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Purple Sweet Potato (*Ipomoea batatas* L.) Peels Extract as an Alternative Dye for Bacteria Gram Staining

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Abstract

Crystal violet and Safranin are dyes in Gram staining, which are carcinogenic. Alternative safe materials are needed to minimize the use of carcinogenic properties. Purple sweet potato (*Ipomoea batatas* L.) peels were the candidate of the alternative dye source because of its high anthocyanin pigment. The purpose of this study was to determine purple sweet potato (*Ipomoea batatas* L.) peels extract as an alternative to Gentian violet in Gram staining of bacteria. Extracts obtained from purple sweet potato peels studied with varying concentrations of 50%, 60%, and 75% for 1, 3, and 5 min as a substitute for Gentian violet on *Bacillus* sp. The parameters observed from this study based on visual field clarity, glass slide cleanliness, contrast, bacterial shape, bacterial colour. Each extract concentration compared with a control group using Gentian violet. The results showed that optimum staining in 50% concentration for 5 min, 60% concentration for 5 min, 75% concentration for 3 min, and 5 min respectively. The present study exhibited the potency of *Ipomoea batatas* L. peels extract as an alternative staining agent.

Keywords

Ipomoea batatas L., Gentian violet, bacteria Gram taining, *Bacillus* sp.

INTRODUCTION

The microorganism is a living organism that is very small in size, so microscopic observation aids are needed. Microorganisms studied in the laboratory for various purposes. The study of these microorganisms intends to facilitate and assist in the examination service in the laboratory (1).

Bacteria have a strong cell wall as an outer part and maintain the shape of bacteria and protect against osmotic pressure. In staining, there are variations in the structure of cell walls. Where the complex structure, semi-rigid with bacterial cell wall thickness

ranges from 10 to 35nm and surrounds the cytoplasmic membrane, serves to give shape to the cell and protect the contents of the cell from outside cell influences (1).

Bacterial cell walls are essentials for growth and division. Based on the chemical composition of the cell wall, which causes cell wall rigidity is peptidoglycan. The mechanism of Gram staining based on the structure and composition of the cell wall (2).

In the laboratory world, especially in the field of staining, microbiology is one of the keys to helping to provide information about the diagnosis of a disease. The existence of the development of staining procedures to assist in roughly observing the morphology of microorganisms helps in identifying parts of the cell structure of microorganisms and helps differentiate similar microorganisms (1).

The importance of observing the morphology of microorganisms, where the appearance of microorganisms in living conditions is quite complex, not only because of their size but also because they are transparent and colourless when suspended in a liquid medium. To study the properties and divide microorganisms into specific groups for diagnostic purposes, the biological dyes and staining procedures with a light microscope have become the main tools in microbiology (3).

The most basic and primary staining methods used are Gram staining. The Gram reaction is related to morphological characteristics in phylogenetic-related forms (4). Synthetic dyes are including Crystal violet, Safranin, Carbol fuchsin, are dyed commonly used in Gram staining. Crystal violet and Safranin are dyes that often used in Gram staining. Crystal violet is a human carcinogen (5).

To minimize the use of carcinogenic properties for gram staining, the research to find alternative materials are needed. To get natural dyes that can be used as alternative dyes, extraction methods are needed to get the best pigment (anthocyanin) from the colour of these natural ingredients, thereby maximizing the quality and quantity. Purple sweet potato peel has an average anthocyanin level of 521.84–729.74 mg / 100g (6). Thus, in this research conducted further testing, using peel extracts of purple sweet potatoes (*Ipomoea batatas* L.).

In the previous study (7), purple sweet potato extract can be obtained manually and can produce reddish-purple colour. For this reason, it needs to develop research to maximize the content of dyes (anthocyanin) in the purple sweet potatoes peels using extraction techniques MAE (microwave-assisted extraction) method. The previous study only examined the coccus Gram-positive group without being compared with other groups.

In this study, gram staining of Bacillus sp tested and also the optimum concentration

and time of gram staining were studied. The purpose of this study was to determine purple sweet potato (*Ipomoea batatas* L.) peels extract as an alternative to Gentian violet in Gram staining of bacteria.

