*Jurnal Biosains Pascasarjana Vol.24 (2022) pp*


# ANALYSIS OF ANTIOXIDANT CONTENT OF ANTHOCYANIN IN THE LOBI-LOBI FRUIT (*Flacourtian inermis*) AND JAMBLANG FRUIT (*Syzygium cumini L Skeel*) USING THE DPPH METHOD WITH SPECTROPHOTOMETRY

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**Abstrak**

Penelitian ini bertujuan untuk mengetahui kadar total antosianin dan nilai IC50 antioksidan dari ekstrak etanol buah Lobi-lobi (Flacourtia Inermis) dan buah Jamblang (Syzygium Cuini L Skeel) yang dianalisis menggunakan metode DPPH secara Spektrofotometri UV-Vis. Penelitian dilakukan di Laboratorium Lingkungan Fakultas Keguruan dan Ilmu Pendidikan Universitas Khairun. Hasil penelitian menunjukkan adanya kandungan antosianin dan aktivitas antioksidan dari buah lobi-lobi dan jamblang yang dianalisis menggunakan metode Perbedaan pH dan DPPH. Untuk kadar antosianin lobi-lobi sebesar 17,99 mg/L dan 4,62 mg/L. Sedangkan untuk IC50 Antioksidan dari buah lobi-lobi didapatkan 50,01 µg/L untuk jamblang didapatkan 91,113 µg/L.

Kata Kunci : Ekstrak Etanol Buah Lobi-lobi dan Jamblang, Metode DPPH dan Perbedaan pH, Kadar Antosianin dan IC50

**Abstract**

*This study aimed to determine the total anthocyanin content and IC50 value) of antioxidants from the ethanol extract of Lobi-lobi (Flacourtia Inermisand Jamblang (Syzygium Cuini L Skeel) fruit which were analyzed using the DPPH method. by UV-Vis spectrophotometry. The research was conducted at the Environmental Laboratory of the Faculty of Teacher Training and Education, Khairun University. The results showed that the anthocyanin content and antioxidant activity of the lobi and jamblang fruit were analyzed using the Differences in pH and DPPH methods. For lobbies anthocyanin levels are 17.99 mg/L and 4.62 mg/L. Meanwhile, for IC50 antioxidants from fruit lobbies, 50.01 g/L was obtained, for jamblang it was 91,113 g/L.*

*Keywords: Lobi-lobi and Jamblang Fruit Ethanol Extract, DPPH Method and Differences in pH, Anthocyanin Levels and IC50*

## 1. INTRODUCTION

The development of science and technology that is increasingly advanced in recent times has invited various researches conducted by experts regarding active compounds sourced from both animal and vegetable sources. One of the studies that is often done is about active compounds including flavonoid compounds.

Flavonoids are a group of secondary metabolites found in plants that have more than one phenolic structure (polyphenols), these compounds are also commonly found in fruit (Kohirunnisa and Sumiwi.2019). Flavonoids themselves have derivative compounds known as anthocyanins. Anthocyanins are found in plants and act as pigment givers which have a flavilum cation structure (AH+) (Tena, Martin, and Asuero.2020) The anthocyanin structure has the characteristics of a carbon skeleton sequentially, namely (C6C3C6) with the basic structure of anthocyanins 2-phenyl-benzophyrylium (Priska et al.2018) The anthocyanin structure can be seen in Figure 1 flavilum is a flavonoid salt that acts as an

acid whose structure is closely related to its antioxidant activity (Tena, Martin, and Asuero.2020).

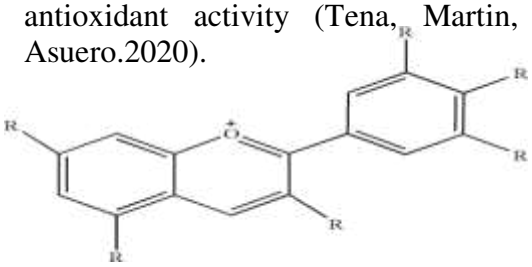

Figure 1. The basic structure of anthocyanin compounds

As it is known that the main benefit of antioxidants itself is to counteract free radical compounds that will enter the body. A free radical is an atom or molecule that has one or more unpaired electrons. The effect of this unpaired electron causes chemically, these free radicals will be reactive. These reactive free radicals can cause chemical changes, can change and also damage various components of living cells (Marliani, 2014). Anthocyanins have an important role in keeping the body healthy because of their antioxidant properties, the benefits of which are being able to prevent cardiovascular health disorders, decreased vision, anti-agents such as anti-diabetes, inflammation, and cancer (Ifadah et al. 2021).

