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Utilization of Purple Sweet Potato Juice As an Alternative Violet Crystal in Coloring *Streptococcus sp* Bacteria and *Escherichia coli*

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ABSTRACT

Gram staining is a staining method used to distinguish bacterial species into two major groups, namely Gram-positive and Gram-negative, Crystal violet is the primary (main) dye that will colour the target microorganisms. Staining in bacteria can be replaced using alternative substances with low cost, readily available and harmless, one of which uses substances that have anthocyanin content, namely purple sweet potato. The purpose of this study was to determine the results of using purple sweet potato as an alternative substance to replace crystal violet in staining *Streptococcus sp* and *Escherichia coli* bacteria. The method used is experimental. Based on the results of research that has been done on the use of purple sweet potato juice as an alternative substance to crystal violet in gram staining of *Streptococcus sp* and *Escherichia coli* bacteria, it shows that purple sweet potato material can be used at pH 6.5 and 5.7 while at pH 5.5 and 5 the bacteria are not perfectly coloured. So it can be concluded that purple sweet potato juice can be used as an alternative substance to Crystal violet in gram staining.

INTRODUCTION

Gram stain is a staining method used to differentiate bacterial species into two major groups namely Gram-positive and Gram-negative. Danish Hans Christian Gram is the name that developed this technique. Gram-positive bacteria retain crystal violet dye while Gram-negative bacteria do not (Lutviandhitarani et al., 2015).

Crystal violet is the primary (main) dye that will color the target microorganisms. Crystal violet is acidic so that it can bind to microorganism cells that are alkaline, so transparent microorganism cells will look purple (Hidayanti et al., 2021).

Streptococcus sp is a Gram-positive bacterium (Fahmi, 2020). *Streptococcus* bacteria are divided into two types, namely type A and type B. Type A *Streptococcus* bacteria can live on the skin and throat, and are transmitted through direct contact, for example through skin touch. Type B *Streptococcus* bacteria can live in the intestines, vagina, and the end of the large intestine (rectum), and do not cause problems (Hendrik, 2006). There are triggering factors, such as age and health conditions, so that these bacteria can cause problems (Eddy & Mutiara, 2015).

Escherichia coli is a Gram-negative bacterium (Juliantina et al., 2009). This bacterium is a member of the normal intestinal flora, under certain conditions it can cause intestinal infections with symptoms of diarrhea due to its penetrating power that damages mucosal cells, the ability to produce toxins that affect fluid secretion in the intestine, and increase the attachment of germs (Bota et al., 2015).

Purple sweet potato (Ipomoea batatas poiret) can be used as an alternative natural dye because of its high anthocyanin pigment content (Misbach, 2016). The purple color in sweet potatoes is caused by the presence of anthocyanin pigments which are spread from the skin to the flesh of the tuber. The high anthocyanin content in purple sweet potatoes has high stability compared to anthocyanins from other sources. Anthocyanins are secondary metabolites of flavonoids and polyphenols that can act as antioxidants (Gunawan et al., 2016). Anthocyanin substances also have amphoteric properties, which are able to react in both acidic and basic conditions. If the acidic atmosphere will be red and in an alkaline atmosphere will be purple (Gustriani et al., 2016).

The management of purple sweet potato flesh as a substitute for the main coloring agent crystal violet can be a very useful thing and can be utilized properly. In addition, purple sweet potato raw materials are easy to obtain compared to crystal violet dyes which generally have an expensive price. Not only from the price, crystal violet dye is a flammable liquid mixture, dangerous if swallowed, causes serious eye damage, and is very toxic to aquatic life with long-term effects (Luziana & Permatasari, 2020).

The purpose of the study was to determine the results of using purple sweet potato as an alternative substance to replace crystal violet in staining *Streptococcus sp* and *Escherichia coli* bacteria.

METHOD

This research is descriptive research with an experimental approach. This study uses two variables, namely independent variables in the form of Streptococcus sp and Escherichia coli bacteria with dependent variables in the form of coloring using sweet potatoes.

The data used in this study used primary data, namely data directly obtained from observations of gram staining results. The instrument used in the study is purple sweet potato juice as an alternative to crystal violet which will then be examined macroscopically between the results of control preparations and experimental preparations and the results will be processed manually for narration.
This research was conducted in June 2021 at the Bacteriology Laboratory of STIKes Muhammadiyah Ciamis.