MATERIALS AND METHODS

The materials used in this study were the pure culture of *Bacillus sp.* obtained from Central health laboratory (Balai Besar Laboratorium Kesehatan/BBLK) in Surabaya, NaCl 0.85% (0,85 gr NaCl [SAP chemicals]/100 mL distilled water), Gram stain hucker method, immersion oil (Olympus), staining from *Ipomoea batatas* L. peel extract, ethanol 96% (SAP chemicals), HCl 1N (8.3 mL HCl 37% (SAP chemicals)/100 mL distilled water), HCl 2N (16.6 mL HCl 37% (SAP chemicals)/100 mL, NH_4OH 1N (16 mL HCl 37% (Merck)/100 mL distilled water), NH_4OH 2N (32 mL HCl 37% (Merck) 100 mL distilled water).

The sample used was the peels of purple sweet potato (*Ipomoea batatas* L.) not other types of sweet potato. This study consisted of several stages. The first stage was the process of sample preparation using MAE (microwave-assisted extraction) method extraction (8), determination of the concentration formula, the staining process, observation under a microscope, and lastly data analysis. Extraction techniques MAE (microwave-assisted extraction) method was selected to maximize the content of dyes (anthocyanin) in the purple sweet potatoes peels.

In sample preparation, the sample extracted using the MAE. The extraction process carried out with a concentration variation of 50%, 60%, 75%, which approved with a combination of staining time of 1 minute, 3 minutes, and 5 minutes.

At each concentration of *Ipomoea batatas* L. peel extract, a formulation was performed to obtain the appropriate color. 50% concentration obtained by adding (extract 0.5 gr/10 mL ethanol 96%, 4mL HCl 2N, 3mL NH_4OH 2 N), 60% concentration obtained by adding (extract 0.6 gr/10 mL ethanol 96%, 5 mL HCl 2N, 3.5 mL NH_4OH 2 N), a concentration of 75% was obtained by adding (extract 0.75 gr/10 mL ethanol 96%, 6 mL HCl 2N, 4.2 mL NH_4OH 2N).

The smear prepared from culture *Bacillus* sp. on a clean glass slide allowed it to air-dried and fixed it by flaming.

For the experimental group, at first, the smear on a glass slide was added with *Ipomoea batatas* L. extracts at each concentration for 1 minute, 3 minutes and 5 minutes. The *Ipomoea batatas* L. extract was poured off and applied lugol for 1 minute and washed with water, the smear decolourized with an organic solvent-alcohol for 30 seconds, or until the colour was oozed from the slide and washed with water.

The Safranin applied for 1-minute washed with water and blot dried.

The slide was observed under magnification 100x after putting a drop of immersion oil then the results were compared with staining using Gentian violet for 1 minute. For the control group, the first step did not use extracts but Gentian violet and the next steps as in the experimental group until the observation was processed.

The results were compared directly between the Gram staining using *Ipomoea batatas* L. peel extract tested on Gram-positive bacteria as an experimental group. Furthermore, in Gram staining using Gentian violet as a control group. The sampling technique in this study was a simple random sampling. The parameters observed in this study included visual field clarity, glass slide cleanliness, contrast, bacterial shape, and bacterial colour.

In the data analysis, the observation was results of each extract concentration of 50%, 60%, and 75% with a staining time of 1 minute, 3 minutes, and 5 minutes compared to the control, coding was carried out based on 5 observation parameters to obtain quantitative data. Then, the calculation carried out with the formula for the total number of coding values with 3 times repetition / 15 (5 parameters repeated 3 times) x 100%.

RESULTS

Ipomoea batatas L. Peel Extract Concentration of 50%, 60%, 75%

Based on observations obtained by the results of colouring where the extraction concentration of 50% showed the best results when staining for 5 minutes, the extract was inadequate to penetrate bacteria, and some bacteria were not completely stained. The extraction concentration of 60% showed the best results when staining for 5 minutes (Figure 1). The dye appears to penetrate the bacterial cell wall, but some bacteria were not completely stained. The extraction concentration of 75% showed the best results when staining for 3 minutes and 5 minutes, although some bacteria were not stained (Figure 2). In the Gentian violet stain, visible dyes penetrate the bacterial cell wall and also the spores of *Bacillus sp.* (Figure 3).

