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"COMBINATION POTENTIAL SOAKING TEAK LEAVES *(Tectona Grandis)* WITH HCl ON STAINING GRAM IN *Escherichia coli* BACTERIA"

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ABSTRACT

Safranin or fuchin is a dye that is widely used in the fields of industry, textiles, histology, cytology and bacteriology. Safranin has a very expensive price in 2019 reaching 3,565,000 / 25 grams. Thus, it is necessary to substitute dyes from natural and much cheaper materials. Teak leaves *(Tectona grandis)*, especially the young ones, contain the pigment pheophitin, ß-carotene, chlorophyll and two other pigments that have not been identified as well as several anthocyanin derivatives, namely, pelargonidin 3-glucoside, pelargonidin 3,7-diglucoside. Anthocyanins contained in teak leaf *(Tectona grandis)* buds are reddish brown. The presence of these pigments can be used in laboratory tests for staining bacteria. The purpose of this study was to determine the soaking variation of teak leaf *(Tectona grandis)* buds for 24 hours, 48 hours, 72 hours, 96 hours in 96% alcohol with concentrated HCl, and the use of safranin as a positive control. The data of the research was in the form of scores of observations. Based on the Kursal Wallis alpha test, it was found that there was a significant difference in the results of staining bacteria among various soaking of teak leaf *(Tectona grandis)* buds in 96% alcohol with concentrated HCl in staining gram technique. Soaking teak leaf *(Tectona grandis)* buds in 96% alcohol with HCl for 72 hours and 96 hours gave the same staining results as safranin. The results showed that soaking teak leaf *(Tectona grandis)* buds in alcohol with concentrated HCl can be used as an alternative natural dye for the method of staining Gram on *Escherichia coli* bacteria.

Keywords: *Safranin, anthocyanin, teak leaf (Tectona Grandis), Gram staining, Eschericia coli*

INTRODUCTION

In laboratory research, especially in the field of microbiology, bacterial identification is often carried out on preparations using a microscope. Identification on the preparations aims to determine the type of bacteria that grow. Microorganisms in nature have distinctive morphology, structure and properties, including bacteria. Bacteria are colorless and contrast with water, so bacterial cells must be suspended. To see and observe living bacterial cells is very difficult, to identify it requires a method, namely by using the method of painting or staining, so that it is easy to see clearly and observe. It can also serve to see its physiological properties. This bacterial cell staining technique is one of the main ways in microbiological research (Hadi, 2016). Bacteria have a transparent color, and if they are in an aqueous medium it will be difficult to see under a microscope. One way to observe small bacteria and difficult to observe, it is necessary to try staining or inserting dyes that can change the appearance from previously transparent to colored (Lindayani et al., 2016). One of the bacterial stains that is often used is Gram staining including crystal violet, safranin of carbol fucshin. *Echericia coli* is a beneficial bacteria and can be pathogenic, can be a major cause of morbidity and mortality worldwide. Methods that can identify bacteria are Gram stain, capsule, and acid-fast stain. Gram stain is a useful and most widely used differential stain in microbiology laboratories (S.A. Rahayu et al., 2017).

One of the stains that is often used is the Gram stain, which is to distinguish the types of Gram negative bacteria from Gram positive bacteria. Gram staining is based on the principle of the difference between Gram negative and positive cell walls. In the laboratory, synthetic dyes are mostly used, one of which is safranin. Safranin is a dye that is widely used in industry, textiles, histology, cytology and bacteria. However, waste from safaranin is very toxic to the human body, which can cause irritation to the mouth, throat, breathing and stomach. The price of safranin is very high and can reach 3,565,000/25gram in 2019. Therefore, a cheaper natural substitute is needed (Edyani, 2020).

