

**IN VITRO PHARMACOLOGICAL ACTIVITY TEST OF TELANG
FLOWER KOMBUCHA AS ANTIBACTERIAL *Vibrio cholerae* AND
Shigella dysenteriae THROUGH FERMENTATION
BIOTECHNOLOGY METHOD**

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ABSTRAK

Diare yang terjadi pada saluran pencernaan merupakan masalah yang besar bagi kesehatan usus manusia. Hal tersebut dapat disebabkan oleh adanya aktivitas bakteri patogen baik berupa *Vibrio cholerae* maupun *Shigella dysenteriae*. Kombucha bunga telang pada penelitian ini telah terbukti memiliki aktivitas farmakologi secara in vitro dalam menghambat pertumbuhan bakteri *Vibrio cholerae* maupun *Shigella dysenteriae* pada seluruh konsentrasi gula. Tujuan penelitian ini adalah untuk mengetahui aktivitas farmakologi secara in vitro pada kombucha bunga telang dalam menghambat kedua pertumbuhan bakteri uji yang terdiri dari konsentrasi gula sebesar 20%, 30%, dan 40%. Metode dalam pengujian daya hambat kedua pertumbuhan bakteri uji adalah difusi cakram. ANOVA satu jalur dan analisis *pos hoc* merupakan salah satu metode uji kuantitatif yang digunakan dalam penelitian ini dari rata-rata diameter zona hambat kombucha bunga telang dari berbagai konsentrasi gula. Hasil penelitian ini telah membuktikan berdasarkan ANOVA satu jalur dengan masing-masing nilai $P > 0,05$ kemudian dilanjutkan melalui analisis *pos hoc* disimpulkan bahwa kombucha bunga telang pada konsentrasi 40% merupakan perlakuan yang terbaik dalam menghambat kedua pertumbuhan bakteri uji dimana konsentrasi tersebut berbeda nyata dengan konsentrasi gula 20% dan 30%.

Kata Kunci: Kombucha, Bunga Telang, *Vibrio cholerae*, *Shigella dysenteriae*.

ABSTRACT

Diarrhea that occurs in the digestive tract is a big problem for human intestinal health. This can be caused by the activity of pathogenic bacteria in the form of *Vibrio cholera* and *Shigella dysenteriae*. In this study, kombucha has been shown to have pharmacological activity in vitro in inhibiting the growth of *Vibrio cholera* and *Shigella dysenteriae* bacteria at all sugar concentrations. The purpose of this study was to determine the in vitro pharmacological activity of the butterfly pea flower kombucha in inhibiting the growth of the two test bacteria consisting of a sugar concentration of 20%, 30% and 40%. The method for testing the inhibition of both bacterial growth tests is disc diffusion. One way ANOVA and post hoc analysis are one of the quantitative test methods used in this study of the average diameter of the inhibition zone of teal flower kombucha from various sugar concentrations. The results of this study have proven based on a one-way ANOVA with each P value > 0.05 and then continued through post hoc analysis concluded that the telang flower kombucha at a concentration of 40% is the best treatment in inhibiting the growth of both test bacteria where the concentration is significantly different from sugar concentration 20% and 30%.

Keyword: Kombucha, Telang Flower, *Vibrio cholerae*, *Shigella dysenteriae*

INTRODUCTION

Telang flower kombucha (*Clitoria ternatea* L.) is a probiotic drink that can boost the immune system [1], especially during the COVID-19 pandemic [2]. The latest butterfly pea flower kombucha has been shown to have pharmacological activity as an antibacterial source [3]; [4]; [5] good for gram positive bacteria [6] as well as in gram-negative bacteria [7], antimicrobial source [8], antifungal source [9], anti-cholesterol [10]; [11]; [12], source of antioxidants [13], and anticancer sources [14].

Telang flower kombucha, as has been described in the results of previous studies, has provided a lot of health effects that are quite good in fighting diseases and infections caused by the activity of pathogenic bacteria [15]; [16]; [17] both pathogenic bacteria originating from gram positive [18] nor negative [19] where the activity of pathogenic bacteria seriously threatens the human immune system which has the potential to be attacked by disease and infection. One of the gram-positive bacteria that can cause cholera through infection in the

digestive tract, one of which is *Vibrio cholera*, and contaminates many food products derived from fishery products [20].