At the percentage of the staining results, at the extraction of 50% concentrations obtained staining approaching control with the results of the staining showed the optimum results when staining for 5 minutes. For extraction of a concentration of 60%, it obtained staining that approached the control with the results of the staining showed the optimum results when staining for 5 minutes. The extraction concentration of 75% attained a colour approaching the control with the results of the staining showed the optimum results when staining for 3 minutes and 5 minutes (Figure 4).

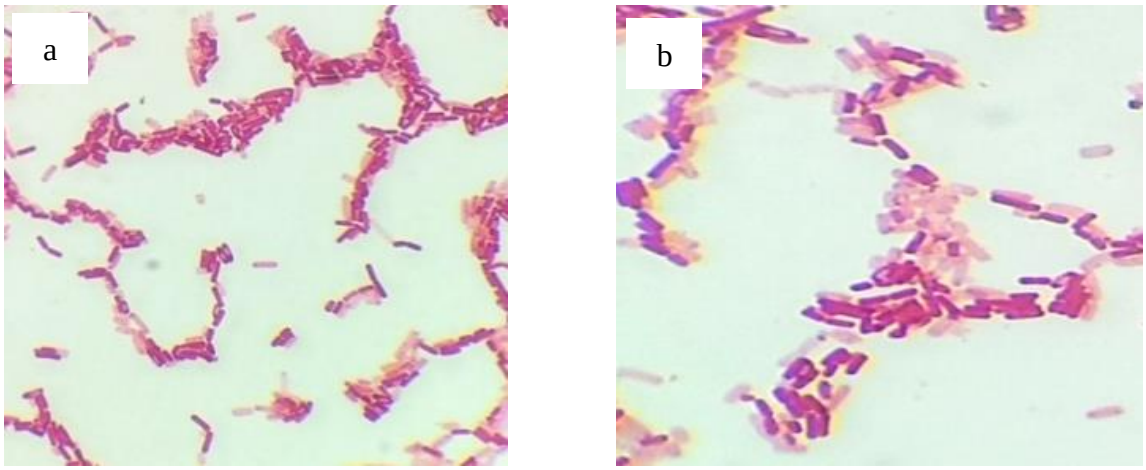

Fig 1. Microscopic observation results of *Bacillus* sp. 100x magnification with Staining of *Ipomoea batatas* L. for 5 minutes. (a) Extract Concentration of 50%, (b) Extract Concentration of 60%

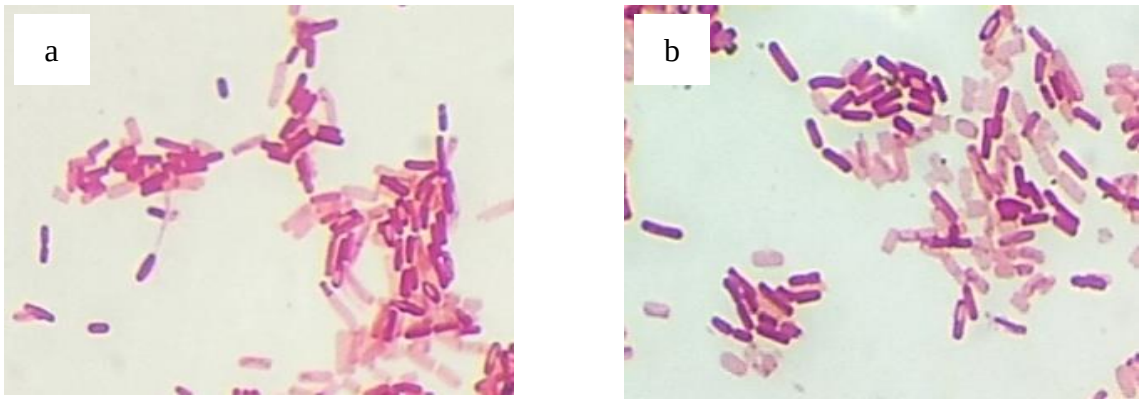

Fig 2. Microscopic observation results of *Bacillus* sp. 100x magnification with Staining of *Ipomoea batatas* L. Extract Concentration of 75%. (a) Stainding for 3 minutes, (b) Staining for 5 minutes

