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Antibacterial Activity of *Staphylococcus capitis, Bacillus cereus, Pantoea dispersa* From Telang Flower (*Clitoria ternatea* L.) Kombucha Bath Soap as a Pharmaceutical Biotechnology Product

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Abstract

Telang lower kombucha is a probiotic drink that has the potential to enhance the immune system, active cosmetic ingredients, and its waste has been proven to be used as an organic liquid fertilizer preparation. The purpose of this study was to make a formulation and preparation of liquid bath soap with an active ingredient in a solution of seagrass kombucha fermentation at a sugar concentration of 20%, 30%, and 40% in inhibiting the growth of S. capitis, B. cereus, and P. dispersa bacteria. via the disc diffusion method. The results of the study have proven that based on the post hoc test analysis, kombucha bath soap at a concentration of 40% is significantly different from the concentration of 20% and 30% but not significantly different from the positive control and the concentration of 40% is the best concentration in inhibiting the growth of the three test bacteria. compared with the treatment and the two comparisons.

Abstrak

Kombucha bunga telang merupakan minuman probiotik yang memiliki potensi sebagai peningkat sistem kekebalan tubuh, bahan aktif kosmetik, dan limbahnya telah terbukti dapat dimanfaatkan sebagai sediaan pupuk cair organik. Tujuan dari penelitian ini adalah untuk membuat formulasi dan sediaan sabun mandi cair yang berbahan aktif larutan fermentasi kombucha bunga telang pada konsentrasi gula sebesar 20%, 30%, dan 40% dalam menghambat pertumbuhan bakteri S. capitis, B. cereus, dan P. dispersa melalui metode difusi cakram. Hasil penelitian telah membuktikan berdasarkan analis uji pos hoc adalah sediaan sabun mandi kombucha bunga telang pada konsentrasi 40% berbeda nyata dengan konsentrasi 20%, dan 30% namun tidak berbeda nyata dengan kontrol positif serta konsentrasi 40% merupakan konsentrasi terbaik dalam menghambat ketiga pertumbuhan bakteri uji dibandingkan dengan perlakuan dan kedua pembandingnya.

Keywords: *Body Wash, Kombucha, Telang Flower*

Kata kunci: Sabun Mandi, Kombucha, Bunga Telang.

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INTRODUCTION

We have entered an era where the era is growing from various sides of life in producing practical efforts for health, food, agriculture, pharmacy, and the development of life technology or biotechnology[1]. The use of living things to produce goods and services on a conventional and modern scale is one of the definitions of biotechnology[2] in responding to environmental challenges.

One of the conventional biotechnology products is kombucha fermentation. It is known that kombucha is a tea-based functional drink and acts as a probiotic to boost the immune system[3], has antioxidant properties[4], anti-cholesterol[5], and anticancer[6]. Another ingredient in making kombucha is Telang flower (*Clitoria ternatea* L) which has a secondary metabolite source as an antibacterial[7].

Qualitatively, butterfly pea flower kombucha contains secondary metabolites from alkaloids, flavonoids, and saponins[8] which have the potential as antibacterial sources[9-11], antimicrobial sources[12], and antifungal sources[13].

Telang flower kombucha (*Clitoria ternatea* L), besides having the ability as a functional drink to enhance the immune system[14], has potential as an active cosmetic ingredient such as a liquid bath soap preparation that has been tested as an antibacterial for *Staphylococcus aureus*[15], *Escherichia coli*[16], *Vibrio parahaemolyticus* and *Klebsiella pneumonia*[17], antifungal *Trichophyton rubrum* and *Trichophyton mentagrophytes*[18], antifungal *Candida albicans*[19], dish soap preparations as gram-positive and negative antibacterials[20], antifungal shampoo[21].

Even the Telang flower kombucha can be used as an organic liquid fertilizer[22-23]. The many benefits of Telang flower kombucha in health, pharmacy, and agriculture made the authors want to be interested in conducting this latest research to make Telang flower kombucha bath soap preparations in inhibiting the growth of *Staphylococcus capitis*, *Bacillus cereus*, and *Pantoea dispersa*.

Previous research conducted by Saddam et al. (2022)[22] has stated that telang flower kombucha (*Clitoria ternatea* L) at a concentration of 40% is the best concentration in inhibiting the growth of *S. capitis* with an average diameter of the resulting inhibition zone of 17.25 mm (strong category), *B. cerreus* with an average inhibition zone diameter of 14.86 mm (strong category), and *P. dispersa* with an average inhibition zone diameter of 12.24 mm (strong category).

This study aims to make formulations and preparations for bath soap with an active ingredient of telang flower kombucha fermented solution as an antibacterial for *Staphylococcus capitis* (a Gram-positive bacterium that lives on the scalp, neck, face, scrotum, and human ears), *Bacillus cereus* (a Gram-positive bacterium that inhabits the soil and causes food poisoning), and *Pantoea dispersa* (a gram-negative bacterium that causes sepsis)[24].

