**INTEK Jurnal Penelitian**
Volume 9, No. 1, 18-24, April 2022 

# Senggani Fruit Anthocyanins (Melastoma Malabathricum Auct, Non-Linn) as Bacterial Dyes Differential Painting Techniques

**Ratih Indrawati[a], Gervacia Jenny R,[b,*], Sri Tumpuk[c]**
[1]Department of Health Analyst, Poltekkes Kemenkes Pontianak, Jalan Dr. Sudarso, Pontianak, Indonesia
[a]indrawati.haykal@gmail.com
[b,*]prodi_d4analis@yahoo.co.id (Corresponding Author)
[c]sritumpuk66@gmail.com

**Abstract— Bacteria are difficult to see with a light microscope, because they do not absorb or refract light. Dyes absorb and refract light so that the contrast of bacteria with their surroundings is enhanced. Anthocyanins are water-soluble pigments that are naturally found in various types of plants. As the name implies, this pigment gives color to flowers, fruits, and leaves of green plants. This research is a type of descriptive research. The treatment consisted of painting bacterial preparations with anthocyanin pigment extract of Senggani fruit with 70% ethanol solvent and control with gram staining. The treatments were: T1: staining of bacterial preparations with gram staining, T2: painting of bacterial preparations with anthocyanin extract of Senggani fruit added with 14% citric acid as a substitute for Safranin and $NH_4Cl$ as a substitute for crystal violet. The results of the study showed that anthocyanin pigment extract was proven to be used as a dye for Staphylococcus Aureus, Escereria coli as a substitute for synthetic dyes Safranin and Crystal violet in Gram staining.**

***Keywords: Anthocyanins; Natural dyes; Staphylococcus aureus; Escherichia coli***

## I. Introduction

Bacterial identification is a laboratory procedure used to determine the morphological characteristics of bacteria, so that bacteria can be examined whether they are alive or dead. Examination of the morphology of these bacteria is necessary, to recognize the name of the bacteria. Besides that, it is also necessary to recognize the physiological characteristics, even these physiological characteristics are mostly certain factors in recognizing the species name of a bacterium. While the confirmation of bacteria is to determine the type of bacteria and their colonies. Confirmation of the type of bacteria can use various stains, enzymatic reactions or biochemical reactions, especially if identification using media is still doubtful/unsatisfactory [1].

Bacteria easily react with simple dyes because their cytoplasm is basophilic (like bases) while the dyes used for simple staining are generally alkaline (the chromophoric component is positively charged). Staining techniques on bacteria can be divided into four types, namely simple painting, negative painting, differential painting and structural painting. Giving color to bacteria or other microorganisms by using a single solution of a dye on a thin layer, or smear, which has been fixed, is called simple staining.

The staining procedure that displays the differences between bacterial cells or parts of bacterial cells is called a differential staining technique. While structural staining only colors one part of the cell so that it can distinguish parts of the cell. Included in this painting are endospore, flagella and capsule staining [2]. Bacteria are difficult to see with a light microscope, because they do not absorb or refract light. This is the reason why dyes are used to color bacteria or their background. The dye absorbs and refracts light so that the contrast of the bacteria with its surroundings is enhanced [3]. Dyes are chemicals, both natural and synthetic, that give color. Based on the source, coloring agents for food can be classified into natural and synthetic dyes [4].

Natural dyes are dyes obtained from animals such as: pink in flamingos and salmon while from plants such as caramel, chocolate and suji leaves. Artificial dyes are also known as synthetic dyes. Synthetic dyes are more expensive than natural dyes. The process of making these synthetic dyes is usually through the treatment of sulfuric acid or nitric acid which is often contaminated [5].

DOI : http://dx.doi.org/10.31963/intek. v9i1.2987

Synthetic dyes on bacteria are gram stains, one of the components is safranin and crystal violet dyes, side effects of synthetic dyes can irritate mucous membranes so that redness or swelling occurs and even necrosis when used at high concentrations. Laboratory studies of synthetic dyes can cause cancer in mice and cases of allergies to synthetic dyes such as extensive redness, swelling, itching and lightheadedness such as fainting and difficulty breathing can occur.

