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Antibacterial Effectiveness Of Extracts And Fractions Of Telang Leaves Against Salmonella Typhi Bacteria

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Abstract. Telang leaves are plants that are very easy to find and contain flavonoids, flavanols, kaempferol, quercetin and myrisetin. From the results of various studies, Telang leaves have pharmacological influences as antimicrobials, one of which is for the treatment of typhoid fever (typhoid). Typhoid fever is still one of the endemic diseases in Indonesia and this disease is caused by infection with the bacterium *Salmonella typhi*. The purpose of this study was to determine the effectiveness of extracts and fractions of telang against the growth of *Salmonella typhi*. The research method used is experimental laboratory. The test was carried out by extracting Telang leaves by maceration method, then evaporated with a rotary evaporator so that concentrated extracts were obtained and then fractionated using organic solvents n hexane, chloroform and ethyl acetate. Test of antibacterial activity of extracts and fractions against *Salmonella typhi* bacteria was carried out by disc diffusion method. with DMSO negative control and Amoxcilin positive control. The results showed that the ethylacetate fraction showed the strongest inhibitory zone when compared to ethanol extract against *Salmonella typhi*. The ethylacetate fraction is able to produce inhibitory power of 18.4 mm, while the striped leaf extract only inhibits 15.5 mm in the medium category. Based on the test results, it can be concluded that the ethyl acetate fraction and ethanol extract are effective in inhibiting the growth of *Salmonella typhi* bacteria, while the n-Hexane, water and chlorophome fractions show weak antibacterial activity.

Keywords : Alginate-clay composite, eco-friendly adsorbent, methylene blue adsorption, methylene blue removal

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Introduction

Salmonella typhi is a class of Gram negative bacteria. *Salmonella typhi* can cause typhoid fever. These bacteria enter through the mouth then go to the gastrointestinal tract. Treatment of typhoid fever generally uses synthetic antibiotic drugs such as chloramphenicol, ampicillin and co-trimoxazole; however, because it has many side effects, treatment is developed through the treatment of natural ingredients [1]. Telang (*Clitoria ternatea*) has long been used as a traditional medicine for healing various diseases. The commonly used parts of *Clitoria ternatea* are flowers and leaves. Striped leaves contain flavonoids, flavanol glycosides, kaempferol glycosides, quercetin glycosides and myricetin glycosides [2]. From the results of various studies *Clitoria ternatea* has pharmacological influences as antimicrobial, antiparasitic, anti-inflammatory, anticancer, antioxidant, antidepressant, antidiabetic, antihistamine, immunomodulator and potential role in the nervous system, Central nervous System (CNS) [3]. Various studies of *Clitoria ternatea* have been conducted on flowers, but the research that has been done is not yet known. The potential of extracts and fractions from leaf parts, especially as antibacterial, so it needs to be further investigated the effectiveness of antimicrobials in extracts and fractions of striped leaves against *salmonella typhi* bacteria.

Experimental

Sample Preparation. Telang leaves are cleaned of dust and dirt by washing. After that it is dried in the oven at 45 °C for 72 hours. Next, it is mashed with a blender and sifted so that a fine powder is obtained.

Extraction of Telang leaves. 500 grams of telang leaf powder was soaked in 1 L of ethanol solvent with a solvent ratio of 1:5 (b/v) made 5 more soaks with the same ratio. Soaking was carried out for 6 days with several stirring [4]. The resulting extract was vacuum filtered and the filtrate was concentrated with a vacuum rotary evaporator to obtain a thick extract and then fractionated and tested for antibacterial activity.

Fractionation of telang leaf extract. Fractionation was carried out by means of concentrated ethanol extract of telang leaves dissolved in water, filtered then fractionated with

several organic solvents n-hexane, chloroform, ethyl acetate, the fractionation process was carried out in a separating funnel in a ratio of 1: 1, shaken sufficiently and allowed to form 2 layers. so that the n-hexane, chloroform, ethyl acetate and water fractions were obtained. The results of the fraction were then evaporated with a rotary evaporator to obtain a thick fraction and then tested for antibacterial activity [5].

Antibacterial Test. A 100 µl suspension of *Salmonella typhi* bacteria was put into a sterile Petri dish, then 10 mL of liquid NA medium was added, and the medium was allowed to solidify. On top of the NA medium, sterile paper discs that have been soaked with concentrated extract and fractions of telang leaf extract with a concentration of 0.1 g/mL (10%) for 30 minutes were placed. The disc paper was placed on the surface of the medium using tweezers and pressed slightly. Then incubated at 37°C for 24 hours. The positive control used was Amoxicillin 10% and the negative control used was DMSO. After 24 hours, it was observed whether or not there was a clear zone around the disc paper [6].

