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by Barolym Tri Pamungkas

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[1]Barolym Tri Pamungkas, [2]Ayu Safitri, [2]Firman Rezaldi, [3]Muhammad Andry, [4]Lucky Dita Agustiansyah, [5]M. Fariz Fadillah, [6]Fajar Hidayanto and [7]Hari Hariadi

[1] Pharmacist Study Program, Faculty of Pharmacy, Mulawarman University, East Kalimantan, Indonesia
[2]Pharmacy Study Program, Faculty of Science, Pharmacy, Healthy, Mathla'ul Anwar University of Banten
[3]Pharnacy Study Program, Faculty of Pharmacy and Health, Helvetia Institute of Health, Medan, North Sumatera, Indonesia
[4]D3 Pharmacy Study Program, Al-Islah Pharmacy Academy, Cilegon, Banten
[5]Food Technology Study Program, Faculty of Tecnology and Informatics, Mathla'ul Anwar University of Banten, Indonesia
[6] Agrotechnology Study Program, Faculty of Agriculture, Merdeka Pasuruan University, East Java, Indonesia
[7] Appropriate Technology Research Center, Subang, West Java, Indonesia

Email: firmanrezaldi417@gmail.com

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ABSTRAK

Kombucha bunga telang (*Clitoria ternatea* L) merupakan minuman probiotik fungsional yang memiliki khasiat sebagai antifungi. Penelitian ini bertujuan untuk menguji aktivitas antifungi formulasi sediaan sabun mandi probiotik fermentasi kombucha bunga telang terhadap *Trycophyton rubrum* dan *Trycophyton mentagrophytes*. Metode penelitian ini dilakukan secara eksperimental dilaboratorium secara *in vitro*. Formulasi sediaan sabun mandi fermentasi kombucha bunga telang dibuat dengan konsentrasi yang bervariasi yaitu 20%, 30%, dan 40%. Sediaan sabun mandi probiotik kombucha bunga telang diuji aktivitas antibfunginya terhadap bakteri *T. rubrum* dan *T. megantrophytes* menggunakan metode difusi cakram. Prosedur kerja meliputi pembuatan sediaan sabun mandi, dan uji aktivitas antifungi pada sediaan sabun mandi probiotik kombucha bunga telang. Hasil penelitian berkolerasi secara positif berdasarkan uji ANOVA satu jalur dengan f hitung lebih besar daripada f tabel dan menunjukkan bahwa kombucha bunga telang dalam bentuk sediaan sabun mandi probiotik memiliki aktivitas sebagai antifungi terhadap *T. rubrum* dan *T. mentagrophytes* secara keseluruhan. Konsentrasi 40% merupakan konsentrasi tertinggi pada sediaan sabun mandi probiotik fermentasi kombucha bunga telang dengan rata-

rata diameter zona hambat 17,70 mm pada spesies *T. rubrum* dan 17,73 mm pada spesies *T. mentagrophytes*. Nilai rata-rata diameter zona hambat tersebut termasuk dalam kategori kuat.

Kata Kunci: Kombucha Bunga Telang, Antifungi, Sabun Mandi, Probiotik

ABSTRACT

Telang flower kombucha (Clitoria ternatea L) is a functional probiotic drink that has antifungal properties. This study aimed to examine the antifungal activity of the probiotic bath soap formulation fermented kombucha telang flower against Trycophyton rubrum and Trycophyton mentagrophytes. This research method was carried out experimentally in an in vitro laboratory. The formulation of the fermented kombucha bath soap for the flower of telang was made with varying concentrations of 20%, 30%, and 40%. The preparation of probiotic bath soap for kombucha telang flower was tested for its antifungal activity against the bacteria T. rubrum and T. megantrophytes using the disc diffusion method. The working procedure includes the manufacture of bath soap preparations, and antifungal activity tests on the probiotic kombucha bath soap preparations of telang flower. The results of the study were positively correlated based on the one-way ANOVA test with f count greater than f table and showed that telang flower kombucha in the form of probiotic bath soap had antifungal activity against T. rubrum and T. mentagrophytes as a whole. The concentration of 40% was the highest concentration in the probiotic soap preparations fermented kombucha telang flower with an average inhibition zone diameter of 17.70 mm in T. rubrum species and 17.30 mm in T. mentagrophytes species. The average value of the diameter of the inhibition zone is included in the strong category.

Keyword: Telang Flower Kombucha, Antifungal, Bath Soap, Probiotic.