Crystal Violet can give a red color to the preparation, its use is practical and the resulting color is stable. The disadvantages of crystal violet are that it is expensive, easily damaged, difficult to store and carcinogenic. Carcinogenic substances in dyes can cause problems for the environment and human health. Therefore, it is necessary to have an alternative to the use of natural dyes made from plant/vegetable materials that are easy to obtain and have the same function and are safe as coloring agents to replace crystal violet and safranin.

Natural dyes have a higher economic value than artificial dyes. One of the natural dyes comes from fruits. Senggani fruit (Melastoma malabathricum Auct, non Linn) is one of the fruits that can be used as a natural dye. Senggani fruit (Melastoma malabathricum Auct, non Linn) is a fruit that is included in the fruit that contains anthocyanins. Anthocyanins can also be found in strawberries, watermelon, apples, raspberries and others. High levels of anthocyanins are found in various plants [6].

Anthocyanins are water-soluble pigments that are naturally found in various types of plants. As the name implies, this pigment gives color to flowers, fruit, and leaves of green plants, and has been widely used as a natural colorant in various food products and various other applications [6].

Senggani fruit has a purple dye that is difficult to remove from cloth or hands. Research on the use of red fruit ethyl acetate extract as a substitute for primary dyes as a substitute for primary dyes in the single staining technique of gram-negative rods, states that ethyl acetate extract cannot be used as a substitute for primary coloring in gram-negative bacteria [7], on the contrary. Research on the use of purple sweet potato peel extract as a natural dye for purslane stem (Puttulaca aleraceae) preparations gave good results where sweet potato peel extract could color the epidermis, parenchyma, xylem and phloem tissues of purslane stems [8].

The difference in response to the gram staining mechanism in bacteria is based on the structure and composition of the bacterial cell wall. Gram-positive bacteria contain protein and gram-negative bacteria contain a higher percentage of fat and have thin cell walls. The administration of alcohol (ethanol) in the bacterial staining practicum causes lipids to be extracted thereby increasing the permeability of the cell wall.

Based on the above opinion, the research technique that will be carried out on the staining of E, coli and Staphylococcus aureus bacteria preparations uses color paints of 70% ethanol solvent anthocyanin pigment extract by painting the preparations with natural dyes, heated over a spirit fire for ± 30 seconds, then color paints on discard and wash with running water, dry at room temperature, then read under a 100x lens microscope. The function of heating on colored preparations is to ensure that there is 70% ethanol and heating can open a layer of wax and fat on the bacterial cell wall, so that the colored paint can absorb the bacterial cell wall.

Maceration is an extraction process by immersing the sample at room temperature using a suitable solvent so that it can dissolve the analyte in the sample. According to research on the effect of maceration time of anthocyanin substances as natural dyes from sweet potatoes. The result is that the longer the extraction time, the higher the anthocyanin content obtained. The types of solvents that are usually used for maceration of dyes are ethanol, methanol, and aquades. The three solvents have polar properties that match the anthocyanins [9].

Nida stated that ethanol is a good solvent for flavonoid extraction, especially anthocyanins because it is polar [10]. Anthocyanin dyes are unstable in neutral or alkaline solutions, so extraction is carried out under acidic conditions. Based on research on the effect of citric acid concentration on the characteristics of anthocyanin extracts and the addition of the best teak leaf anthocyanins affect the color stability of ice cream [11].

DOI : http://dx.doi.org/10.31963/intek. v9i1.2987

## II. Research Methodology

This research is a type of descriptive research. Treatments were: T1: staining of bacterial preparations with gram staining, T2: painting of bacterial preparations with anthocyanin extract of senggani fruit added with 14% citric acid as a substitute for safranin and added $NH_4CL$ as a substitute for viole crystals production of senggani fruit anthocyanin pigment extract.

*A. Making Senggani Fruit Anthocyanin Pigment Extract*

1. The research sample used was Senggani fruit (Melastoma malabathricum Auct, non-Linn) which was obtained in the vicinity of Wonodadi Village, Kubu Raya. Samples of senggani fruit (Melastoma malabathricum Auct, non-Linn) which had been collected were weighed as much as 150 grams, then washed and separated from the skin.
2. Samples of Senggani fruit (Melastoma malabathricum Auct, non Linn) which were clean were ground and then put into a glass jar and 250 ml of 70% ethanol solvent was added and then covered with aluminum foil.
3. The solution was left (macerated) for 30 minutes. After that, the solution was filtered using filter paper. Then, the filter (extract) is used as a bacterial dye
4. To give the desired red color, the extract was added with 14% citric acid.
5. To give a bluish purple color, the extract was added with 25% $NH_4Cl$.