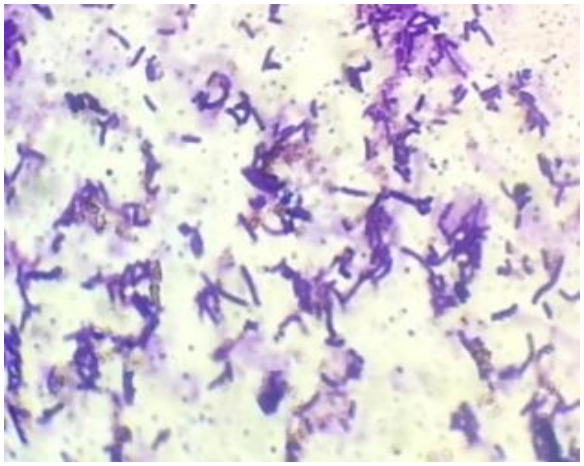

Fig 3. Microscopic Observation Results of *Bacillus* sp. 100x magnification with Gentian violet for 1 minute

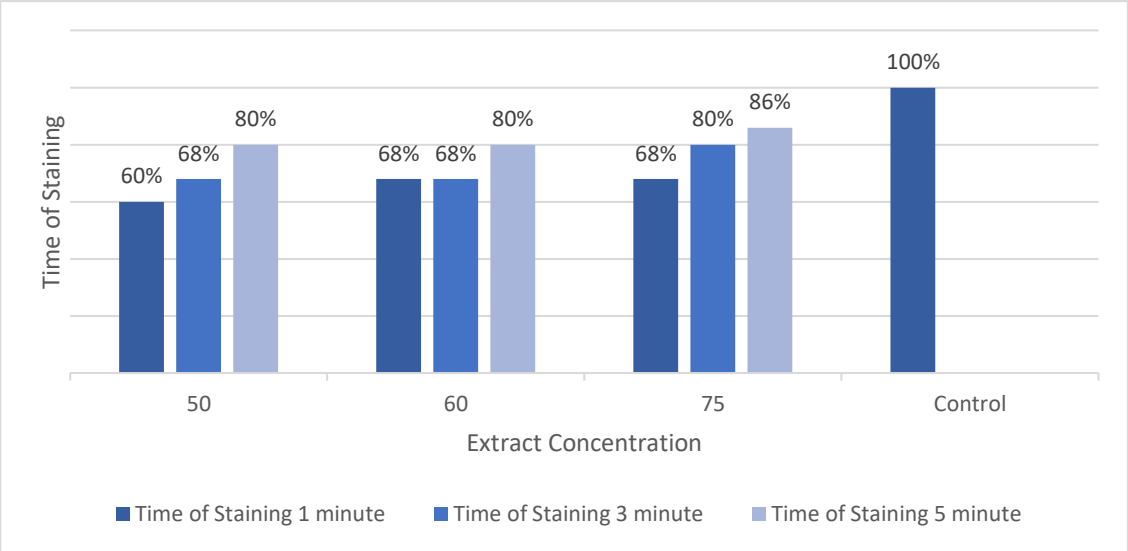

Fig 4. Comparison Percentage of Staining Results of *Ipomoea batatas* L. Extract Concentration and Time of Staining of Gentian violet

The results analyzed statistically using the one-way ANOVA test with SPSS instrument version 16.0. Where the concentration of 50% obtained α value of 0.06, a concentration of 60% obtained α value of 0.227, a concentration of 75% obtained α value of 0.297, α value of >0.05. There was no significant difference between the results of staining using *Ipomoea batatas L.* extracts and control (Gentian violet). The determination of colour tendency was using the colour matching with the RGB table, then compared with the control (Table 1).

Table 1. RGB Code for Bacterial Gram Staining (RGB Color Chart.cdr - AWS)

Bacteria	Time of Staining (minute)	RGB Code for Bacterial Gram Staining				Color Name
		Concentration of Staining			Control	
		50%	60%	75%		
Bacillus sp.	1	RGB: 180 4 133	RGB: 173 14 145	RGB: 180 4 133 – 153 0 122	RGB: 70 20 111	Purple
	3	RGB: 173 14 145	RGB: 152 4 110	RGB: 153 0 122	-	
	5	RGB: 152 4 110	RGB: 180 4 133 – 153 0 122	RGB: 141 4 123	-	

DISCUSSION

The extraction concentration of 50% showed the best results when staining for 5 minutes. The extract was inadequate to penetrate bacteria, and some bacteria were not completely stained. The extraction concentration of 60% showed the best results when staining for 5 minutes. The dye appears to penetrate the bacterial cell wall, but some bacteria were not completely stained. The extraction concentration of 75% showed the best results when staining for 3 minutes and 5 minutes, although some bacteria were not stained.