One of the plants that can be used as a substitute for coloring is teak leaves (Tectona grandis). Teak leaf (Tectona grandis) is a plant that is widely cultivated in Indonesia. Teak leaves (Tectona grandis) are included in the Verbenaceae family which can be used as natural dyes because they contain anthocyanin pigments. Ati's research (2006) stated that teak adaun extract contains anthocyanin type pelagornidin as its natural pigment. Pelagornidine is an anthocyanidin pigment group, namely anthocyanin aglycones that are formed when anthocyanins are hydrolyzed with acid. This content will function as a blood red pigment in teak leaves (Tectona Grandis) (Virgianti et al., 2017).

Anthocyanins are flavonoid pigments. Flavonoid compounds are polar and can be dissolved in polar solvents such as ethanol, ethyl and ethyl acetate. Anthocyanins can be extracted using methanol and concentrated HCl 1%. However, methanol has very high toxic properties, so it is recommended to replace it with ethanol. Therefore, researchers are interested in conducting this research by making an alternative to safranin using teak (Tectona Grandis) + concentrated HCl leaf immersion against Gram staining (Surianti et al., 2019).

RESEARCH METHODS

This type of research is an experimental laboratory research that aims to determine whether there are differences in the results of bacterial staining of various lengths of soaking teak leaf extract (Tectona grandis) in alcohol with HCl. The design of this research is the Posttest Only Control Group Design .

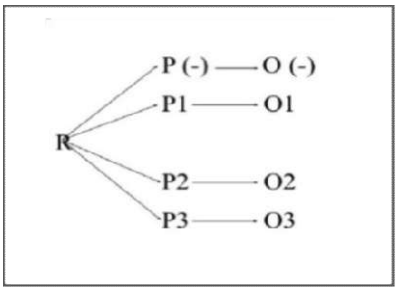


Figure 1. posttest Only Control Design (Soekidjo, 2012)

The population in this study was Escherichia coli preparations. The sample in this study were 30 preparations of Escherichia coli bacteria which were stained with teak leaf buds. So in this study the number of repetitions of each group was 6 times. Because in this study there were 5 different treatment groups, so a total of 30 preparations were used in the experiment.

The location of the research was carried out at the Microbiology Laboratory, D3 Study Program in Medical Laboratory Engineering, Faculty of Health Sciences, University of Muhammadiyah Surabaya. The time of the research was carried out on June 1, 2022 until June 30, 2022. While the examination time was on July 1, 2022.

The variables of this study were the independent variables: the duration of immersion of teak leaf buds (Tectona grandis) in alcohol+HCl. Bound Variable: Bacterial staining results. Control Variables: Method or technique of staining and concentration of dye.

a. Method of collecting data

Examination of Eschericia colii preparations stained with teak leaf bud staining (Tectona Grandis) was carried out using the Gram staining technique. Gram staining is a staining that uses a solution of crystal violet, lugo, 96% alcohol, and safranin or carbol fuchin which is poured in the last stage over the entire surface of the preparation (Kurniawati, 2005).

b. Tools and materials

The tools used are beaker glass, funnel, filter, measuring flask, chocolate bottle, scale, watch glass, measuring pipette, and measuring cup. The materials used in the study were 100g teak leaves, 99ml 96% alcohol + 1ml concentrated HCl, crystal violet, lugol, 96% alcohol, safranin, and Ecoli bacteria.

c. Inspection Method

Methods Examination of Eschericia colii preparations stained with teak leaf bud staining (Tectona Grandis) was carried out using the Gram staining technique. Gram staining is a staining that uses a solution of crystal violet, lugo, 96% alcohol, and safranin or carbol fuchin which is poured in the last stage over the entire surface. preparation (Kurniawati, 2005).

d. Inspection procedure

Making Teak Leaf Coloring (Tectona Grandis) Preparation of tools and materials needed.Alat : Gelas ukur, pipet ukur, pushball, aluminium foil, botol coklat. Bahan : Alkohol 96%, HCl pekat.