*B. Preparation of test preparations*

1. Clean the slide with a cotton swab soaked in alcohol.
2. The organism code is written on the right corner of the slide with a marker.
3. Dropped 2 PZ ose eyes in the center of the slide.
4. Touched the ose on the bacterial culture, then mixed with NaCl-Faali until evenly distributed. Spread this mixture on the slide.
5. Allow the preparation to dry on the slide for a while.
6. Fixed on fire. Preparations that have been dried and fixed are placed on a painting bath.
7. The methylene blue dye solution is dripped as much as 2-3 drops and allowed to stand for 1 minute.
8. The preparations were given flowing distilled water and dried
9. Lugol's solution is dripped and left for 1 minute then washed with running water and dried
10. Acid alcohol solution is given for 30 seconds, then washed with running water and dried
11. Crystal Violet solution is given for 20 seconds
12. Washed with running water and dried
13. Do it also by replacing the dye methylene blue and Crystal Violet using anthocyanin extract of the senggani fruit.
14. The stained glass preparations were observed using a microscope with a magnification of 10x100
15. Especially for preparations colored with anthocyanin pigment extract, heating was carried out for ± 30 seconds.
16. The paint is removed, washed with running water until clean. Dry with a tissue.
17. Observed under a microscope with a magnification of 100x using anisol/emersion.

The data obtained in the form of bacterial painting by using a comparison of methylene blue paint, Crystal Violet and anthocyanin pigment extract of Senggani fruit were analyzed using a test with painting criteria, namely:
a. Good, if the results of the absorption of the dye on the preparations look contrasting against the background, the staining preparations look clean of paint deposits and stained bacteria well on observation under a microscope.
b. Not good, if the results of the absorption of the dye on the preparations look less contrasted against the background and the staining preparations look clean of paint deposits and stained bacteria on observation under a microscope.

Analysis of the data used is descriptive qualitative data analysis including contrast and clarity of bacterial painting preparations with natural dyes from Senggani fruit extract.

## III. Result and Discussion

*A. Research Results*

The extraction of senggani fruit (Melastoma malabathricum Auct, non-Linn) using 70% ethanol produces a purplish red (violet) liquid then divided into 2 bottles, 1 bottle is added with a few drops of 14%

DOI : http://dx.doi.org/10.31963/intek. v9i1.2987

citric acid until a red color that resembles the color of Safranin is formed. In the second bottle, add a few drops of 25% $NH_4Cl$ until a bluish purple color is formed.

The resulting staining was then tested on each of the Escherichia coli and Staphylococcus aureus bacteria preparations, this stage of testing also could not color the bacteria, then the test was repeated by adding dye to the preparations then heated for ± 30 seconds, and then staining according to the staining method bacteria simply.

The results of staining on Eschericia coli and Staphylococcus aureus bacteria using Gram dye and senggani fruit extract (Melastoma malabathricum Auct, non-Linn) can be seen in the table 1.

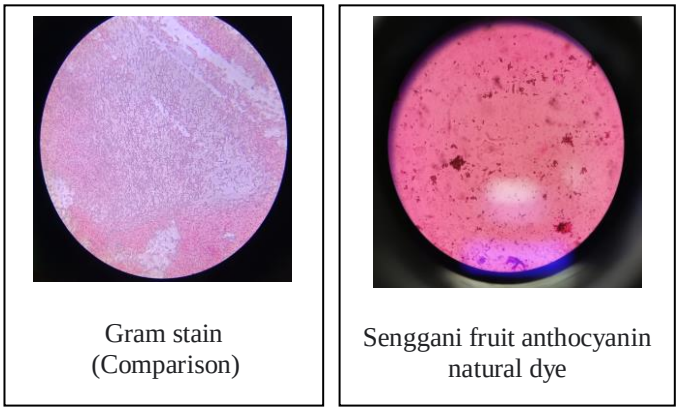
Table 1. Staining Results of E-Coli Bacterial Preparations using Salt and Anthocyanin staining of senggani fruit (Melastoma malabathricum Auct, non-Linn)