Humans do not have antioxidant reserves in excess, so when free radicals are formed the body will need exogenous antioxidants (antioxidants that come from outside the body). However, there are concerns about the possible unknown side effects of synthetic antioxidants, causing natural antioxidants to become an alternative that is needed by humans (Sayuti and Yenrina, 2015). However, humans can obtain antioxidants naturally from vegetables and fruits (Faisal and Handayani.20019) and jamblang fruit (Zyzygium cumini L.Skee) is one of the local Indonesian fruits. Jamblang (Zyzygium cumini L. Skeel) contains compounds that have antioxidant activity that plays a role in counteracting free radicals (Widyastuti, Hilaliyati, and Rahmi.2021). Besides jamblang fruit (Zyzygium cumini L. Skeel), Lobi-lobbi fruit (Flacourtia Inermis) is also thought to contain antioxidant compounds. The antioxidant activity of fruit can be carried out by several methods, one of the most commonly used methods is using 1,1-diphenyl-2-picrylhydrazyl (DPP) (Chen et al.2022).

Therefore, researchers are interested in conducting research by analyzing the content of antioxidants and anthocyanins in lobi fruit (*Flacourtia inermis*) and Jamblang fruit (*Zyzygium cumini*) with the DPPH method using UV-Vis spectroscopy.

## 2. RESEARCH METHOD

The materials used in this study were tome-tome fruit extract, aquades, ethanol, HCl, NaOH, KCl, sodium citrate, and DPPH. The tools used in this study were blenders, jars, beakers, balance sheets. analyte, aluminum foil, spray bottle, filter paper, dark reagent bottle, pH meter, rotary vacuum evaporator, stir bar, micro pipette, dropper, measuring pipette, measuring flask, measuring cup, reaction tube and UVVis SHIMADZU 1800. .Sample Preparation The tome - tome fruit is cleaned then drained and then cut into small pieces and then in a blender until smooth.
Analysis of the water content of the sample. Analysis of the water content of tome-tome (Flacourtia inermis) fruit using the oven drying method at 100oC. Empty plates were dried in the oven for 1 hour after which they were cooled at room temperature. Then weighed with an analytical balance (W0). A total of 1 gram of the sample was put into a dry cup and then weighed quickly (W1). The cup and the sample were dried in an oven at 105oCfor3 hours. After being in the oven for 3 hours, put it in a desiccator for 20-30 minutes, then weigh it again. The cup was put back into the oven until a constant weight was obtained (W2).
Extraction of tome-tome (flacourtia inermis) fruit. A total of 1000 g samples of tome -

tome fruit that has been finely extracted by wet maceration technique using 95% ethanol and 1% HCl with a ratio of 9: 1 as much as 1000 mL. Maceration was carried out for 24 hours, then filtered and the filtrate was collected. Extraction was carried out until the anthocyanins in tome fruit were completely extracted. The filtrate obtained was evaporated using a rotary vacuum evaporator to obtain a thick ethanol extract and then weighed using an analyte balance.

Anthocyanin phytochemical test. The color test was carried out on the anthocyanin compound group on fruit samples containing anthocyanins, where Harborne said that if 0.5 grams of ethanol extract of dragon fruit peel was added as much as 2M HCl then heated at 1000C for 5 minutes. The result is said to be positive if the solution appears red. In addition, if 2M NaOH is added dropwise while observing the color change. The results are said to be positive if in the phytochemical test process a blue green color appears which fades slowly

Method DPPH. Antioxidant activity was determined by the DPPH free radical method. The DPPH solution was prepared by dissolving 1 mg DPPH crystals in a 50 mL volumetric flask and then adding ethanol to obtain a dpph solution with a concentration of 20 ppm. Determination of the absorption of DPPH was measured using a UVVis spectrophotometer at a maximum wavelength of 517 nm. The ethanol extract from each sample was diluted to various concentrations of 3, 5, 7, and 10 ppm. Each of these test solutions was taken as much as 0.3 mL which was then added with 3.7 mL of DPPH solution, antioxidant measurements with UV-Vis were carried out after the solution mixture was allowed to stand for 25 minutes (Karisma and Hidajati., 2016).