1) Weigh the teak leaf buds using a scale of 100 g.
2) Dissolve in a beaker glass that has been added 96% alcohol which has been measured with a measuring cup as much as 99ml.
3) Add 1 ml of concentrated HCl.
4) Soak the teak leaf buds in a glass bottle that has been dissolved according to the predetermined soaking time, namely 24 hours, 48 hours, 72 hours, 96 hours.
5) Waiting for teak leaf bud dye until the soaking time is according to the predetermined, dye.
6) The teak leaf buds are squeezed with a coffee squeezer.
7) Teak leaf bud dye is ready to use.

d. Data Analysis Techniques

The data analysis technique used in this study was the Kruskal Wallis test with an error rate of 5% to test differences in the staining results of Escherichia coli preparations on various variations in the length of time for soaking teak (Tectona Grandes) leaves in alcohol

+ HCl.

RESULT AND DISCUSSION

The results of gram staining using teak leaf bud soak (Tectona Grandis) + HCl which has been carried out at the Microbiology Laboratory of the D3 ATLM Study Program which was held in June 2022. The results of the staining of teak leaves are as follows:

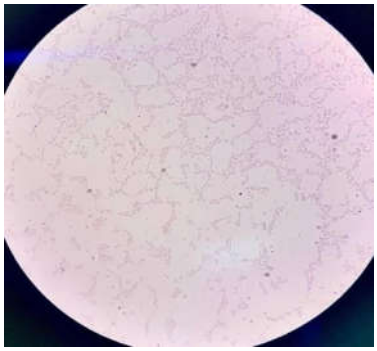
Score 1: The cell walls of E-coli bacteria are pink, the cells are clearly visible and there are no clusters of color when viewed under a microscope.

Score 2: The cell walls of E-coli bacteria are pink, the cells are clearly visible and there are clusters of color when viewed under a microscope.

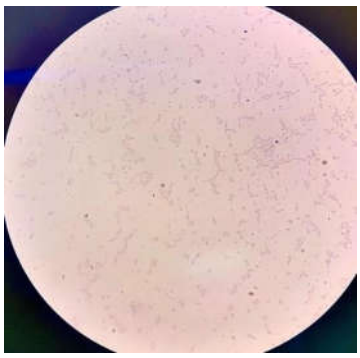
Score 3: The cell walls of E-coli bacteria are pale (+) cells are clearly visible, the color is not clustered when viewed under a microscope.

Score 4: The cell walls of E-coli bacteria are paler in color (++), the cells are clearly visible and there are clusters of color when viewed under a microscope**.**

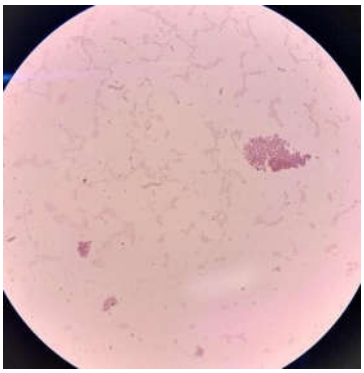
This research was conducted to test whether soaking teak (Tectona Grandis) leaves with the addition of 1 ml of concentrated HCl can be a substitute for safranin or fuchin staining in Ecsherichia coli preparations with immersion time of 24 hours, 48 hours, 72 hours, 96 hours and positive control using safranin or fuchin.

