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Innovation of extract (*Lawsonia Inermis L*) as alternative dye for *Escherichia Coli* bacterial staining

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Abstract

Escherichia coli (E.coli) is a group of gram-negative bacteria that are part of the normal flora in the body. Under certain conditions, these bacteria can be pathogenic by producing enterotoxins that can cause serious infections such as diarrhea. Establishing the diagnosis of infection due to *E.coli* bacteria, namely through laboratory tests by identifying the bacteria through the bacterial staining method. So far, the most widely used bacterial dyes are synthetic dyes such as safranin. Safranin is a dye in Gram stain which can be carcinogenic and in the long run has a negative impact on health. To overcome this problem, it is necessary to innovate natural dyes that can be used as alternative dyes. Leaves of henna nails (*Lawsonia inermis* L.) is one of the natural ingredients which has an orange-orange lawsone pigment that is able to color the cell walls of gram-negative bacteria such as *E.coli*. The purpose of this study was to determine the ability of henna leaf extract (*Lawsonia inermis* L.) as an alternative to safranin staining. This research is a laboratory experiment. The experimental group was stained with henna leaf extract, using ethanol solvent concentrations of 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml. The control group used safranin dye. All research groups were carried out 3 repetitions. The parameters used were the clarity of the visual field, the shape of the bacteria, the cleanliness of the preparation and the color of the bacteria compared to the safranin control. The results showed that henna leaf extract was effective enough to be used as a coloring agent to replace safranin because it can color *E.coli* bacteria well.

Keywords:Bacterial identification, Safranin, Escherichia coli, Lawsonia inermis L

Introduction

Escherichia coli (E.coli) is a Gram negative bacterium, coccobacillus with size 2.4 x 0.4 -0.7 µm, has petriticus flagella so it is motile, and cannot form spores. This bacterium belongs to the normal human flora, but under certain conditions it can cause serious illnesses such as

hemolytic uremic syndrome (HUS), hemorrhagic colitis (HC), food poisoning, and diarrhea (Hemeg, 2018). One example of an infection caused by e.coli is diarrhea. The incidence of diarrhea in toddlers in the city of Padang in 2019 was 3648 cases (Padang City Health Service, 2019). In order for a diagnosis of disease infection caused by E. coli bacteria to be made, a laboratory examination must be carried out. The method used to identify bacteria in the laboratory is the staining method.

Gram staining is a staining method that is often used to differentiate Gram Positive or Gram Negative bacteria. The principle of gram staining is that there are differences in the physical and chemical properties of the cell walls (Nainggolan et al 2019). In gram staining, four reagents are needed, namely the main dye (gentian violet), Lugol, alcohol, and a counter dye (safranin). Safranin functions to bind gram-negative bacteria, producing a red color in the bacteria (Yusdiani, 2016). E.coli is a gram-negative rod-shaped bacteria that is red when seen from gram staining. So far, the bacterial dyes that are widely used are synthetic dyes such as safranin. Safranin is a dye that is widely used in industry, textiles, histology, cytology and bacteria (Malekbala et al, 2017). Long-term use of safranin can have an impact on health because it is carcinogenic. Coloring substances are considered safe if the arsenic content cannot be more than 0.00014% and not more than 0.001%. Apart from that, the price of safranin in 2022 will reach Rp. 3,565,000/ 25 grams and safranin is also easily damaged in storage (Chowdhury et al, 2017).

To overcome this problem, it is necessary to innovate alternative dyes from cheaper natural ingredients. Pigments in natural dyes are safer to use even though their stability to light, heat and acidity is uncertain (Kwartiningsih et al, 2016). One type of plant that can be used as an alternative to coloring from natural ingredients is henna leaves (Lawsonia inermis L) (Hafiz, 2017). The leaves of henna nails (Lawsonia Inermis L) contain a dye lawsone (2-hydry-1,4-naphtaquinone) with a concentration of 1.0-4.0% which gives a yellow-orange or orange color (Pujilestari, 2015). Supriningrum, et al, 2018 explained that henna nail leaves can be used as a bacterial stain.

