant activity is intended as a control. When antioxidant compounds are added to the DPPH solution, DPPH free radicals will interact and undergo reduction into more stable compounds. These changes can be observed through a decrease in the area or height of the peak on the chromatogram. Therefore, the peak intensity at 2.263 min in the blank solution became the main reference for assessing the radical reducer activity by the test compound. This method conforms to the basic principles of DPPH antioxidant activity analysis introduced by Blois (1958) and further modified by Brand-Williams et al. (1995).

### Bioactivity of Antioxidant

The Moringa sample showed the most ideal data with a strong linear relationship, while the Belimbing sample needed more data for trend analysis, and the Keres sample needed method optimization to improve linearity. Further research with data replication and additional validation can help obtain more accurate and representative results for all samples (Wilson & Carter, 2022). In addition, environmental factors such as exposure to light and temperature can also accelerate the degradation of active compounds, thereby decreasing antioxidant activity (Fahey, 2005; Lobo et al., 2010).

### Bioactivity of Antimicroba

The test results showed a significant difference between the two sets of star fruit extracts used, with Set 1 showing a larger inhibition zone (0.291 mm) than Set 2 (0.103 mm). This shows that star fruit extract has antibacterial potential, which is in accordance with the results of previous studies that show that star fruit has bioactive compounds with antibacterial properties in a weak response (Ulum et al., 2022; Tiwari et al., 2011). According to research by Ulum et al. (2022), the flavonoids and ascorbic acid found in star fruit extract are known to have strong antibacterial activity against various types of bacteria, including Staphylococcus aureus. These compounds work by disrupting bacterial cell membranes and damaging their internal structures. This may explain the larger inhibition zone on Set 1 if the extract concentration is higher, containing more active compounds that can inhibit bacterial growth (Ulum et al., 2022).

## IV. CONCLUSION

This study evaluated the antioxidant and antibacterial bioactivity of Moringa (Moringa oleifera), Starfruit (Averrhoa bilimbi), and Keres (Muntingia calabura) extracts. The results showed that Moringa had the best antioxidant activity, even though its IC50 was above 100 ppm, while Star Fruit and Keres had lower activity. In antibacterial tests, Starfruit Wuluh extract showed potency against Staphylococcus aureus with significant inhibitory zones, although the response was weak. Optimization of extraction methods and identification of active compounds is needed to improve the effectiveness of the bioactivity of this plant as a pharmaceutical raw material.

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