Determination of total anthocyanin levels using the pH difference method. The appropriate dilution factor for the sample must be determined in advance by dissolving the sample with KCl buffer pH 1 until an absorbance of less than 1.2 is obtained at a wavelength of 510 nm. The next step is to measure the water at the wavelength that will be used (510 and 700 nm) to find point no. A wavelength of 700 nm was used to correct for the precipitate present in the sample. The absorbance will reach 700 nm if the sample is completely clear is 0 (zero). Prepared a solution with a sample, used KCl buffer with a pH of 1 in the first sample and Na-citrate buffer with a pH of 4.5 in the second sample. Each sample is dissolved in a buffer based on DF (*Dilution Factor*). The sample was dissolved using pH 1 buffer and left for 15 minutes before being measured, the sample dissolved in 4.5 buffer was ready to be measured after being allowed to mix for 5 minutes. The absorbance of each solution at wavelengths of 510 and 700 nm was measured with pH 1 and pH 4.5 buffers as blanks (Meydayanti Putri., 2015).

## 3. RESULTS AND DISCUSSION

The research was carried out at the Environmental Laboratory of the Faculty of Teacher Training and Education, Khairun University, Ternate. The research that has been carried out is a comparative test of antioxidant activity and total anthocyanin levels in ethanol extract samples from Jamblang fruit (Zyzygium Cumini L. Skeel) and lobi fruit (Flacourtia Inermis) using DppH and Differential pH methods by UV-*Vis spectrophotometry*.

Table 1. Results of measurement of water content, extract yield, and anthocyanin content test on samples of lobbies and jamblang

| . Parameters of Test | Samples | |
|---|---|---|
| | Lobby | Jamblang |
| Moisture Content | 0.2 | 0.3 |
| Extract Yield | 91.748 | 79.336 |
| Anthocyanin Test (Harbourne Test) | (+ ) | (+) |
| Rf Anthocyanin | 0.44 | 0.42 |
| Total Anthocyanin Content | 17.99 | 4.62 |
| *IC50* Antioxidant | 66.85 | 91.113 |

Determination of the water content of the sample. Determination of the moisture content of lobbies and jamblang samples using the drying method with an oven as the tool used. Determination of water content was carried out by weighing 1 gram of each sample and then placing it in an oven at 100°C for 12 hours. The moisture content of the samples of 0.2% and 0.3%, respectively, can be said to be very good and can be stored for a long time because based on the threshold value of water content from 5 to 30%, it is relatively resistant to microorganisms (Ulfah, Kurniawan, et al. and Erny.2020).

Extraction and Maceration of Tome-tome and Jamblang Fruit Samples. Extraction of lobbies and jamblang fruit was carried out using the maceration method by weighing 50 grams of each sample that had been mashed. with a blender then dissolved in 100 mL of solvent with a comparison of 90 mL of 95% ethanol and 10 mL of 2 molar HCl, then left for 24 hours. The next step is the ethanol extract of the two samples is evaporated using a rotary vacuum evaporator. The purpose of this treatment is to obtain a thick extract from the extraction. The results obtained from the evaporation of a thick red-brown extract for the ethanol extract from the lobbies and a purplish-red color for the jamblang.

Anthocyanin Identification Test. The researcher's self-identification test carried out two stages, namely the phytochemical screening (color change based on the solvent) and separation using Thin Layer Chromatography (TLC).

Anthocyanin Phytochemicals (Harbourne Test) This test was carried out by weighing 0.5 grams of each sample, where for jamblang and lobbies there was no color change (red color) after being added with 0.5M HCl. While in the next treatment the two samples changed color after being added with 0.5M NaOH, where in the Lobby samples the color changed to yellow on the top layer of the solution then green to blue which faded slowly in 5 minutes. Satyatama said, the factors that affect the color of Anthocyanins are changes in the pH of the acids and bases of each solution used. The red color is due to the acidic nature of HCl, while the alkaline nature of NaOH causes anthocyanins to turn blue (Meidayanti et al. 2015).

TLC (Thin Layer Chromatography). This process is carried out by spotting the extracts of lobbies and jamblang on a silica plate that has been lined 1 cm, the extract is put in chamber containing the eluent and then the extract is allowed to separate. This process used forestal solvents and BAA, but the separation used forestal eluent with a composition of concentrated HCl:acetic acid:aquader 5:2:3 the results obtained were less than optimal when the elution process was carried out. As a result, the use of this mobile phase cannot determine the Rf value of each sample. However, the use of BAA eluent with the same composition as the forestal eluent n-butanol:acetic acid:aquades (5:2:3) showed a separation that occurred when the extract was spotted on a previously activated plate. The results of the separation formed spots with a brownish red color for the lobbies extract and a purple color for the jamblang extract. With this spot separation, the calculation of the Rf value of the two sample extracts was carried out by dividing the distance traveled by the stain by the distance traveled by the solvent during the elution process. (Firtiyani et al2018). The Rf value obtained in each sample was 0.42 for tome-tome extract and 0.4 for jamblang extract and was an anthocyanin type Definilin.