The colour produced by the *Ipomoea batatas* L. peel is purple. The colour produced by *Ipomoea batatas* L. peel is more stable under acidic conditions and the increase in pH affects the resulting colour. Besides the use of polar solvents makes it easier to dissolve the peel content of *Ipomoea batatas* L. (6).

Based on the study results of Yuniarti and Misbach (7) that *Ipomoea batatas* L. can give colour to bacteria. On microscopic observations, *Staphylococcus aureus* shaped coccus with reddish-purple. In this study, the formulation was needed as an anthocyanin proof test that was appropriate in processing *Ipomoea batatas* L., so that the colour produced under the microscope can resemble the purple colour produced by Gentian violet.

Phenolic compounds of *Ipomoea batatas* L. peel which included in the flavonoid group and the content of phenol compounds which is *Ipomoea batatas* L. peel is the same as *Gentian vielot* content. Gentian violet is a triaryl methane dye with three phenol groups arranged by methane groups. The composition contained in Gentian violet is (2 gr crystal violet, 20 mL ethanol 95%, 0.8 gr ammonium oxalate, 100 mL distilled water).

Based on a study conducted Virgianti and Lucyana (9), $KMnO_4$ used as an oxidizer when added to the solution of Angkak and teak leaves as a cover dye in Gram staining. The alleged positive charge of potassium and dye complexes forming dissociation, so that it can be bound to bacterial cell components which have a negative charge. For this reason, the process of extraction results requires the addition of ethanol to match the composition of Gentian violet. Besides, the addition of acid-base was useful to get the appropriate violet colour.

Violet colour obtained when it was at anthocyanin equilibrium, which was a quinoidal base for the addition of NH_4OH and HCl serves as a counterweight when trial and error. *Bacillus sp.* bind dyes from *Ipomoea batatas* L., after the formulation and was obtained purple colour, which comes from the reaction between HCl and NH_4OH, forming compounds NH_4Cl.

In this study, the dyes formed tend to be alkaline dyes, to maintain the purple colour of the extraction of the peel of *Ipomoea batatas* L. Alkaline dyes are substances that produce cations. The ionization of the chromogen component shows a positive charge that can bind to the cell component, which is a negative charge. The nucleic acid is a negatively charged cell component (9, it also can accept positively charged dyes such as the *Gentian violet*, or in this study, the dyes derived from the peel extract of *Ipomoea batatas* L.

Assumes that the positive charge of NH4 ions dissociates with anthocyanin dyes in the form of cyanide which has a negatively charged OH group from the results of *Ipomoea batatas* L. peel extracts. Positive charge ions resulting from dissociation with anthocyanin dyes bind with protoplasm ions of bacterial cells that have negatively charged nucleic acids in the results of the *Ipomoea batatas* L. peel extract form of phosphate groups (9). In this study, there was a control group and an experimental group. The control group *Bacillus* sp. *w*ere stained with Gentian violet, lugol, alcohol, and Safranin.

This study has limitations. The further research is needed to use *Ipomoea batatas* L. peel extract as a substitute for Gentian violet in Gram stain. The author assumes that the dye produced from the peel extract of *Ipomoea batatas* L. has not maximally penetrated the bacterial wall so that the colour of bacteria did not entirely derive from the extract. The author assumes this was coming from the residual colouring of Safranin. To minimize information bias, the authors recommend that further study on this topic, especially regarding the mechanism of alternative dyes in penetrating bacterial cell walls, so that we can find out more about the ability of alternative dyes to stain bacterial cell walls.

CONCLUSIONS

The present study demonstrated that *Ipomoea batatas* L. peel extract can be used as an alternative staining agent, but to substitute Gentian violet in Gram staining of bacteria, need conducting further studies on this topic and observe to several aspects. Besides, the matter that needs to be considered is the storage method and storage time for the extraction of *Ipomoea batatas* L. peel. The author recommended conducting further studies use the extract 100% concentration and the staining time used for 5 minutes.

CONFLICT OF INTEREST

There are no conflicts of interest.

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