METHODOLOGY

Types of Research

Making as many as four bodies wash preparations consisting of a body wash base as a negative control that tends to be designed without active substances. The soap base is added with a solution of fermented Telang flower kombucha with a concentration of 20%, 30%, and 40% as the active substance. This research is an experimental laboratory providing liquid body wash circulating in the market as a comparison (positive control).

Materials

The materials used in this study included *Staphylococcus capitis*, *Bacillus cereus*, and *Pantoea dispersa*. *Muller Hinton Agar* (MHA) media, a solution of fermented Telang flower kombucha at a sugar concentration of 20%, 30%, and 40% as an active substance (antibacterial).

The main ingredients of liquid body soap consist of 40% KOH (Potassium Hydroxide) of 8 g as a foaming agent. Na-CMC of 1 g as a thickener. Sodium Lauryl Sulfate (SLS) of 1 g as a surfactant. 0.5 mL of Olive Oil Infused as a fatty oil. Phenoxyethanol of 0.5 g as a preservative. BHT of 1 g as an antioxidant. 1 mL of essential oil as a perfume[17].

Other ingredients as additional ingredients for liquid bath soap include 1 mL of castor oil as a fluid. Sodium lactate of 1 g as a moisturizer. White sugar of 1 g as a foam enhancer. Yoghurt of 1 g as a softness enhancer. Kaolin Klay of 1 g as an addition to the slip and silky effect when bathing. 100 mL of distilled water as a solvent[20].

Stages of Making Bath Soap

Weigh the ingredients as a whole. Put 15 mL of olive oil into a beaker glass, then add 8 mL of 40% KOH little by little while heating it at 60-70^{o}C until getting a paste of soap and add 15 mL of distilled water (first mixture).

Add hot water and Na-CMC, then let stand until it swells. Add SLS, then stir until homogeneous. Add the ingredients for soap and stir until homogeneous. Add the active substance as a fermented butterfly pea flower kombucha solution with a sugar concentration of 20%, 30% and 40%. Enter the distilled water up to

100 mL in each soap preparation, and put it in the soap bottle (Pertiwi *et al*., 2022).

Bath Soap Preparation Formulation

Bath soap formulations and preparations consist of 5 formulas. The bath soap preparations referred to include F0, namely as a soap base / comparison / negative control / without active substance. F1, namely bath soap, has been stable and is circulating in the market as a positive control. F2 is a bath soap base that is added to the active substance as a fermented butterfly pea flower kombucha solution with a concentration of 20%. F3 is a bath soap base added to a solution of fermented butterfly pea flower kombucha with a concentration of 30%. F4 is a bath soap base added to a solution of fermented Telang flower kombucha with a concentration of 40%. Bath soap formulations and preparations are listed in table 1 below.

Table 1. Bath Soap Formulation and Preparation[18]

Ingredients	Function	F0 (-)	F1 (+)	F2 (20%)	F3 (30%)	F4 (40%)
Fermentation Solution Telang Kombucha	Active substance	0	X	20	30	40
Olive oil	Soap base	15	15	15	15	15
KOH 40%	Foam maker	8	8	8	8	8
Na-CMC	Thickner	1	1	1	1	1
SLS	Surfactant	1	1	1	1	1
Infused in olive oil	Oil	0.5	0.5	0.5	0.5	0.5
Phenoxyethanol	Preservative	0.5	0.5	0.5	0.5	0.5
BHT	Antioxidants	1	1	1	1	1
Essence oil	Parfume	1	1	1	1	1
Castor oil	Additional liquid	1	1	1	1	1
Sodium lactat	Moisturizer	1	1	1	1	1
Sugar	Foam booster	1	1	1	1	1
Yoghurt	Soft enhancer	1	1	1	1	1
Kaolin clay	Slik and silky effect enhancer	1	1	1	1	1
Aquadest	Solvent	100	100	100	100	100

Bacterial Inhibitory Test of Bath Soap Preparation

The inhibition test on the growth of *Staphylococcus capitis*, *Bacillus cereus*, and *Pantoea dispersa* from liquid soap preparations with an active ingredient of kombucha-fermented Telang Flower is generally carried out to determine the clear zone of an antibacterial agent in the form of an active substance. This

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study refers to the results of previous research conducted by Rezaldi et al. (2022)[25], namely in the form of a liquid bath soap with an active ingredient of telang flower kombucha fermented solution.

The tool used to measure the growth of the diameter of the inhibition zone is the analytic caliper. The formula for measuring the average diameter of the inhibition zone is as follows:

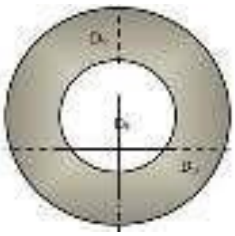

Figure 1. Calculation of the Average Diameter of the Inhibition Zone.

Description:

DV: Vertical Diameters

DH: Horizontal Diameters

DC: Cakram Diameters[13].

Data Analysis

Data obtained from the average diameter of the inhibition zone of all soap preparations in inhibiting the growth of *S. capitis*, *B. cerreus* and *P. dispersa* were processed through one-way ANALYSIS OF VARIANCE (ANOVA) with a 95% confidence level. If there is a significant difference in all bath soap preparations in inhibiting the growth of the three test bacteria, it will be followed by a post hoc analysis[19].