The lawsone dye in nail henna (Lawsonia Inermis L) is a phenolic compound and is included in the protein group which provides good coloring capabilities. The leaves have a variety of dye substances, namely red, burgundy (burgundy), dark yellow, reddish brown to brown (Setiana, 2015). Nail henna leaves have high anthocyanin levels, namely 500g/L. Apart from that, henna nail (Lawsonia Inermis L) leaves have not been widely used in the laboratory because they are still considered an ornamental plant (Ratnawati et al, 2018). Based on the problem above, henna nail leaves (Lawsonia Inermis L) are thought to have potential as an alternative dye to replace safranin for staining bacteria, so research needs to be carried out with the title "Innovation of Henna Kuku Leaf Extract (Lawsonia inermis L) as an alternative dye to Safranin for staining Escherichia coli bacteria.

Literature Review

Escherichia coli is a normal flora bacteria that is often found in the human intestine. It is unique because it can cause primary infections such as diarrhea. Escherichia coli or E.coli is a Gram-negative bacteria belonging to the Enterobacteriaceae family, which is present in

the human body. They move using flagella and are short rods or commonly called coccobacilli (Radji, 2011).

Escherichia coli belongs to the Enterobacteriaceae family. E. coli is a short rod-shaped gram-negative bacterium or often called coccobacillus. This bacterium (Figure 2.1) has a flagellum, which measures 0.4-0.7 μm x 1.4 μm and has a loop (Radji, 2011). E. coli is about 2 μm long, 0.7 μm in diameter, 0.4-0.7 μm wide, and is a facultative anaerobe. And forms round, convex and smooth colonies with distinct edges (Hidayati et al, 2016).

Lawsonia inermis L contains tannins which can prevent the injured skin layer from being attacked by bacteria which will form new tissue on the injured skin. Henna leaf extract also has astringent properties which can reduce wounds on the skin. Phytochemical screening carried out on henna leaf extract also contains glycosides, phytosterols, tannins, flavonoids and curcumin. The methanol extract of henna leaves has the potential for high biological activity, due to the presence of nophtoquinone in the extract (Luftinor, 2017).

The henna plant produces a reddish yellow molecule called lawsone (2-hydroxy-1:4-napthoquinone). This molecule has the ability to bind proteins so it can be used to color skin, hair, nails, silk and wool. This compound is a phenolic compound which is included in the nophtoquinone content in the extract. a group of proteins that have good coloring abilities. Phytochemical screening carried out on henna leaf extract found that it contained glycosides, phytosterols, steroids, tannins and flavonoids (Raja and Ovais, 2013).

Research Method

This research will be carried out in June-August 2023 at the Stikes Syedza Saintika Microbiology Laboratory. The population in this study was the extract (Lawsonia inermis L) which was found around the Stikes Syedza Scientika campus. While the samples in the study were 3 kg of henna leaves and pure cultures of E. Coli bacteria. This research is a laboratory experiment. The staining experimental group was carried out with henna nail leaf extract, using ethanol solvent concentrations of 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml. The control group used safranin dye. All research groups were carried out 3 repetitions. The parameters used were clarity of the visual field, shape of the bacteria, cleanliness of the preparation and color of the bacteria compared using the safranin control.

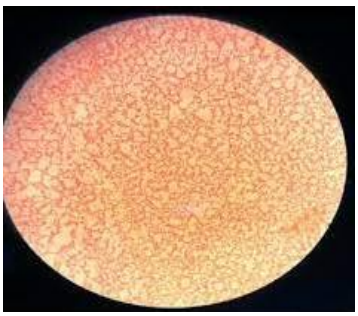
Result

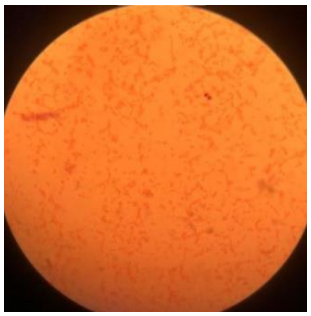
The innovative use of (Lawsonia inermis L) leaf extract as an alternative coloring agent for gram staining was carried out in the Stikes Syedza Saintika microbiology laboratory. This experiment was carried out using henna nail leaf extract using a maceration technique.