This result is reinforced by Harborne's opinion that the anthocyanin type Definilin will have an Rf value ranging from up to 42 with a purple-red color if eluted using BAA eluent (Fitriyani et al.2018). The results of identification and determination of the Rf value from testing using thin layer chromatography can be strengthened by

Harborne's opinion that anthocyanins will follow the color of the aglycone type, and peralgonidine itself has a red aglycone.

Determination of IC50 Antioxidants. Antioxidant levels were determined by the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method. The use of the DPPH method is one of the tests to determine the antioxidant activity of scavenging free radicals. According to Sunarni, this method provides information on the activity of an anti-radical test compound. DPPH gives strong absorption at a wavelength of 517 nm with a violet colored solution. After 15 minutes of being left in the incubator, there was a change from purple to yellow, this indicates a process of catching free radicals.

The color change that occurred after the reaction was carried out, indicated that the DPPH underwent a reduction process so that the color changed from purple to yellow. This change can be determined by the level of antioxidant activity content using a UVVis spectrophotometer which is plotted at various concentrations of the decrease in color intensity that occurs, this process occurs due to double bonds that are conjugated in the DPPH solution.

After making observations, it was found that with the process of capturing radical compounds by antioxidants, the decrease in DPPH color also decreased. In addition, the absorbance of DPPH also decreased during the reaction process. It is from the process of decreasing color and absorbance that it is possible to determine. Determination of antioxidant levels can be seen in the magnitude of the IC50. This value of IC50 is the effective amount of the concentration of the substance found in the lobbies and jamblang fruit. The IC50 value is also the percentage value of the activity of antioxidant compounds that can ward off free radicals by 50%. The smaller the IC50value, the stronger the antioxidant activity. Specifically, a compound has strong antioxidant activity if the IC5050 ppm, it is said to be 50-100 ppm strong, 101-150 ppm is moderate, and it is said to be weak if the antioxidant is > 150 ppm. variations in concentration of 3 ppm, 5 ppm, 7 ppm, and 10 ppm respectively. All of which also have various inhibition percentages, where for the tome-tome itself has a value variation of 82.919%, 84.061%, 85.088% and 85.773%. Jamblang absorbance measurement data were 78.125%, 80.751%, 81.550% and 84.061% and from this data the regression equation was determined which can be seen in Figures 2 and 3 below.

Figure 2. Lobby-lobby linear regression equation (Flacourtia Inermis)

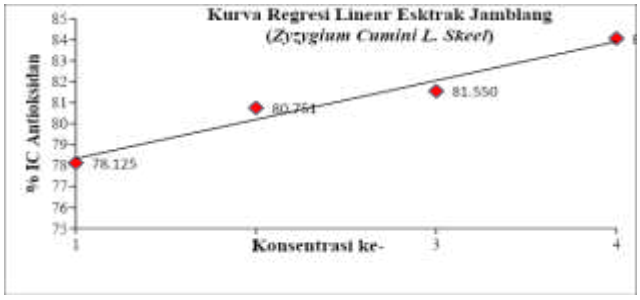


Figure 3. Jamblang linear regression equation (Zyzygium Cumini L. Skeel)

The amount of antioxidant activity is indicated by the IC50. The concentration of the sample when scavenging DPPH radicals can be determined by the concentration value of IC50. The comparison between the concentration and absorbance values can then be determined by a straight line graph so that the straight line equation of a test sample can be determined (Widyastuty., 2015).

The lobbies extract has a regression equation y = 0.958x + 82.06 with a value of $R^2$ = 0.988 so that the obtained value IC50 is 50.011 g/L. As for the jamblang fruit extract, the regression equation y = 1.860x + 76.47 with a value of $R^2$ = 0.964 so that the IC50 of the jamblang fruit extract sample is 91.113 g/L.

The results obtained from the results of testing the IC50 antioxidant value for the two samples, there is a difference in the

number of IC50 where for the lobbies extract itself is at a very strong intensity as an antioxidant because the IC50 from the lobbies is 50.011 g/L and for the fruit extract Jamblang is also still at a strong intensity because IC50 of the test sample is 91.113 g/L.

Determination of Anthocyanin Levels with Differential (Giusti and Worlstad) The total anthocyanin content of each sample was determined by the pH comparison method. In this determination, the dilution factor was first determined by dissolving the sample in buffer with a pH of 1.0 and Sodium Citrate with a pH of 4.5 which were analyzed using a UV-Vis spectrophotometer at a wavelength of 510 nm so that the absorbance obtained was below 1.2.