INTRODUCTION

Telang flower kombucha (*Clitoria ternatea* L) is a probiotic drink made from telang flower and produced through biotechnology methods [1] fermentation by a consortium of bacteria and yeast or known as Scoby (Symbiotic Colony Bacteria & Yeast) which can produce various by-products by utilizing the substrate as its nutrition. The substrate used in the kombucha fermentation process is generally known as sugar which will be used as nutrition for scoby Different sugar concentrations

have the potential to inhibit the growth of pathogenic bacteria, both gram-positive [2] negative bacteria, microbes in both bacterial and fungal species, and fungi.

This statement is in accordance with the results of research conducted by Rezaldi et al., (2021) which has proven that the concentration of white granulated sugar of 40% in kombucha flower telang (*Clitoria ternatea* L) is the optimum concentration in inhibiting the growth of gram-positive bacteria, namely Staphylococcus aureus with The average diameter of the resulting inhibition zone is 13.2 mm and is included in the strong category, *Staphylococcus epidermidis* with the average diameter of the resulting inhibition zone is 10.78 mm and is included in the strong category. Gram-negative bacteria, namely the *Pseudomonas aeruginosa* species with an average inhibition zone diameter of 7.1 mm, were in the medium category and *Eschericia coli* with an average inhibition zone of 6 mm in the medium category [3].

The results of another similar study conducted by Rezaldi et al., (2022) have proven that the concentration of palm sugar in telang flower kombucha of 40% is the best concentration in inhibiting the growth of *Salmonella typhi* bacteria with an average diameter of the resulting inhibition zone of 18 .23 mm and is in the strong category. *Vibrio parahaemolyticus* with an average diameter of the resulting inhibition zone was 15.31 mm and was included in the strong category [4].

The results of research conducted by Puspitasari et al., (2022) have proven that the sugar concentration of 40% in telang flower kombucha is the best concentration in inhibiting microbial growth in both bacterial and fungal pathogenic species. The average value of the resulting inhibition zone is 20.62 mm, which is in the strong category for *Listeria monocvytogenes* bacteria. The average value of the resulting inhibition zone was 24.07 mm which was categorized as very strong for *Staphylococcus hominis* bacteria. The average diameter of the resulting inhibition zone was 18.07 mm for the fungus *Trycophyton mentagrophytes*. The average diameter of the resulting

inhibition zone was 17.20 mm for the fungus Trycophyton rubrum [5].

The results of research conducted by Rezaldi et al (2022) have also proven that the sugar concentration of 40% in telang flower kombucha is the best concentration in inhibiting the growth of various pathogenic fungi. The average diameter of the resulting inhibition zone was 21.24 mm for the *Candida albicans* fungus species. The average diameter of the resulting inhibition zone was 19.84 mm in the *Malasezia furfur* species. The average diameter of the resulting inhibition zone was 20.89 in *Pitosporum ovale* species. The average diameter of the resulting inhibition zone was 18.76 mm in *Aspergillus fumigatus* species [6].

Kombucha has the potential as a source of antimicrobial, antibacterial [7][8], a source of antioxidants [9], a source of cholesterol [10][11] and a source of anticancer [12] in addition to has the potential as a functional drink to enhance the immune system [13] and has the potential to be developed in the world of pharmaceutical biotechnology as an active ingredient in the manufacture of liquid bath soap.

The results of previous studies conducted by Fatonah et al., (2022) proved that probiotic liquid bath soap with active ingredient fermented kombucha telang flower solution at a concentration of 40% was the best treatment in inhibiting the growth of *Escherichia coli* bacteria with an average diameter of the inhibition zone. Produced is 11.60 mm and is included in the strong category [14].

The results of another study conducted by Rezaldi et al (2022) proved that probiotic liquid bath soap with active ingredient fermented kombucha telang flower at a concentration of 40% was the best treatment in inhibiting the growth of *Staphylococcus aureus* bacteria with the average inhibition zone produced was of 15.5 mm and is included in the strong category [15].

Kombucha flower telang according to the results of research by Abdilah et al (2022) has proven that it contains secondary metabolites such as alkaloids, flavonoids, and saponins, so that it has the potential to inhibit microbial growth [16]. Even in previous research conducted by Ma'ruf et al (2022) Kombucha flower telang

has been shown to have the potential to be used as a dish soap preparation that is bactericidal in both gram-positive and gram-negative bacteria and at a concentration of 40% is the best treatment in inhibiting the growth of gram-negative bacteria, positive or negative [17].