RESULT AND DISCUSSION

Preparation of liquid bath soap made from telang flower kombucha fermented solution, which includes a sugar concentration of 20%, 30%, and 40%, has been shown to correlate positively in inhibiting the growth of the three test bacteria, namely *S. capitis*, *B. cerreus*, and *P. dispersa*.

The average diameter of the inhibition zone produced from telang flower kombucha bath soap at a concentration of 20% in inhibiting the growth of *S. capitis* bacteria was 7.56 mm (moderate), 30% 8.17 mm (moderate), and 18.78 mm (strong). 0 mm body soap base (no response) and 17.67 mm commercial soap (strong)

The average diameter of the inhibition zone produced from telang flower kombucha bath soap at a concentration of 20% in inhibiting the growth of *B. cereus* bacteria was 7.40 mm (moderate), 30%, namely 8.05 mm (moderate), and 40%, namely 15.20 (strong). Bath soap base 0 mm (no inhibition), and market soap as a positive control, namely 14.90 mm (strong)

The average diameter of the inhibition zone produced from telang flower kombucha bath soap in inhibiting the growth of *P. dispersa* bacteria at a concentration of 20% was 7.09 mm (medium), 30% was 7.15 mm (moderate), and 40% 13.52 mm (strong). The body wash base is 0 mm, and the commercial body wash is 12.70 mm (strong).

The results of the one-way ANOVA analysis have proven that each bath soap preparation inhibiting the growth of the three test bacteria has a lower F table value than the calculated F, namely with a p value> 0.05 mm, so that it can be continued through post hoc analysis.

Post hoc analysis has proven that the concentration of 20% in preparing butterfly pea kombucha bath soap significantly differs from the concentration of 30%, 40%, soap base, and market soap. The concentration of 30% in the preparation of telang flower kombucha bath soap was significantly different from the 40% concentration, bath soap base, and commercial body wash as a positive control. However, it was not significantly different from the butterfly pea kombucha bath soap at a concentration of 20%. The Telang flower kombucha bath soap at 40% was significantly different from the eggplant kombucha bath soap at a concentration of 20% and a 30% soap base. However, it was not significantly different from market soap as a positive control in inhibiting the growth of *S. capitis*, *B. cereus*, and *P. dispersa* bacteria.

The results of this study have proven that the higher the concentration of bath soap, the higher its potential to inhibit the growth of the three test bacteria. The concentration of 40% is the best in inhibiting the growth of the three test bacteria and exceeds that of the market bath soap as a positive control.

The aim of using positive controls in the form of bath soap preparations that are spread on the market is to compare the average diameter of the inhibition of the preparations that have been circulating with the bath soap preparations used in this study[26].

The results of research conducted by Pertiwi et al. (2022) regarding the strength of antibiotics (antibacterial), which refers to the David-Stout method, states that if the clear zone is formed < 5 mm, the inhibition zone criteria are as weak antibacterial if the average value of the diameter of the inhibition zone is produced by 6 to 10 mm has inhibition zone criteria as moderate antibacterial, if the average diameter of the resulting inhibition zone is 10 to 20 mm, has inhibition zone criteria as strong antibacterial, and if the average diameter of the resulting inhibition zone exceeds 20 mm, then the criteria as an antibacterial inhibition zone are very strong.

Referring to this standard, it has been proven that formulations II and III in inhibiting the growth of the three tested bacteria have a moderate category, and Formulation IV has a strong category. The higher the concentration of the telang flower kombucha bath soap, the higher it is potential to inhibit the growth of the three test bacteria.

Antibacterial compounds that enter will ideally result in a higher

osmotic pressure inside the cell, thus causing cell lysis or rupture in pathogenic bacteria[2]. This is due to the influence of the secondary metabolites in the telang flower kombucha's fermentation[27].

The secondary metabolites contained in the telang flower kombucha fermented solution have been qualitatively studied by Abdilah et al. (2022)[28], namely from the alkaloids, flavonoids, and saponins, each of which has a different potential to inhibit the growth of pathogenic bacteria.

The alkaloid group contained in the telang flower kombucha fermented solution works cellularly in inhibiting the growth of pathogenic bacteria, including inhibiting the synthesis of enzymes and proteins, resulting in disturbances in the metabolism of pathogenic bacteria[29].

The cellular mechanism of the secondary metabolites contained in the telang flower kombucha fermentation solution acts as an antibacterial from the flavonoid group, namely by inactivating proteins and enzymes in the cell membrane of pathogenic bacteria[8].

The cellular mechanism of the secondary metabolites contained in the telang flower kombucha fermentation solution as an antibacterial from the saponin group is to synthesize complex compounds in pathogenic bacterial cells through hydrogen bonds until damage to the protein structure occurs and has the potential to influence imbalances in the pathogenic bacterial cell membrane[23].

CONCLUSION

This study concluded that the telang flower kombucha bath soap positively inhibited the growth of the three test bacteria. The concentration of 40% is the best in inhibiting the growth of the three test bacteria compared to the concentration of the two concentrations, namely 20% and 30%, and the two comparisons are positive and negative controls.

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