One of the gram-negative bacteria that causes shigellosis or bacillary dysentery and acute inflammation of the digestive tract is *Shigella dysenteriae*. Telang flower kombucha (*Clitoria ternatea* L.) is an effort that can be given as a solution to inhibit the growth of both *Vibrio cholerae* and *Shigella dysenteriae* bacteria where in previous research results, especially butterfly pea flower kombucha has potential or pharmacological activity in vitro through fermentation biotechnology methods as an anti-bacterial. The results of research that has been conducted, it has been proven that tepu flower kombucha at sugar concentrations of 20%, 30%, and 40% correlated positively in inhibiting the growth of *Vibrio parahaemolyticus* and *Salmonella thypi* bacteria through the disc diffusion method [21];[22]. A sugar concentration of 40% is the best concentration that has pharmacological

activity in vitro in inhibiting *Vibrio cholera* and *Salmonella typhi* bacteria.

This is due to the butterfly pea flower kombucha as has been revealed through the results of research conducted by [23] It is proven that the butterfly pea flower kombucha qualitatively contains secondary metabolites in the form of alkaloids, flavonoids, and saponins. Where the three groups of secondary metabolites contained in telang flower kombucha are secondary metabolite compounds that are polar in nature and have been proven from various research results to have pharmacological activity in vitro as antibacterial [24]; [25].

Explanation regarding the potential or pharmacological activity of telang flower kombucha from the results of previous studies as a source of antibacterial [26], made the authors more interested in conducting research entitled in vitro pharmacological activity test of butterfly pea flower kombucha as an antibacterial for *Vibrio cholerae* and *Shigella dysenteriae*.

RESEARCH METHOD

This research is experimental in nature by making 3 kombucha preparations which are made from butterfly pea flowers and include sugar concentrations of 20%, 30%, and 40% which have been tested in previous research results to have the ability in the form of pharmacological activity as a source of antibacterial pathogens both gram positive and negative [27]. Providing sterile distilled water as a comparison or negative control and providing black tea-based kombucha as a positive control.

Preparation of Butterfly Pea (*Clitoria ternatea* L.)

Butterfly pea flower (*Clitoria ternatea* L) 500 grams which will be made in 1 liter of fermented butterfly pea flower kombucha from Pekuncen village located in Ciwedus village, Cilegon city, Banten province, then washed in running water until clean and dried to store in a clean container and covered in black cloth for Scoby to ferment [28].

Fermented Telang Flower kombucha

The first steps in fermenting butterfly pea flower kombucha are preparing priority tools and materials including a glass jar as an incubator, white granulated sugar as a substrate, Scoby or baby scoby as a starter or initial kombucha culture. The second step in the fermentation of the butterfly pea flower kombucha is to weigh the butterfly pea flower as much as 17.2% for 1 liter. The third step in the butterfly pea flower kombucha fermentation is to weigh 7.2% of the water until 2.4% of the water remains. The fourth step in the butterfly pea flower kombucha fermentation is adding the sugar concentration according to the treatment which includes 20%, 30%, and 40%. The fifth step in the butterfly pea flower kombucha fermentation is to heat the sugar until it boils within 10 minutes and then put it in a glass jar for each sugar concentration treatment. The sixth step in the butterfly pea flower kombucha fermentation is to put the cooking water on the inside of the glass jar where sugar has been added based on the concentration of each. The seventh step in the fermentation of

butterfly pea flower kombucha is to cool the cooking water at 25⁰C then add the initial kombucha culture with 1 week of age as much as 8% (v/v) in each treatment. The eighth step in the butterfly pea flower kombucha fermentation is to cover the glass jar using a cloth cover so that the kombucha fermentation process runs statically for 2 weeks at room temperature conditions [28].

Test of Inhibitory Power on the Growth of *Vibrio cholerae* and *Shigella dysenteriae*

The steps in testing the inhibition of *Vibrio cholera* and *Shigella dysintreae* growth were preparing 24 petri dishes to be poured into Muller Hinton Agar (MHA) media in each 15 mL petri dish. Silence the media until it solidifies. Dipping a sterile cotton swab into the suspension of *Vibrio cholerae* and *Shigella dysenteriae* bacteria. Wipe the MHA media until it is completely covered on the surface. Attaching the disk that has been soaked to the preparation in the form of a solution of fermented eggplant kombucha at various

concentrations, namely the first petri dish containing the eggplant kombucha at a sugar concentration of 20%. The second petri dish contains tethera kombucha at a sugar concentration of 30%. The third petri dish contains tethera flower kombucha at a sugar concentration of 40%. The fourth petri dish contains kombucha fermented black tea as a positive control and the fifth petri dish contains sterile distilled water as a negative control. Each treatment was repeated 3 times. Incubate the media containing the antibacterial agent and the test bacteria for 1 day. Measuring the average diameter of the inhibition zone from each concentration of sugar in telang flower kombucha [29].

Data Analysis

The results of this study were in the form of data regarding the average diameter of the inhibition zone produced from each telang flower kombucha based on sugar concentrations including 20%, 30%, and 40% in inhibiting the growth of the two test bacteria analyzed through one way ANOVA at a confidence level of

95 %. If there is the smallest significant difference (LSD) in each butterfly pea flower kombucha which includes a sugar concentration of 20%, 30%, and 40% in inhibiting the growth of the two test bacteria, then it will proceed through a further test, namely post hoc analysis [30] ; [31].