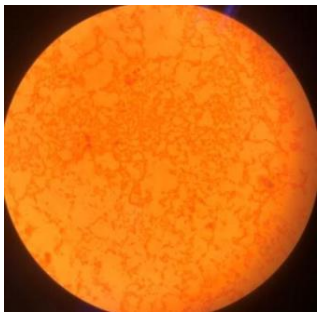
Table 1. Results of microscopic observations of staining with leaf extract against Eschericia Coli bacteria

Bacteria	**Extract Concentration**			
	25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
Eschericia Coli	Red Bacil	Red Bacil	Red Bacil	Red Bacil

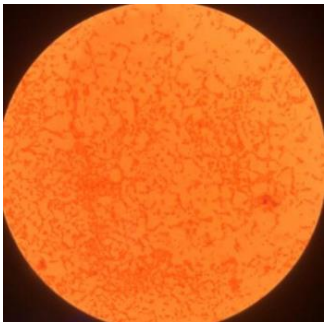
Based on table 1, the results of observations of staining of Eschericia Coli bacteria using henna leaf extract were viewed under a microscope at concentrations of 25 mg/ml, 50 mg/ml, 75 mg/ml, and 100 mg/ml, the results obtained were bacteria in the form of red bacilli and there were differences which in each concentration is at a concentration of 100 mg/ml. The following is a picture of the results of observations under a microscope using control and experiments using henna nail leaf extract.

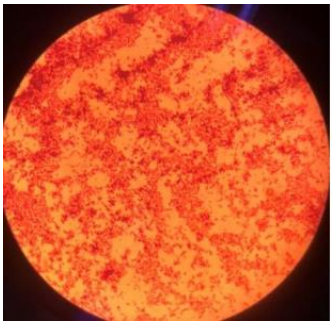






(a) Control + (b) Concentration 25 mg/ml (c) Concentration 50 mg/ml





(d) Concentration 75mg/ml (e) Concentration 100 mg/ml

Discussion

In this study, the sample used was henna leaf extract (Lawsonia Inermis L) containing a dye lawone (2-hydry-1,4-naphtaquinone) with a concentration of 1.0-4.0% which gives an orange or yellow color. as a bacterial stain (Supriningrum, et al, 2018). In this research, it was started by making henna leaf extract as an alternative dye to replace safranin in staining gram-negative bacteria, namely Eschericia coli. Controls and experiments were subjected to the same treatment, namely using pure Escherichia coli cultures, preparations were made which were then individually stained, control preparations were stained with gentian violet, Lugol, 96% alcohol, and safranin. While the experimental preparations were stained using

henna leaf extract which had been divided into several concentrations including 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml, gentian violet, lugol, 96% alcohol. The staining results are then viewed with a microscope with 100x magnification. The results of bacterial staining using henna nail leaf extract were significant, namely at a concentration of 100 mg/ml.

The characteristics of the Escherichia coli bacteria in the form of gram-negative bacilli are where these bacteria bind to the covering dye (safranin) so that it is red. Based on the results of the picture above, the results of staining of Escherichia coli bacteria preparations in the form of bacilli or rods are red in color because the bacteria bind to the covering dye, namely safranin (Rahayu et al, 2018). The stability of lawsone dye can also affect coloring. The stability of lawsone dyes is influenced by several factors, including pH, temperature, light and oxygen which can also damage the color of lawsone dyes during the maceration process (Herwin et al, 2022).

Lawsone dye in henna (Lawsonia Inermis L) is a phenolic compound and belongs to the protein group which provides good staining ability. The leaves have a variety of color substances, namely red, burgundy (wine red), dark yellow, reddish brown to brown (Setiana, 2015). Henna kuku leaves have high anthocyanin levels, namely 500g/L. Apart from that, henna nail leaves (Lawsonia Inermis L) have not been widely used in the laboratory because they are still considered an ornamental plant (Ratnawati et al, 2018). The results of the study using a Systematic Review of literature studies on 10 journals concluded that the most effective natural coloring material used as a substitute for safranin in Gram Negative Staining was henna nail leaves (Lawsonia inermis L) (Edyani, 2020). Henna leaf extract can be used as a substitute for safranin which is commonly used in Gram staining reactions (Hafiz et al, 2018).

Conclusion

Based on the results of research that has been done using extract (Lawsonia inermis L) is effective and can be used as a substitute for the main dye in gram staining, so it can be concluded that henna leaf extract can be used as an alternative to the main dye, namely safranin in gram staining.

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