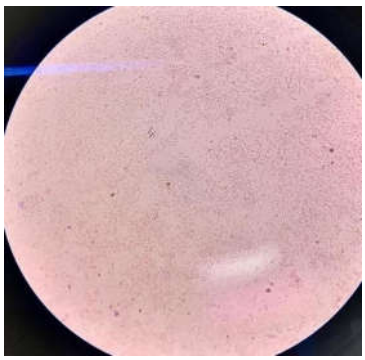


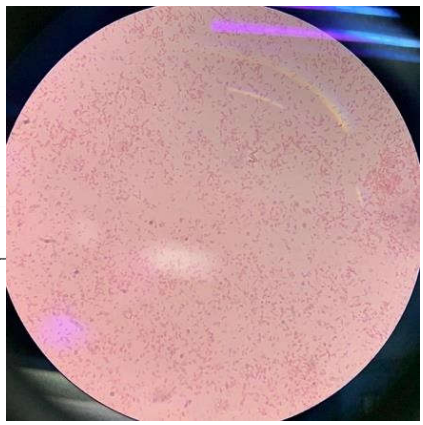
24 hours



48 hours







The results of staining of Escherichia coli bacteria with a soaking time of 24 hours are at a score of 3, where a score of 3 indicates that the cell walls of Escherichia coli bacteria are pale red and there are no clusters of color when viewed under a microscope. This proves that soaking teak (Tectona Grandis) leaves for 24 hours has not been able to color Escherichia coli optimally. The results of staining of Escherichia coli bacteria with a duration of immersion of 48 hours are at a score of 3, where a score of 3 indicates that the cell walls of Escherichia coli bacteria are pale red and there are no clusters of color when viewed under a microscope. This proves that soaking teak (Tectona Grandis) leaves for 48 hours has not been able to color Escherichia coli optimally.

The results of staining of Escherichia coli bacteria with a soaking time of 72 hours are scored 1 and 2, where a score of 1 indicates that the cell wall of Escherichia coli bacteria is red

and there are no clusters of color when viewed under a microscope and a score of 2 indicates that the cell wall of bacteria Escherichia coli is red and has clustered colors, this proves that soaking teak (Tectona Grandis) leaves for 72 hours is good enough to color Escherichia coli.

The results of staining of Escherichia coli bacteria with a soaking time of 96 hours are at a score of 1, where a score of 1 indicates that the cell walls of Escherichia coli bacteria are red and there are no clusters of color when viewed under a microscope. This proves that soaking teak (Tectona Grandis) leaves for 96 hours is highly recommended as an alternative to safranin or fuchin for staining Escherichia coli bacteria.

The results of this study indicate that the duration of immersion of teak leaf buds (Tectona Grandis) in 96% alcohol + concentrated HCl has varied results. Where the longest immersion of 96 hours showed the best and significant results with the results of safranin or fuchin staining, this was due to the immersion of teak leaf buds (Tectona Grandis) in 96% alcohol + HCl for 96 hours was able to dissolve quite a lot of crystal violet color. Based on previous research, soaking teak (Tectona Grandis) leaves with only 96% alcohol produced a less concentrated anthocyanin color because anthocyanins can dissolve in acidic conditions and are unstable in neutral or alkaline conditions.

Safranin is an alkaline and strong chloride and dye. This safranin substance will color very well if the tissue is fixed with a flaming solution. Safranin is also used in secondary schools in practical activities, especially as a dye in the observation of the mitotic phase in onion roots. Although safranin is often used in practical activities, there are obstacles faced by schools to obtain safranin, namely the price of safranin is expensive, easily damaged, and difficult to store. In addition to the high price, safranin also has other drawbacks such as not easy to use and very slow in the coloring process. Due to the limitations of schools in obtaining safranin and the weakness of safranin, alternative dyes from plants that have the same function as safranin are needed, namely by using young teak leaves (Tectona grandis) and guava leaves (Annacardium occidentale L.) (Wahyuni, 2010).

Safranin dye in previous studies showed that young teak leaf extract can also be used as an alternative dye in preparations. This is because there is a fairly high anthocyanin content in young teak leaves which functions as a coloring pigment. Different types of solvents and immersion time have an effect on color absorption in plant tissues. The quality of the preparations produced is quite good. As for the color contrast of 14% citric acid and 96% ethanol solvents, the results were different but both were good. Young teak leaf extract with 96% ethanol solvent type at 26 hours of immersion has the best quality because the color

produced is very close to the synthetic dye, namely safranin (Elayanti, 2018).

