Antibacterial activity of pasteurized milk supplemented with binahong leaf extract (*Anredera cordifolia* (Ten) Steenis) and sukrose toward *Escherichia coli* and *Staphylococcus aureus*

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Abstract. The functional quality of pasteurized milk need to be improved by giving natural ingredients such as the addition of binahong leaf extract and sucrose. The purpose of this study was to determine the concentration of binahong leaf extract and the appropriate sucrose on the antibacterial activity of *Escherichia coli* and *Staphylococcus aureus*. This study uses a completely randomized design (CRD) factorial pattern with 2 (two) factors. The first factor (A) is the concentration of Binahong leaf extract (0%, 4%, 5%, 6%) and the second factor (B) is the concentration of sucrose (0%, 5%, 7%, 9%) of each treatment. repeated 3 times. Antibacterial activity of binahong leaf extract significantly affected the inhibition of *Escherichia coli* and *Staphylococcus aureus*. Inhibition of pasteurized milk against *Escherichia coli* and *Staphylococcus aureus* was not affected by sucrose antibacterial activity and the intrusion between the two treatments (binahong leaf extract and sucrose). Binahong leaf extract concentration of 5-6% can increase inhibition of *Escherichia coli* and *Staphylococcus aureus*.

1. Introduction

Pasteurized milk is a dairy product that undergoes a heating process at a certain temperature and time, where the heating function is to kill all pathogenic microorganism contained in milk without affecting the physical and chemical properties of milk. In the conventional pasteurized process, the temperature cannot be controlled properly so that the milk produced is susceptible to bacterial contamination. Therefore, it is necessary to improve the quality of milk added with natural ingredients, the purpose of which is to improve the functional properties of pasteurized milk such as binahong leaf extract is an alternative to improve the nature of pasteurized milk.

Binahong (*Anredera cordifolia* Ten. Steenis) is a potential medicinal plant that can overcome certain types of diseases. Antimicrobial compounds found in binahong leaves are flavonoids, saponins, alkaloids, terpenoids and essential oils that can inhibit pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus* [1,2,3]. The addition of binahong leaf extract into pasteurized milk can provide a defense effect from contamination of microorganisms that are resistant to pasteurization temperature heating. In addition this product has antimicrobial compounds against certain pathogenic bacteria.

The process of pasteurized milk binahong leaf extract needs to study the amount of addition of sucrose (sugar). Sucrose is known to be able to minimize the flavor of the binahong extract to improve
texture and can prevent the growth of microorganisms. Determining the concentration of sucrose is needed in determining the taste and liking of consumers. The purpose of this study is to find out the appropriate concentration of Binahong leaf extract and sucrose in relation toward antibacterial activity Escherichia coli and Staphylococcus aureus.

2. Materials and methods

The materials which is used in this study are constituted fullcream powder milk (SSR) 10%, binahong leaf extract (Anredera cordifolia (Ten) Steenis), bacteria Escherichia coli, bacteria Staphylococcus aureus, Tryptone Soya Broth (TSB), Nutrient Agar (NA), Muller Hilton Agar (MHA), sucrose, alcohol 70%, distilled water, and tissue.

This research used a completely randomized design (CRD) factorial pattern 4 x 4 with 3 replications, consisting with two (2) factors. The first Factor (A) was the concentration of Binahong leaf extract (0%, 4%, 5%, 6%), while the second factor (B) was the concentration of sucrose (0%, 5%, 7%, 9%).

2.1 Research procedure

The selected binahong leaves are washed and then dried at room temperature. The dried binahong leaves were weighed and added to aquadest. This immersion is done for 5 days. Furthermore, immersion results are used in making pasteurized milk [4](Salikin, et al., 2014).

Pasteurized milk is made from 10% reconstituted milk. Powdered milk was added with concentration of binahong extract 0%, 4%, 5% and 6%. Furthermore, the solution mixture was added with a concentration of sucrose (sugar) as much as 0%, 3%, 5% and 7% then pasteurized with the HTST method (± 72°C for 15 seconds)[5].

2.2 Parameters measured

The parameters measured in this study were the testing of the antibacterial activity toward Escherichia coli and Staphylococcus aureus [6,7].

2.3 Data analysis

The data obtained in this study were analyzed by analysis of variance according to factorial completely randomized design (CRD). If the treatment has significant effect, further testing is performed using the Duncan test. Data is processed using the SPSS 16 program.