The results of this study are in accordance with Rezaldi et al (2022) which stated that a 40% concentration in the probiotic shampoo preparation of kombucha flower telang has the potential to inhibit the growth of pathogenic fungi such as *Candida albicans, Pitosporum ovale*, and *Aspergillus fumigatus* [18].

Departing from the results of previous relevant studies that support this research, the authors conducted a study on the growth inhibition of the fungi *Trycophyton rubrum* and *Trycophyton mentagrophytes* on probiotic liquid bath soaps with active ingredients in the fermented kombucha solution of telang flower with sugar concentrations of 20%, 30%, and 40% as pharmaceutical biotechnology products and the latest breakthroughs.

METHOD

Research Materials

The materials used in this study include pathogenic fungi from the species *Trycophyton rubrum*, and Trycophyton mentagrophytes. Sabouraoud Dextrose Agar (SDA) Media. The main ingredient for making soap is fermented kombucha telang flower solution at a sugar concentration of 20%, 30%, and 40% as the active substance. 15 mL of olive oil which serves as the basic ingredient. KOH 40% as much as 8 grams as a foam producer. Na-CMC as much as 1 gram as a thickener. SLS (*Sodium Lauryl Sulfate*) as much as 1 gram which functions as a surfactant. Olive oil infused at 0.5 mL which functions as a fat oil. *Phenoxyethanol* of 0.5 grams which functions as a preservative. 1 gram of BHT which functions as an antioxidant. Essence oil of 1 gram which functions as a fragrance. The additional ingredients for soap consist of 1 gram of castor oil which functions as a fluid. Sodium lactate as much as 1 gram which functions as a moisturizer. 1 gram of sugar which serves as a foam enhancer. 1 gram of yogurt which serves as a softness enhancer. Kaolin

Clay of 1 gram which functions as a slip and silky effect when bathing. Aquades 100 mL as solvent [15].

Stages of Making Probiotic Liquid Bath Soap Kombucha Bunga Telang (*Clitoria ternatea* L)

The steps in making probiotic liquid bath soap with active ingredients from the fermented kombucha solution of telang flower include weighing the ingredients as a whole. Enter the olive oil into a glass beaker as much as 15 ml. Add 8 mL of 40% KOH little by little while heating at a temperature of 60^0C to 70^0C until you get a soap paste and add 15 mL of distilled water (Mixture 1). insert Na-CMC then let stand until it expands, stir until homogeneous, add *Sodium Lauryl Sulfate* (SLS). Add BHT (*Butyl Hydroxy Toluene*) and stir until homogeneous. Add *Phenoxyethanol* and stir until homogeneous and add Essense oil, stir until homogeneous. After becoming a bath soap base, add enough water to 100 mL. Put it in a clean container that has been provided. Then add the additional soap ingredients. Add the telang flower kombucha fermentation solution according to their respective concentrations after the soap base is available and the active substance is ready to be added [14].

Antifungal Testing of the Telang Flower Kombucha Liquid Bath Soap (*Clitoria ternatea* L) against *Trycophyton rubrum* and *Trycophyton mentagrophyes*

The stages in this study include preparing 24 petri dishes to be poured on 15 mL SDA (*Sabouraud Dextrose Agar*) media in each petri dish. Let the media arrive at a solid state. Dipping a sterile cotton swab into the suspension of the fungi species *Trycophyton rubrum* and *Trycophyton mentagrophytes*.

Wiping the SDA media until the surface is tightly closed. Attaching a disc that has been soaked in a probiotic liquid bath soap with an active ingredient fermented kombucha telang flower solution with a certain concentration variation, namely in cup I 20%, cup II 30%, cup III 40%, cup IV containing positive control in the form of soap market, and Cup V containing a negative control in the form of a soap base. Repeat 3 times. Incubate for 48 hours. Measuring the diameter of the

inhibition zone at each concentration of telang flower kombucha fermentation along with positive and negative controls [5].

Data analysis

The research data measured were the average diameter of the inhibition zone on the fungi *Trycophyton rubrum* and *Trycophyton mentagrophytes* in each formulation of probiotic liquid bath soap with active ingredients in the kombucha fermentation solution of telang flower, soap base as a negative control, and market soap as a positive control. The research data were processed using statistical analysis, namely one-way ANOVA at the 95% confidence level. Research data that has the smallest significant difference (BNT) will be followed up through a post hoc test [19].