This study is in accordance with previous studies, namely by making teak leaf buds (Tectona Grandis) it requires as much as 100 grams of teak leaf buds (Tectona Grandis) soaked in 96% alcohol + 1ml concentrated HCl. Teak leaves (Tectona grandis) with the criteria of leaves in the bud to the third node of the stem, either leaves that are still curled or that have opened and are reddish green. Then the teak leaves are cut into small pieces and weighed as much as 100 grams. Then put into a beaker, add 99 ml of 96% alcohol and add 1 ml of concentrated HCl. Soaked for 24 hours then filtered using filter paper. And stored in a cola bottle (Hastuti & Haryatmi, 2021). The color of anthocyanins depends on the structure and acidity. At very acidic pH (1-2) the dominant form of anthocyanins is the flavilium cation. In this form, the anthocyanin condition is the most stable and the most colorful. When the pH increases above 4, anthocyanins take the form of yellow chalcones, blue quinoids and colorless carbinol bases (Sulistiawati et al., 2017).

Gram staining on Escherichia coli bacteria showed that the bacteria were short bacilli and red in color after the staining process. This is due to the lipid concentration and thickness of the peptidoglycan layer on the cell wall. This means that Escherichia coli is a Gram negative bacterium. In Gram-negative cells, alcohol can increase the porosity of the cell wall by dissolving the lipid which is the outer layer. So the crystal violet dye can be more easily removed from the thick peptidoglycan layer. Therefore, alcohol washing aids in the release of unbound crystal violet. Which makes the cells lose the crystal violet dye. Because only the negative bacterial cells will lose the dye from crystal violet, so the cells that absorb are the counter color. So, soaking teak (Tectona Grandis) leaves with the addition of concentrated HCl will affect the stability of anthocyanin pigments. Because when the dye is in an acidic condition, it will produce a lot of extract from soaked teak leaves (Tectona grandis) + concentrated HCl. One of the factors affecting the stability of anthocyanins is pH. So the soaking of teak (Tectona Grandis) + HCl leaves does not affect the lipid solubility of Escherichia coli bacteria (S. A. Rahayu et al., 2017).

When carrying out the staining process on Eschericia coli bacteria Gram staining technique using teak leaf buds (Tectona Grandis) there are several obstacles to determining the optimal solvent when used as a solvent for soaking teak leaf buds, but from various experiments and sufficient references this research can be completed with good. The development of natural dyes in bacteria is currently still low, therefore innovation is needed to make alternative dyes from natural materials such as teak leaf plants (Tectona grandis).

Because teak leaves are plants that are cultivated in Indonesia (Hermayati et al., 2015). Teak leaves (Tectona Gandis) are included in the Verbenaceae family which can be used as natural dyes because they contain anthocyanin pigments (Virgianti et al., 2017)

Through the results of this study, the researchers found that teak (Tectona Grandis) leaves were able to stain Escherichia coli bacteria using the Gram staining technique. So to overcome the problem of the price of safranin or expensive synthetic dyes, the solution is to replace it with alternative dyes from plants that have high anthocyanin levels. Researchers also hope that this research can be useful for education and the community about the use of plants that are around us and increase knowledge about bacteria.

CONCLUSION

Based on this study, it was concluded: There was a significant difference in the results of bacterial staining between various variations in the duration of immersion of teak (Tectona grandis) leaf buds in 96% alcohol and concentrated HCl stained using gram staining technique. The results of bacterial staining on the gram staining technique between the treatment of soaking teak leaf buds (Tectona Grandis) in 96% alcohol with concentrated HCl for 96 hours were not different from that of safranin or fuchin.

For Further Researchers Can conduct further research using other methods, namely the extraction method, or used as a substitute for safranin or fuchsin so that it can be Gram staining. For Laboratory Practitioners Can add knowledge and insight and can be applied in areas that still often have difficulty in obtaining colors such as safranin or fuchsin. For Educational Institutions Can add references and information regarding the use of teak leaf bud soak (Tectona Grandis) as an alternative to safranin or fuchsin dyes in Gram staining.

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