RESULTS AND DISCUSSION

Telang flower kombucha fermentation solution which includes sugar concentrations of 20%, 30%, and 40% correlated positively in inhibiting the growth of *Vibrio cholerae* bacteria as gram-positive bacteria and *Shigella dysenteriae* as gram-negative bacteria. The results of this study are listed in table 1 below.

Table 1. Mean Measurement Results of the Inhibition Zone Diameter of Muller Hinton Agar (MHA) Media

Type of Bacteria	Diameter obstacles zone (mm)	Negative Control (mm)	Positive Control (mm)	Sugar Concentration in Fermented Telang Flower Kombucha (mm)		
				20%	30%	40%
<i>Vibrio cholerae</i>	I	0	10,23	7,34	8,23	12,50
	II	0	10,25	7,35	8,24	12,50
	III	0	10,26	7,40	8,25	12,55
	Average	0	10,24	7,36	8,24	12,51
<i>Shigella dysenteriae</i>	I	0	10,12	7,12	8,05	12,07
	II	0	10,12	7,15	8,07	12,08
	III	0	10,15	7,20	8,12	12,08
	Average	0	10,13	7,15	8,08	12,07

Table 1 listed above is the result of a study regarding the average diameter of the inhibition zone formed from an antibacterial agent, namely kombucha fermented eggplant solution which has proven its pharmacological activity in vitro in inhibiting the growth

of the two test bacteria used in this study and includes *Vibrio cholerae* and *Shigella dysenteriae*. The average diameter of the inhibition zone produced from telang flower kombucha at a sugar concentration of 20% to inhibit *Vibrio cholerae* bacteria was

7.36 mm and *Shigella dysenteriae* bacteria was 7.15 mm. The average diameter of the inhibition zone produced from butterfly pea flower kombucha at a sugar concentration of 30% to inhibit the growth of *Vibrio cholerae* bacteria was 8.24 mm and to inhibit the growth of *Shigella dysenteriae* bacteria was 8.08 mm. The average diameter of the inhibition zone produced from butterfly pea flower kombucha at a concentration of 40% to inhibit the growth of *Vibrio cholerae* bacteria was 12.51 mm and to inhibit the growth of *Shigella dysenteriae* bacteria was 12.07 mm.

The results of this study showed that the higher the concentration of sugar as a substrate that was broken down by scoby during the telang flower kombucha fermentation process, the more proven its pharmacological activity in vitro as an antibacterial against the growth of the two test

bacteria used in this study. This is due to the different concentrations of the substrate, especially sugar, which greatly affect its pharmacological activity as a source of antibacterial [32].

Data from subsequent studies were analyzed using one-way ANOVA where before carrying out the one-way ANOVA analysis two stages of the test were needed which included a data normality test which aims to identify data from a study that ideally is parametric or normally distributed/distributed with a higher F table. Compared to the calculated F value of 0.05 which has been determined based on statistical rules. Both data variance tests aim to identify a data that is homogeneous with the same requirements as the data normality test [33]. The data normality test through the Shapiro Wilk test in this study is in table 2 below.

Table 2. Data Normality Test Through the Shapiro Wilk Test

No.	Test Bacteria Species	Statistical Test Name	Sig
1.	<i>Vibrio cholerae</i>	The Shapiro-Wilk test	0,76
2.	<i>Shigella dysenteriae</i>	The Shapiro-Wilk test	0,66

Table 2 listed has explained the data normality test using the Shapiro Wilk test where the results of previous studies have been carried out by [30] if the resulting table F value exceeds the calculated F value that has been determined based on statistical rules, namely 0.05, it can be concluded that the data from this study are parametric or normally distributed. The data normality test in this study listed in table 2 above is in accordance with the research results [30] where each of the F tables has a value above 0.05, so that it can be concluded that the data from this study are parametric in nature and can be continued with the data variance test before the one-way ANOVA test is carried out. The data variance test in this study is listed in Table 3 below.

Table 3. Data Variance Test

No.	Test Bacteria Species	Statistical Test Name	Sig
1.	<i>Vibrio cholerae</i>	Data Variance Test	0,58
2.	<i>Shigella dysenteriae</i>	Data Variance Test	0,48

Table 3 listed above is a data variance test which aims to identify research data that is homogeneous in all treatments in inhibiting the growth of both test bacteria. The requirements of the data variance test are the same as the data normality test where the resulting table F value is ideally higher than the calculated F value that has been determined based on statistical rules, namely 0.05. The results of this study have proven that each treatment has the same value for inhibiting the growth of the two tested bacteria as the dependent variable, so that the one-way ANOVA test can be carried out which is listed in table 4 below.