RESULTS AND DISCUSSION

Probiotic liquid bath soap with active ingredient fermented kombucha telang flower solution that has been produced at concentrations of 20%, 30%, and 40% showed positive correlation results as antifungals *Trycophyton rubrum* and *Trycophyton mentagrophytes*. These results can be listed in Table 1 below.

Table 1. Results of Measurement of Inhibitory Zone Diameter that has been Formed on Sabouraud Dextrose Agar (SDA) Media

Types of Fungi	**Replication**	**Negative Control (mm)**	**Positive Control (mm)**	**Concentration of Kombucha Fermented Telang Flower Soap (mm)**		
				20%	30%	40%
Trycophyton rubrum	I	3	14,23	9,80	12,60	16,21
	II	5,7	16,34	10,70	13,34	17,89
	III	6,9	18,90	12,22	15,67	19,00
	Avarage	5,2	16,49	10,90	13,87	17,70
Trycophyton mentagrophytes	I	2,2	13,60	8,6	10,67	15,40
	II	4.4	15,52	8,8	12,33	17,78

Types of Fungi	Replication	Negative Control (mm)	Positive Control (mm)	Concentration of Kombucha Fermented Telang Flower Soap (mm)		
				20%	30%	40%
	III	5,7	16,30	9.33	13,78	18,83
	Avarage	4,1	15,14	8,9	12,26	17,33

Table 1 listed above has proven that the concentration of probiotic liquid bath soap with active ingredients from the fermented kombucha solution of telang flower has the potential as antifungal agents for *Trycophyton rubrum* and *Trycophyton mentagrophytes*. These data have shown that at a concentration of 40% is the concentration of probiotic liquid bath soap with active ingredients from fermented kombucha telang flower solution and has the potential to form an inhibition zone for each fungal culture. The average value of the diameter of the inhibition zone on the fungi species *Trycophyton rubrum* from a probiotic liquid bath soap with active ingredient fermented kombucha telang flower solution was 10.90 mm at a concentration of 20% with a strong category, 13.87 mm at a concentration of 30% with strong category, and 17.70 mm at 40% concentration with strong category. The average value of the inhibition zone on the fungi species *Trycophyton mentagrophytes* from a probiotic liquid bath soap with active ingredients in the fermented kombucha solution of telang flower was 8.9 mm at a concentration of 20% with a medium category. 12.26 mm at a concentration of 30% with a strong category. 17.33 mm at a concentration of 40% with a strong category.

The data obtained from the next research were statistically tested using one-way ANOVA. The steps before the one-way ANOVA test are needed to perform the normality test. The data normality test aims to further ensure that the data from the research results are parametric or normally distributed and the data variance test aims to make the data homogeneous.

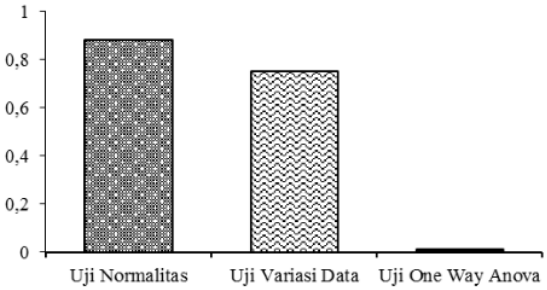

Figure 1. One Path Anova Test

Figure 1 listed above is the result of the normality test in the form of Saphiro-wilk which shows the data has a p value> 0.05, meaning that the data is parametric (normally distributed/distributed). Then the data variance test is also displayed in the form of a variance test which shows the data in this study have the same variance so that it can be tested using one-way ANOVA. In Figure 1, the results of the one-way ANOVA test are shown which states that the results of the one-way ANOVA test against the probiotic liquid bath soap treatment group with the active ingredient in the telang flower kombucha fermentation solution has a P value <0.05. The average value of the probiotic liquid bath soap treatment group with active ingredients in the fermented kombucha solution of telang flower has a significant difference or the smallest significant difference test (BNT) so that it can be done by post-hoc analysis

Table 2. Post Hoc Analysis Test

Types of Fungi		**20%**	**30%**	**40%**	**Positive Control**	**Negative Control**
Trycophyton rubrum	20%	-	0,444	0,004*	0,000*	0,000*
	30%	0,444	-	0,122	0,000*	0,000*
	40%	0,004*	0,444	-	0,000*	0,000*
	Positive Control	0,000*	0,000*	0,000*	-	0,000*
	Negative Control	0,000*	0,000*	0,000*	0,000*	-