3. Result and discussion

The results of variance analysis showed that the addition concentration of binahong leaf extract had a significant effect (P <0.05) on the inhibition of Escherichia coli and Staphylococcus aureus while the sucrose antibacterial activity and between the two treatments (binahong leaf extract and sucrose) had no significant effect (P > 0.05) on the inhibition of Escherichia coli (table 1).

| Table 1. Antibacterial activity of pasteurized milk with a concentration of binahong leaf extract and concentration of sucrose showed inhibition of Escherichia coli and Staphylococcus aureus. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Inhibitory zone (mm) | Concentration of binahong leaf extract | Concentration of sucrose (sugar) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Escherichia coli | 2.48±1.10a | 3.40±1.58a,b,c | 4.01±1.93a,b,c | 4.50±1.93a,b,c | 3.17±1.82a | 3.40±1.97a | 3.19±1.19a,b,c | 4.63±1.70a,b,c |
| Staphylococcus aureus | 10.55±1.73a,b,c | 12.11±1.17a,b,c | 13.27±1.48a,b,c | 14.94±1.22a,b,c | 12.55±2.92a,b,c | 12.62±2.12a,b,c | 12.60±2.06a,b,c | 13.09±2.12a,b,c |

Description : Different superscripts in the same row and concentration show significant differences (P<0.05)

This study shows that the addition of binahong leaf extract, sucrose and the interaction of both treatments (concentration of binahong leaf extract, and sucrose) in different concentration can improve
the quality of pasteurized milk such as increased inhibition of *Escherichia coli* and *Staphylococcus aureus*.

The inhibitory zone showed growth barriers to indicator bacteria (*Escherichia coli* and *Staphylococcus aureus*) due to antibacterial activity from both binahong leaf extract and sucrose.

Pasteurized milk without the addition of binahong leaf extract and sucrose has inhibitory ability against *Escherichia coli* and *Staphylococcus aureus*. Milk contains an enzyme that functions as an antibacterial so it can inhibit the growth of certain bacteria. [8] said that milk contains bioactive components, one of which functions as an antimicrobial agent. Some of these bioactive components are the enzyme lactoperoxidase (LPO) and lactoferrin (LF). Both of these enzymes play an active role in inhibiting microbial growth in milk.

The higher concentration of binahong leaf extract used in pasteurized milk the greater inhibition activity are produced. Binahong leaves have antibacterial compounds such as flavonoids, saponins, alkaloids, terpenoids and essential oils. These antibacterial compounds play a role in inhibiting *Staphylococcus aureus*. [1,2,3] said that the components of antimicrobial compounds found in binahong leaves are flavonoids, saponins, alkaloids, terpenoids and essential oils. These components play a role in inhibiting bacterial growth by damaging the permeability of bacterial cell walls.

Using sucrose in manufacture the pasteurized milk can inhibit the growth of *Escherichia coli* with inhibitory zones of 1.07-3.79 mm. Sucrose can also inhibit the growth of *Staphylococcus aureus*. The amount of inhibition against *Staphylococcus aureus* shown by the inhibitory zone due to sucrose 9.54-11.47 mm. Sucrose can inhibit the growth of *Staphylococcus aureus*. [9] said that sucrose at certain concentrations can prevent the growth of microorganism.

The broad of the inhibitory zone from the addition of binahong leaf extract and sucrose in inhibition of *Escherichia coli*‘s growth was in the weak to moderate category of 0.15 mm to 7.79 mm while the inhibition of *Staphylococcus aureus* was in the moderate to strong category of 6.64 mm up to 16.72 mm depending on the antibacterial that works. According to [10], the criteria for antibacterial power strength are as follows: 5 mm inhibition zone diameter or less categorized as weak, 5-10 mm inhibition zones categorized as moderate, 10-20 mm inhibition zones are categorized as strong and 20 mm inhibition zones are categorized very strong.

4. Conclusions and recommendations

The concentration of binahong leaf extract and sucrose can improve the quality of pasteurized milk such as an increase in inhibition of *Escherichia coli* and *Staphylococcus aureus*. The concentration of binahong leaf extract used in making pasteurized milk should be 6% and 9% sucrose because at this concentration pasteurized reconstituted milk can inhibit the growth of pathogenic bacteria (*Escherichia coli* and *Staphylococcus aureus*).

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