| Code | Bakteria color | Against the Background | Cleanlinesof preparations | Coloring Result Quality | |
|---|---|---|---|---|---|
| | | | | Good | Bad |
| T1 | Red | Contrast | Clean | Good | - |
| T1 | Red | Contrast | Clean | Good | - |
| T1 | Red | Contrast | Clean | Good | - |
| T1 | Red | Contrast | Clean | Good | - |
| T2 | Red | Contrast | Clean | Good | - |
| T2 | Red | Contrast | Clean | Good | - |
| T2 | Red | Contrast | Clean | Good | - |
| T2 | Red | Contrast | Clean | Good | - |

The dye test on the preparations showed different results depending on the ability of the dye to penetrate the bacterial cytoplasm, the dyes used for gram staining were generally alkaline (the chromophore component was positive).

The results of staining 4 preparations of E-Coli bacteria using senggani fruit pigment extract as a substitute for Crystal Violet and Safranint gave good coloring to the preparations, the results of observations under a microscope magnification of 1000 X the bacteria were clearly visible in the form of bacilli, E. coli bacteria is one of the gram negative bacteria . In Gram staining, gram-negative bacteria will lose crystal violet dye after washing with alcohol, and when treated with a counter-staining agent, namely safranin, will appear red.

The results of staining 4 preparations of S.aureus bacteria using anthocyanin of senggani fruit as a substitute for Crystal Violet and Safranint gave good staining on the preparations, the observations under a microscope magnification of 1000 X the preparations lacked contrast, but for bacteria it can clearly be seen under a microscope, the bacteria should be purple in color bluish to grayish, Anthocyanin is a compound that is amphoteric, which has the ability to react both with acids and bases. In acidic media, anthocyanins are red, and in alkaline media they turn purple and blue [12].



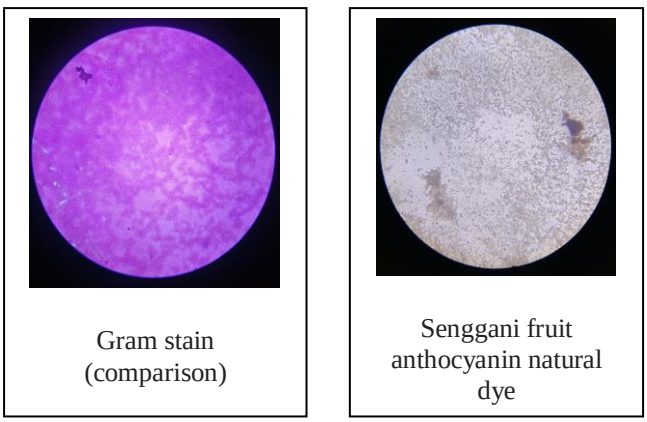
Gram stain (Comparison) | Senggani fruit anthocyanin natural dye

Figure 1. Staining Results on E coli Bacteria

Table 2. Staining Results of S-Aureus Bacterial Preparations using Salt and Anthocyanin staining of senggani fruit (Melastoma malabathricum Auct, non-Linn)

| Code | Bakteria Color | Against the Backgrount | Cleanlinesof preparations | Coloring Result Quality | |
|---|---|---|---|---|---|
| | | | | Good | Bad |
| T1 | Blue | Contrast | Clean | Good | - |
| T1 | Blue | Contrast | Clean | Good | - |
| T1 | Blue | Contrast | Clean | Good | - |
| T1 | Blue | Contrast | Clean | Good | - |
| T2 | Gray | Enough | Clean | - | - |
| T2 | Gray | Enough | Clean | - | - |
| T2 | Gray | Enough | Clean | - | - |
| T2 | Gray | Enough | Clean | - | - |

S. aureus is a Gram positive and cocci-shaped bacterium that produces a purple color on Gram stain. The purple color is caused by the bacteria retaining the first color.