Types of Fungi		20%	30%	40%	Positive Control	Negative Control
Trycophyton mentagrophytes	20%	-	0,333	0,003*	0,000*	0,000*
	30%	0,333	-	0,111	0,000*	0,000*
	40%	0,006*	0,111	-	0,000*	0,000*
	Kontrol Positif	0,000*	0,000*	0,000*	-	0,000*
	Kontrol Negatif	0,000*	0,000*	0,000*	0,000*	-

Note: *) states that there is a significant difference ($p<0.05$)

The Post-Hoc test listed in table 2 above has proven that the diameter of the inhibition zone on the fungus *Trycophyton rubrum*, the concentration of probiotic liquid bath soap with an active ingredient of 20% telang flower kombucha fermentation solution has a difference or significant difference in the concentration of probiotic liquid bath soap with an active ingredient of fermented solution. Telang flower kombucha 40%, but there was a significant difference in the concentration of probiotic liquid bath soap with active ingredients from 30% telang flower kombucha fermentation solution, soap base as a negative control, and commercial soap as a positive control. There was no significant difference in the concentration of probiotic liquid bath soap with an active ingredient of 30% telang flower kombucha fermentation solution, 40%, soap base as a negative control, and market soap as a control. positive. However, it was significantly different at concentrations of 20% and 30%.

Table 2 above has also proven that the diameter of the inhibition zone in the fungus *Trycophyton mentagrophytes*. The concentration of probiotic liquid bath soap with an active ingredient of 20% telang flower kombucha fermentation solution had a significant difference or difference in the concentration of probiotic liquid bath soap with an active ingredient of 40% telang flower kombucha fermented solution, but there was a significant difference in the concentration of probiotic liquid bath soap with an active ingredient of solution. 30% telang flower kombucha

fermentation, soap base as a negative control, and commercial soap as a positive control. There was no significant difference in the concentration of probiotic liquid bath soap with an active ingredient of 30% telang flower kombucha fermentation solution, 40%, soap base as a negative control, and market soap as a control. positive. However, it was significantly different at concentrations of 20% and 30%.

Antifungal testing on probiotic liquid bath soap with active ingredients in the form of fermented kombucha telang flower solution used the same treatment as in previous studies using kombucha telang flower solution as the active ingredient. The results of previous studies have proven that the preparation of probiotic liquid bath soap with active ingredients in the form of a fermented kombucha solution of telang flower has the potential to produce inhibition zone diameters against *Staphylococcus aureus* bacteria using the disc method [15] and Escherichia coli bacteria [14].

The use of this method is due to the general and practical method in testing, as well as having aerobic antibacterial sensitivity and facultative anaerobic bacteria, antifungal and fast reading results, making it suitable for use in research [20]. The results of research conducted by Prestiandri et al (2018), stated that the disc method has a high success rate and a small failure rate when compared to other methods. This is because the inoculated media such as bacterial or fungal suspensions can be positioned upside down. The position that is placed upside down aims to prevent droplets in the form of water vapor from falling on the media that has been cultivated by the tested bacteria and fungi, so that these droplets have the potential to affect the final results of the media incubation process. This method is also more efficient in terms of time used in a study [21].

The results of the average measurement of the diameter of the inhibition zone around the paper disc on the soap base preparation and which was added to the telang flower kombucha solution during incubation for 1 day, namely in formula 1 (20% concentration) 10.90 mm, formula 2 (30% concentration) 13 .87 mm and formula 3 (40% concentration) 17.70

mm in *Trycophyton rubrum* species. Formula 1 (concentration 20%) 8.9 mm, formula 2 (concentration 30%) 12.26 mm, and formula 3 (concentration 40%) 17.33 mm in Trycophyton mentagrophytes species. In addition, a negative control was used which is a soap base with an average diameter of the inhibition zone of 5.2 mm which is the result of the average diameter of the inhibition zone of *Trycophyton rubrum* and 4.1 mm which is the result of the average diameter of the inhibition zone of the species *Trycophyton mentagrophytes* and market bath soap preparations used as positive controls. The market soap preparation used as a positive control contains *benzalkonicum chloride* which plays a role in fighting the growth of bacteria, fungi, and viruses (Pertiwi et al., 2022) and has produced an average inhibition zone diameter of 8.40 mm in the medium category. The use of positive control on soap that has been circulating in the market ideally is to compare it with soap preparations that have been produced [22].