Table 4 One way ANOVA test

No.	Test Bacteria Species	Statistical Test Name	Sig
1.	<i>Vibrio cholerae</i>	One Way Anova Test	0,03
2.	<i>Shigella dysenteriae</i>	One Way Anova Test	0,02

Table 4 listed above contains further tests can be carried out through information about the one-way post hoc analysis listed in table 5 ANOVA test where the resulting F below. tables are below 0.05 each, so that

Table 5 Post Hoc Analysis

		20%	30%	40%	Positive Control	Negative Control
<i>Vibrio cholerae</i>	20%	-	0,777	0,007*	0,000*	0,000*
	30%	0,777	-	0,333	0,000*	0,000*
	40%	0,007*	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*	-
<i>Shigella dysenteriae</i>	20%	-	0,555	0,005*	0,000*	0,000*
	30%	0,555	-	0,111	0,000*	0,000*
	40%	0,007*	0,222	-	0,000*	0,000*
	Positive Control	0,000*	0,000*	0,000*	-	0,000*
	Negative Control	0,000*	0,000*	0,000*	0,000*	-

*: State that there is a significant difference (p<0,05)

Table 5 listed contains concentration of 40% and positive information regarding further tests in control in inhibiting the growth of both the form of post hoc analysis where the test bacteria [34].

results of this study have proven that Likewise, the butterfly pea each research data which includes flower kombucha at a sugar independent variables, controlled concentration of 30% was significantly variables has been tested in answering different from the sugar concentration of 40% and the positive control in each of the two dependent variables used in this study. Table 5 listed above inhibiting the growth of both test bacteria, but not significantly different from the butterfly pea flower has proven that kombucha with a sugar kombucha at a sugar concentration of 20% is not significantly different from a sugar concentration of 30%, but significantly different from kombucha with a sugar 20% in inhibiting the growth of the two test bacteria. The butterfly pea flower

komucha at a concentration of 40% was significantly different from the butterfly pea flower kombucha at a sugar concentration of 20% and 30% in inhibiting the growth of both test bacteria, but not significantly different from the positive control in the form of black tea-based kombucha in inhibiting the growth of both test bacteria.

The results of this study have proven that the higher the concentration of sugar as a substrate, the greater the in vitro pharmacological activity in inhibiting the growth of both test bacteria. The statements in this study are in line with the results of research conducted by [35] who stated that kombucha butterfly pea flower at a sugar concentration of 40% was the best treatment in inhibiting the growth of *Streptococcus mutans* and *Klebsiella pneumoniae*.

Butterfly pea flower kombucha used as an active substance in this study has been shown to have pharmacological activity in vitro to inhibit the growth of both test bacteria. This is because telang flower kombucha contains several secondary metabolites consisting of alkaloids, flavonoids, and saponins which have

the potential to inhibit pathogenic bacteria both from gram-positive and gram-negative bacteria [36].

The flavonoid group contained in kombucha butterfly pea flowers has a mechanism of action as an antibacterial including inhibiting nucleic acid synthesis, cell membrane function, energy metabolism which can cause permeability damage to the bacterial cell wall, microsomes, lysosomes which are the result of interactions with bacterial plasmids. The formation of complex compounds in extracellular proteins capable of bacterial cell membranes is one of the working mechanisms of flavonoids as an antibacterial source in inhibiting the function of cell membranes of pathogenic bacteria [36].

The alkaloid group contained in telang flower kombucha has a mechanism of action as an antibacterial source, including by interfering with the peptidoglycan component in bacterial cells, so that the cell wall layer is not completely formed and causes lysis and even cell death in pathogenic bacteria. The class of secondary metabolite compounds in the form of saponins contained in telang

flower kombucha as an antibacterial source, among others, is by causing a leak in the protein and enzyme parts found in the cell walls of pathogenic bacteria. Saponins are also able to reduce surface tension which can lead to increased permeability and cell leakage as a result of released intracellular compounds [37].

Saponins have the potential to diffuse through the outer membrane

CONCLUSION

From this study it can be concluded that the butterfly pea flower kombucha has been shown to have pharmacological activity in vitro in inhibiting the growth of both test bacteria and a sugar concentration of 40% in the butterfly pea flower

and cell wall which are prone to bind to the cytoplasmic membrane until disturbance or decrease in the stability of the cell membrane of pathogenic bacteria occurs. This causes the cytoplasm to leak, resulting in cell death for the pathogenic bacteria themselves [31].

kombucha is the best treatment in inhibiting the growth of both test bacteria compared to the butterfly pea flower kombucha at the sugar concentration. by 20% and 30% as well as a positive control.

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