Gram stain (comparison) | Senggani fruit anthocyanin natural dye

Figure 2. Staining Results on S. aureus Bacteria

DOI : http://dx.doi.org/10.31963/intek. v9i1.2987

Table 3. Results of Identification of Bacteria

| Replication | **Bakteria color** with code (T1-T2) | | | |
|---|---|---|---|---|
| | T1 | | T2 | |
| | *E. Coli* | *S.Aureus* | *E. Coli* | *S.Aureus* |
| 1 | + | + | + | + |
| 2 | + | + | + | + |
| 3 | + | + | + | + |
| 4 | + | + | + | + |

Description (+) : Bacterial morphology seen under a microscope
(-) :Bacterial morphology is not visible under the microscope

The results of the dye test against bacteria for E-coli and S. aureus all types of dyes used can give positive results, meaning that the bacteria are clearly visible under a microscope, although the staining for S. aureus bacteria does not match the color in the comparison because the resulting color is not purple but grey.

*B. Discussion*

Bacterial staining aims to make it easier to see bacteria with a microscope, clarify the size and shape of bacteria such as cell walls and vocuoles, produce physical and chemical properties that are typical of bacteria with dyes, and increase the contrast of microorganisms with their surroundings [13].

Simple staining is staining using one type of dye with the aim of only seeing the shape of bacterial cells and to determine the morphology and arrangement of the cells. This staining can use basic stains in general, including crystal violet, methylene blue, carbolic acid, fuchsia, and safranin [13].

The simple staining procedure is easy and fast, so this staining is often used to see the shape, size and arrangement of bacterial micro-organisms in bacteria known as round (coccus), rod (bacilli), and spiral shapes. With simple staining can also be seen the arrangement of bacteria. On cocci can be seen staining like chains (stertococcus), grapes (staphylococcus), pairs (diplococcus), cubes consisting of 4 or 8 (saranae) [3].

Colored paints can absorb and refract light, thereby enhancing the contrast of the bacterial cell with its surroundings. The color paint used is acidic or basic. In basic color paints, the part that plays a role in giving color is called a chromophore and has a positive charge. On the other hand, in acid colored paint, the part that plays a role in providing the dye has a negative charge. alkaline color paints are more widely used because negative charges are found on the surface of bacterial cells

The results of the study for preparations of E. coli (gram negative) bacteria that were painted with Safranint color resulted in poor quality staining. As for the preparations painted with Crystal Violet, good quality coloring was produced. Staphylococcus aureus bacteria (gram positive) stained with methylene blue paint produced good quality staining because according to Pelczar, the cell walls of positive bacteria generally have a thick cell wall structure (15-80mm) and a little fat (1-4%). The cell wall of gram-positive bacteria has more peptidoglycan which is able to retain the blue dye, so that the blue color that appears on microscope observation looks contrast [3].

In the use of crystal violet paint, poor quality was obtained because the red color absorbed by the pores of the thicker cell wall peptidoglycan was not perfect so that when using a microscope it looked less contrasted. In the preparation of E.coli bacteria (gram negative) stained with crystal violet obtained good quality, because according to Pelczar, gram negative bacteria have thinner cell walls (10-15 nm) with a higher fat percentage (11-24%) than gram-positive bacteria, because gram-negative bacteria have less peptidoglycan which is able to absorb the red color to the red color that appears on The results of the dye test against bacteria for E-coli and S. aureus all types of dyes used can give positive results, meaning that the bacteria are clearly visible under a microscope, although the staining for S. aureus bacteria does not match the color in the comparison because the resulting color is not purple but grey.

Microscopic observations show contrast. The use of Safranint paint is not good because the blue color absorbed by the peptodoglycan pores of the cell wall is not perfect so that on microscope observation it looks less contrasted, the staining results are not good [13].

Gram negative bacteria are bacteria that cannot retain the purple metal dye in the gram staining method. Gram-positive bacteria will retain a dark purple metallic dye.

DOI : http://dx.doi.org/10.31963/intek. v9i1.2987

In preparations of E, coli and Staphylococcus aureus bacteria that were painted using an anthocyanin pigment extract from the senggani fruit, when using water solvent the preparations could not be colored, both showed poor quality, due to the inability of colored paint to absorb bacterial cell walls, whereas when using ethanol as solvent In the anthocyanin pigment extract, preparations of E, coli and Staphylococcus aureus bacteria can be painted and produce stains with poor quality because they cannot show the morphology of the bacteria. This is possible because bacteria are acidic and have a layer of wax and fat that is difficult for paint to penetrate, in contrast to synthetic paints/dyes such as basic fuchin which are able to penetrate layers of wax and fat.