Based on the results of a study conducted by Prayoga (2013), that the measurement of the strength of antibiotics or antibacterials based on the David-Stout method, stated that if the diameter of the clear zone was < 5 mm, it showed activity as a weak antibacterial, the diameter of the inhibition zone ranged from 5 to 10 nmm. , then the activity as an antibacterial is moderate, if the diameter of the inhibition zone ranges from 10 to 20 mm, then the activity as an antibacterial is strong, and if the diameter of the resulting inhibition zone is > 20 mm, then the activity as an antibacterial is very strong. Referring to these standards, the inhibitory activity of probiotic liquid bath soap with active ingredients in the form of fermented kombucha telang flower solution in formula 1, formula 2 and formula 3 is included in the strong category, namely the fungi species Trycophton rubrum, while the species *Trycophyton mentagrophytes* in formula 1 is included in the sedan category, and formula 2, formula 3 is included in the strong category [19]. The larger the diameter of the clear zone, the greater the inhibition. Compounds that have high inhibitory power as antibacterials cause greater

osmotic pressure in the cells and lysis or rupture [21] and are also antifungal.

These results have proven that the preparation of liquid bath soap with active ingredients in the form of fermented kombucha solution of telang flower showed activity against the fungi species *Trycophyton rubrum* and *Trycophyton mentagrophytes*. The inhibition zones produced in formulas 1 and 2 are not as large as the inhibition zones found in market soaps or positive controls, but at a concentration of 40% probiotic liquid bath soap with active ingredients from the telang flower kombucha fermentation solution has the potential to produce the largest inhibition zone when compared with formula 1, 2, negative control, and positive control on both *Trycophyton rubrum* and *Trycophyton mentagrophytes* species. *Trycophyton rubrum* is one of the pathogenic fungi that macroscopically has a white cotton-like surface, dark red pigment, while microscopically it forms many small microconidia, thin walls, and oval in shape. *Microconidia* are formed in short conidiophores and are arranged one by one on the hyphae side (*en thyrse*) and in groups (*en grappe*). *Macroconidia* are pencil-shaped and consist of several cells [23]. Trichophyton rubrum usually has teardrop-shaped microconidia along the sides of the hyphae, in some strains these *microconidia* may be numerous.

Trycophyton mentagrophytes is the most common cause of fungal infections in Indonesia. The picture of the mold colony begins with fine, flat and yellow hairs, then changes to powdery and brownish cream in color and shows concentric rings. *T. mold macroconidia* have a cigar-like shape, consist of 3-6 cells, thin-walled and smooth, hyphae have septa. *Macroconidia* attach to hyphae with short stalks. *Microconidia* are teardrop-shaped, arranged along the hyphae. The fungus *T. metagrophytes* attacks the superficial layers of the body such as skin, hair and nails. Symptoms of attack are characterized by the appearance of lesions that form circles on the head and face. Lesions can spread to other parts of the body [24].

The antifungal activity is also due to the influence of secondary metabolites found in probiotic liquid bath soap which has active ingredients from the telang flower kombucha

fermentation solution. The results of research by Abdilah et al., (2022) have proven that kombucha flower telang contains secondary metabolites such as alkaloids, flavonoids, and also saponins [16]. The three secondary metabolites have the potential to inhibit the growth of fungi [25]. *Alkaloids* work as antifungals in cellular/molecular biology by inhibiting nucleic acid synthesis, so that fungi do not have the potential to develop [26]. The flavonoid group works as antifungal cellular/molecular biology by disrupting *mitochondrial* homeostasis and fungal cell integrity [27;28] The saponins work as antifungals in cellular/molecular biology by destroying the phospholipid structure found in fungal cell membranes [25].

CONCLUSION

The results of this study have proven that telang flower kombucha can be formulated into probiotic liquid bath soap and fermented telang flower kombucha in probiotic liquid bath soap preparations in the 40% formula has an effective fungal inhibition diameter with an average inhibition zone diameter of 17, 70 mm with a strong category in the Trycophyton rubrum species and 17.33 mm in the Trycophyton mentagrophytes species.

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ANTIFUNGAL Trycophyton rubrum AND Trycophyton mentagrophytes IN LIQUID BATH SOAP FERMENTED PROBIOTIC KOMBUCHA TELANG FLOWER (Clitoria ternatea L) AS A PHARMACEUTICAL BIOTECHNOLOGY PRODUCT

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