Work Procedure
1. Preparation of yam juice
   a. Preparation of purple sweet potato
   b. Peel the purple sweet potato and remove the skin
   c. Wash the peeled purple sweet potato thoroughly
   d. Then grate the sweet potato
   e. Squeeze the grated purple sweet potato with a planel cloth
   f. Filter using filter paper
   g. Measure pH using universal pH using pH variations
   h. Purple sweet potato juice is ready to be used as an alternative crystal violet substance (Misbach & Yuniarty, 2016).

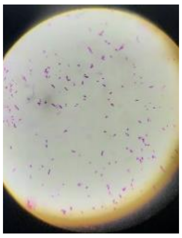
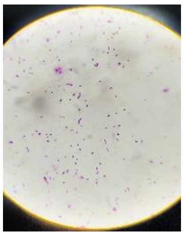
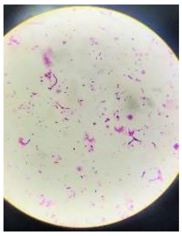
2. pH measurement
Purple sweet potato juice obtained from the previous process was measured with a pH meter and obtained a pH of 6.5 and a Crystal Violet pH of 5.7. After that, pH variation was carried out on purple sweet potato juice by decreasing the pH with the addition of Citric Acid.

3. Coloring Using Purple Sweet Potato as Alternative Crystal Violet Zalt
   a. Making preparations using pure cultures of Streptococcus sp and Escherichia coli
   b. Preparations are inundated with purple sweet potato juice for 1 minute, rinsed with running water,
   c. Then inundated with lugol's solution for 1 minute, rinsed with running water, inundated with alcohol solution for 30 seconds and inundated with carbol fuchsin solution for 1 minute.
   d. Then microscopic observations were made with 100x objective magnification.
   e. The results of observations on preparations using Crystal violet solution were compared with the results of observations on preparations using purple sweet potato juice solution (Arrachman, 2016).

RESULTS AND DISCUSSION

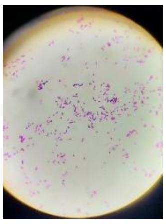
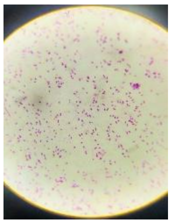
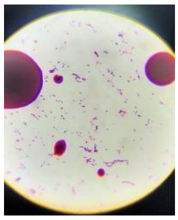
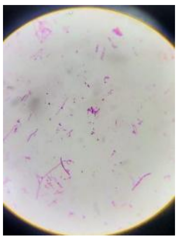
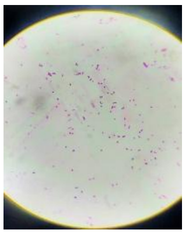
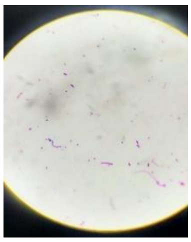
Streptococcus sp and *Escherichia coli* bacteria were used as controls by performing gram staining using Crystal violet, lugol, alcohol and carbolic fuchin. Control results were obtained:

TABLE 1. Observation Results of Control Bacteria

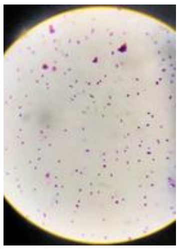
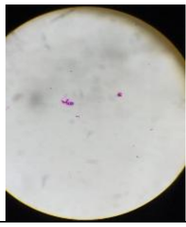
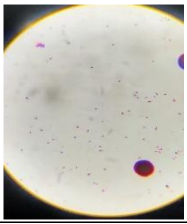
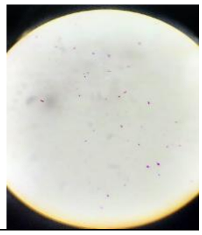
pH	Result		Figure		
			Streptococcus sp	***Escherichia coli***	***Streptococcus sp and Escherichia coli***
5,7	*Streptococcus sp :* Gram stain results coccus shape, purple, gram-positive bacteria	*Escherichia coli :* Bacillus shape gram stain results, red in color, gram-negative bacteria			

Streptococcus sp and *Escherichia coli* bacteria used for the study were identified by performing gram staining using purple sweet potato juice, lugol, alcohol and carbolic fuchin with pH variations. The following results were obtained:

Tabel 2. Microscopic Observation Results with Variation of pH

No	pH	Result		Figure		
				Streptococcus sp	***Escherichia coli***	***Streptococcus sp and Escherichia coli***
1	6,5	*Streptococcus sp* : stained bacteria (chain-shaped cocci, purple in color and gram-positive)	*Escherichia coli* : stained bacteria (bacilli-shaped, red in color and gram-negative)			
2	5,7	*Streptococcus sp* : stained bacteria (chain-shaped cocci, purple in color and gram-positive)	*Escherichia coli* : stained bacteria (bacilli-shaped, red in color and gram-negative)			

Tabel 2. Microscopic Observation Results with Variation of pH

No	pH	Result		Figure		
				Streptococcus sp	***Escherichia coli***	***Streptococcus sp and Escherichia coli***
3	5,5	*Streptococcus sp* : stained bacteria (chain-shaped cocci, purple in color and gram-positive)	*Escherichia coli* : stained bacteria (bacillus-shaped, purplish-red and gram-negative)			
4	5	*Streptococcus sp* : stained bacteria (chain-shaped cocci, red in color)	*Escherichia coli* : stained bacteria (bacilli-shaped, red in color and gram-negative)			

Based on table 2, the results of the above research show that purple sweet potato natural ingredients can be used as an alternative substance for crystal violet at pH 6.5 and 5.7, while at pH 5.5 and 5 the bacteria are not perfectly colored.

Based on the results of research on the use of purple sweet potato juice as an alternative substance to Crystal violet in gram staining of *Streptococcus sp* and *Escherichia coli* bacteria starting with the determination test. The results of the determination test show that the type of purple sweet potato is the species Ipomoea batatas (L.) poir.

In this study begins with the preparation of purple sweet potato juice as an experimental material that becomes an alternative substance in gram staining of *Streptococcus sp* and *Escherichia Coli* bacteria. Purple sweet potato juice is obtained from grated purple sweet potatoes that have been peeled and washed thoroughly and then dried.

Alternative substances in natural materials that contain anthocyanins and betacarotene can be used and those that cannot be used as a substitute for bacterial staining (Irianti & Pramono, 2022). Betacarotene contained in red fruit can be used for staining *Klebsiella pneumonia* bacteria as a substitute dye for carbol funchin in capsules, red fruit is able to color capsules because it contains high levels of beta carotene which can produce an excellent red color resembling funchin water (Fitriyah et al., 2020).

The anthocyanin content contained from the skin to the flesh of purple sweet potatoes can be an alternative substance that can color *Staphylococcus aureus* and *Bacillus sp* bacteria in gram staining instead of Crystal violet, because bacteria will bind to anthocyanin substances contained in purple sweet potatoes (Jannah et al., 2023).

Anthocyanins contained in purple sweet potatoes belong to the flavonoid group which are phenol compounds, phenol substances contained in anthocyanins as much as 4.9-6.9% so that the same as the content in Crystal violet contains phenol substances. The dye caused by this sweet potato is able to color gram-positive bacteria, because the bacteria will bind the first dye used, namely purple sweet potato juice (Marbun et al., 2020).

The color difference in gram-positive and gram-negative bacteria shows that there are differences in cell wall structure between the two types of bacteria. Gram-positive bacteria have a cell wall structure with a thick peptidoglycan content while gram-negative bacteria have a cell wall structure with high lipids (Fatisa, 2013).

In basic dyes, the role in providing color is called chromophore and has a positive charge, while in acidic dyes the part that plays a role in providing color has a negative charge (Apriani, 2016). Basic dyes are more widely used because in the cell wall, cell membrane and cytoplasm more negative charges are found, during the coloring process the positive charge on the basic dye will be related to the negative charge in the cell, so that microorganisms are more clearly visible. Acidic dyes that are negatively charged are usually not used to color microorganisms, but are usually used to color the background of coloring preparations (Padmaningrum et al., 2012). These negatively charged acidic dyes cannot be associated with the negative charge contained in the cell structure (Santoso & Estiasih, 2014). Sometimes negative dyes are used to color positively charged parts of the cell, it should be noted that the charge and binding force of the dye to the cell structure can change depending on the surrounding pH during the staining process (Rahmatullah et al., 2021).

CONCLUSIONS AND RECOMMENDATIONS

Based on the research that has been done, it can be concluded that purple sweet potato juice can be used as an alternative substance to Crystal violet.

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