The difference in response to the gram staining mechanism in bacteria is based on the structure and composition of the bacterial cell wall. Gram-positive bacteria contain protein and gram-negative bacteria contain a higher percentage of fat and have thin cell walls. The administration of alcohol (ethanol) in the bacterial staining practicum causes lipids to be extracted thereby increasing the permeability of the cell wall.

Based on the above opinion, the preparations of bacteria E, coli and Staphylococcus aureus were then stained using color paint of 70% ethanol solvent anthocyanin pigment extract which was carried out by means of bacterial preparations flooded with senggani fruit pigment extract paint, then heated over spiritus fire for ± 30 seconds, then painted The color is discarded and washed with running water, dried at room temperature, then read under a 100x lens microscope.

The heating function on preparations colored with senggani dye with 70% ethanol solvent and heating for 30 seconds aims to dissolve the wax and fat layer on the bacterial cell wall, so that the colored paint can absorb the bacterial cell wall.

The results of the study for 4 preparations of E. coli bacteria (gram negative) which were painted with pigment extract of Senggani fruit with 14% citric acid added, the staining was of good quality and the shape/morphology of E.coli bacteria could be seen under a microscope with 1000x magnification. Meanwhile, preparations painted with senggani fruit pigment extract dye added with 25% $NH_4Cl$ resulted in poor quality coloring. Although this staining is not good, it can show the shape/morphology of bacteria under a microscope.

For 4 preparations of Staphylococcus aureus bacteria (gram positive) which were stained with senggani fruit pigment extract added with 14% citric acid instead of safranin, the staining was of poor quality. Meanwhile, for preparations painted with senggani fruit pigment extract dye added with 25% $NH_4Cl$ as a substitute for crystal violet, staining of poor quality was also produced. But this staining is able to show the shape and morphology of Staphylococcus aureus bacteria.

The quality of the antiocyanin pigment extract dye added with $NH_4Cl$ is less good than the synthetic methylene blue dye for E.coli and Staphylococcus aureus bacteria here, not due to the ability of the dye to absorb bacterial cell walls but because the color produced after adding 25% $NH_4Cl$ is unstable, there is a change the color from blue to gray causes the staining result is not good. Anthocyanin stability can be influenced by light, low temperature, copigment, metal ions and pressure to be a factor in maintaining stability. In general, the addition of hydroxyl will decrease the stability, while the addition of methyl will increase the stability [14]. The pH factor does not only affect the color of anthocyanins but also affects their stability. Anthocyanins are more stable in acidic solutions than in basic solutions [15].

Anthocyanins are in the form of red flavium cations, whereas when the solvent is combined with a weak acid, the anthocyanin color changes to a fading red color at pH 3, purplish red at pH 4, purple at pH 5-6 and purple blue at pH 7 [16].

## IV. Conclusion

Anthocyanin pigment extract is proven to be used as a colorant for bacteria. Anthocyanin pigment extracts give less good results for use as a stain for Staphylococcus aureus bacteria, compared to Gram stai and t.he anthocyanin pigment extract gave good results to be used as a dye for E.coli bacteria when compared to Gram's dye

DOI : http://dx.doi.org/10.31963/intek. v9i1.2987

## Acknowledgement

We would like to thank the Director of Poltekkes, Ministry of Health Pontianak, Head of the Pontianak Health Analyst Department and fellow lecturers at the Pontianak Health Analyst Department who have provided guidance, assistance, support and motivation in writing this journal and thank the entire team of enumerators who have help complete this research.

## References

[1] Y. Jiwintarum, Rohmi, and I. D. P. M. Prayuda, "Buah Naga (Hylocereus Polyrhizus) Sebagai Pewarna Alami Untuk Pewarnaan Bakteri", (Dragon Fruit (Hylocereus Polyrhizus) As Natural Dyes For Bacterial Coloring), *J. Kesehat. Prima*, Vol. 10, No. 2, pp. 1726–1734, 2016.