The dilution factor was 10 times with absorbance obtained for tome-tome for 3 repetitions of 0.107 and 0.111. Meanwhile, the jamblang extract itself had absorbance variations for 3 repetitions, namely 0.027 and 0.029. Based on the calculation results obtained, it was found that the average total anthocyanin level was 18.00 mg/L for lobbies and 4.62 for jamblang.

The results obtained are because they are influenced by various factors including the time of the sample preparation process, the storage conditions of the samples used. At the time of evaporation, a fairly high temperature of 65-700C was used gradually and the presence of other impurities such as metal ions contained in the test sample so that the stability of anthocyanins was disturbed. This is in line with Niendyah who said that temperature, changes in pH, light, oxygen and other impurities such as metal ions can affect the stability of anthocyanins in a test sample.

## 4. CONCLUSIONS AND SUGGESTIONS

There are differences in the antioxidant activity content of the Jamblang fruit samples (Syzygium Cumini L.Skeel) which are in the category of strong antioxidants and Lobi-lobbi fruit (Flacourita Inermis) is very very strong. Meanwhile, the anthocyanin content between Jamblang fruit (Syzygium Cumini L.Skeel) is 4.64 mg/L and Lobi - lobby fruit (Flacourita Inermis) is 17.99 mg/L.

## REFERENCES

Chen Tao et al.2022. Evaluation Of the Chemical Composition and Antioxidant Activity of Mulberry (Morus alba L.) Fruits from Different varieties in China. Molecules, Journal of MDPI, Base, Switzerland.2022.

Faisal Hendri, Handayani Sri.2019. Comparison of Antioxidant Activity of Methanol Extract of Fruit and Okra Leaves (Abelmoschus esculentus L. Moench) by DPPH and ABTS Methods. Faculy of Pharmacy and Health, Instits Kesehatan Helvetia, Medan, Indonesia. Indonesian Journal of Pharmaceutical and Clinical Research. Vol.02, No.2.2019.

Firiyani Rema, Lestario Lydia Ninan, dan Martono Yohanes.2018. Jenis dan Kandungan Antosianin Buah Tomi – Tomi. Program Studi Kimia, Fakultas Sains dan Matematika, Universitas Satya Wacana, Salatiga, Jawa Tengah. Jurnal Teknologi dan Industri Pangan Vol.29(2).2018

Ifadah Raida Amelia, Wiratra Pinasthika Rizkia Warapsari, dan Afgani Chairul Anam.2021. Usulan Ilmiah : Antosianin dan Manfaatnya untuk Kesehatan. Jurnal Teknologi Pengolahan Pertanian, Vol 3 nomor 2. 2021.

Khirunnisa Izzatul, Sumiwi Sri Adi. 2019. Peran Flavanoid Pada Berbagai Aktivitas Farmakologis.Fakultas Farmasi Universitas Padjadjaran. Jurnal Farmaka Volume 17 Nomor 2. 2019.

Marliani Lia, Kusriani Herni, Sari Indah Nur.2014. Aktivitas Antioksidan Daun Dan Buah Jamblang (Syzygium Cumini) Skeel. Sekolah Tinggi Ilmu Farmasi Bandung. Bandung.

Meidayanti Ni Ketut Putri,dkk.2015. Aktivitas Antioksidan Antosianin Dalam Ekstrak Etanol Kulit Buah Naga Super Merah (Hylocereus costaricensis) dan Analisis Kadar Totalnya. KIMIA FMIPA Universitas Udayana, Bukit Jimbaran. Bali.

Sayuti Kesuma, MS. Yenrina Rina.2015. Antioksidan, Alami dan Sintetik.Padang. Andalas University Press.

Ulfah Maria, Kurniawan Ricky Chandra, Erny Metalia.2020. Standarisasi Parameter Non Spesifik dan Spesifik Ekstrak Etanol Daun Jamblang (Syzygium cumini L.Skeel). Jurnal Ilmu Farmasi dan Farmasi Klinik (JIFFK), Vol.17 No.2.2020

Widyastuti, Hilayati Najim, Rahmi Suci Intan Nisa.2021. Potensi Ekstrak Buah Jambu Jamblang (Syzygium cumini L.Skeel) Sebagai Antioksidan dan Tabir Surya. Jurnal Ilmia Farmasi Farmasyifa. Vol 4(1) : 112-119.2021.ISSN:2599-0047.

Widyastuti, Fratama Rizqi ikhwanda dan seprialdi ade.2015. Pengujian aktivitas antioksidan dan tabir surya ekstrak etanol kulit buah naga super merah

(hylocereus costaricensis (f.a.c. Weber) britton & rose). Akademi Farmasi Imam Bonjol Bukittinggi.