[2] L. Waluyo, "Mikrobiologi Umum", (General Microbiology), Cetakan 5. Malang: UMM Press, 2019.

[3] B. W. Lay, "Analisis Mikroba di Laboratorium", (Microba Analysis in Laboratorium). Jakarta: Raja Grafindo Persada, 1994.

[4] J. H. von Elbe and S. J. Schwartz, "Colorants," in Food Chemistry, 5th ed., Owen R. Fennema, Ed. United States Of America: Imprint, 2017.

[5] F. G. Winarno, "Kimia Pangan dan Gizi", (Food Chemistry and Nutrition). Bogor: M. Brio Press, 2008.

[6] F. Cosme *et al.*, "Red Fruits Composition and Their Health Benefits — A Review to Nile," *Foods*, vol. 11, no. 5, 2022.

[7] A. D. Rahayuningtyas and W. Dewi, "Pemanfaatan Ekstrak Etil Asetat Buah Merah Sebagai Zat Pengganti Pewarna Primer Pada Teknik Pengecatan Tunggal Bakteri Gram Negatif Batang", (Utilization of Red Fruit Ethyl Acetate Extract as a Substitute for Primary Dyes in Single Staining Techniques for Gram-Negative Bacteria), J. Kedokt. Gigi Univ. Padjadjaran, Vol. 29, No. 2, pp. 148–144, 2017.

[8] I. D. Saputri, "Pemanfaatan Ekstrak Kulit Ubi Jalar Ungu Sebagai Pewarna Alami Preparat Section Batang Tumbuhan Krokot (Portulaca oleraceae)", (Utilization of Purple Sweet Potato Peel Extract as Natural Colorant Preparation of Purslane Plant Stem Section (Portulaca oleraceae), Universitas Muhammadiyah Surakarta, 2018.

[9] R. S. Armanzah and T. Y. Hendrawati, "Pengaruh Waktu Maserasi Zat Antosianin Sebagai Pewarna Alami dari Ubi Jalar Ungu (Ipomoea batatas L. Poir)", (Effect of Anthocyanin Maceration Time as Natural Colorant from Purple Sweet Potato (Ipomoea batatas L. Poir), Semin. Nas. Sains dan Teknol., 2016.

[10] N. El, M. Novita, and S. Rohaya, "Kandungan Antosianin dan Aktivitas Antioksidan Ubi Jalar Ungu Segar dan Produk Olahannya", (Anthocyanin content and antioxidant activity of fresh purple sweet potato and its processed products), Agritech, Vol. 33, No. 3, pp. 296–302, 2013.

[11] Y. Hermawati, A. Rofieq, and P. Wahyono, "Pengaruh Konsentrasi Asam Sitrat Terhadap Karakteristik Ekstrak Antosianin Daun Jati Serta Uji Stabilitasnya Dalam Es Krim", (Effect of Citric Acid Concentration on the Characteristics of Teak Leaf Anthocyanin Extracts and Stability Tests in Ice Cream), Prodising Semin. Nas. Pendidik. Biol., pp. 301–308, 2015.

[12] J. M. DeMan, "Kimia Makanan", (Food Chemistry), Kedua. Bandung: ITB PRESS, 1997.

[13] J. Michael J. Pelczar and D. E. C. S. Chan, "Dasar-Dasar Mikrobiologi", (Fundamentals of Microbiology). Jakarta: UI PRESS, 2009.

[14] Harborne, "Encyclopedia of Food and Color Additives". New York: CRC Press,Inc, 2005.

[15] P. Markakis, "Anthocyanins as Food Additives," in Anthocyanins As Food Colors, P. Markakis, Ed. Academic Press, 1982, pp. 245–253.

[16] R. Sitepu, Heryanto, T. H. P. Brotosudarmo, and L. Limantara, "Karakterisasi Antosianin Buah Murbei Spesies Morus alba dan Morus cathayana di Indonesia", (Anthocyanin Characterization of Mulberry Fruit Species Morus alba and Morus cathayana in Indonesia), Online J. Nat. Sci., Vol. 5, No. 2, pp. 158–171, 2016.

DOI : http://dx.doi.org/10.31963/intek. v9i1.2987