Result and Discussion

Extraction of Telang Leaf. At this stage, telang leaf extraction is carried out using Maceration Extraction. Maceration is the process of extracting simplisia using a solvent with several stirring at room temperature [7]. In this study, the solvent used in the maceration process was 96% ethanol. Ethanol is used as a solvent because it is polar, universal and easy to obtain. In addition, ethanol is also a solvent for organic and inorganic substances [8]. The purity of the lowest ethanol solvent that can dissolve a secondary metabolite compound is 66%, so 96% ethanol is expected to extract more secondary metabolite compounds. Because the higher the ethanol concentration, the easier it will be in the process of separating secondary metabolite compounds from the sample [9]. The maceration process was carried out for 5 days and the extract obtained was then separated from the solvent using a vacuum rotary evaporator and obtained a thick green ethanol extract.

Fractionation of telang leaf extract. After obtaining the concentrated extract, the next process is fractionation. Fractionation is a separation process between liquid and liquid substances. Fractionation is carried out in stages based on the level

of polarity, namely from non-polar, semi-polar, and polar. Non-polar compounds will dissolve in non-polar solvents and polar ones will dissolve into polar solvents [10]. This fractionation process is carried out using a separating funnel. In this fractionation, solvents that have a higher density will be in the lower layer, and those with a smaller density will be in the upper layer [11]. The compounds contained in the extract will be separated according to the level of solubility of the solvent used. The first fractionation is between the ethanol extract water filtrate with n-hexane solvent in a 1:1 ratio and formed two layers, namely the upper layer of the n-hexane fraction and the lower layer of the water fraction. The difference in solvent density causes the n-hexane fraction to be on top because the density of n-hexane (0.655 g/ml) is smaller than the density of water (1 g/ml) [12]. Further fractionation using chloroform and ethyl acetate solvents [5]. The fractions obtained were evaporated using a rotary evaporator until the concentrated n-hexane fraction was obtained. The fractionation results were then tested for antibacterial activity

against salmonella typhi.

Antibacterial activity test of telang leaf extracts and fractions. This study was conducted to test the antibacterial activity of telang leaf extracts and fractions against *Salmonella Typhi* bacteria in vitro. against *Salmonella Typhi* bacteria in vitro. When the test culture is given a certain substance that is antimicrobial, its growth will be inhibited [13]. The zone of inhibition is a clear zone around the disc paper on media that has been inoculated with bacteria or a zone where there is no growth of *Salmonella Typhi* [1]. In this study, microbes will be inhibited by telang leaf extract with several concentrations of 10%, 20%, 30%, 40%, 50% using disc paper. The results of the inhibition zone of telang leaf extract against *Salmonella Typhi* bacteria can be seen in Figure 1. In the inhibition test, variations in concentration were made, the aim was to find the concentration of the extract that was effective in inhibiting the growth of *Salmonella Typhi* bacteria, the higher the concentration of the extract the higher the inhibition. The diameter of the inhibition zone can be seen in Table 1.

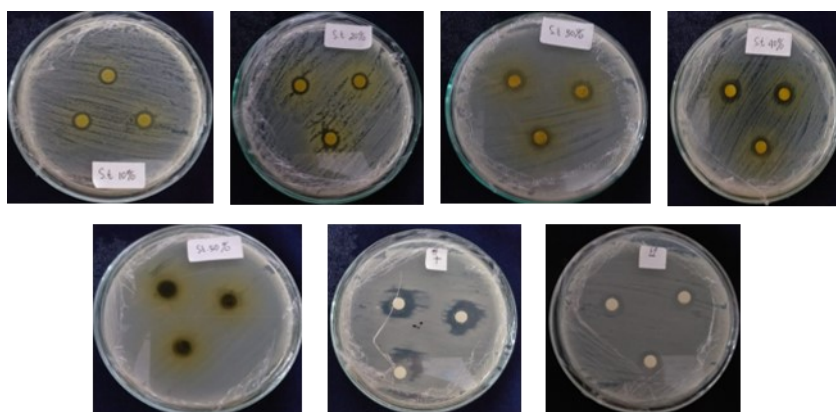


Figure 1. Zone of Inhibition of Extracts against *Salmonella Typhi* bacteria

Table 1. Results of the inhibitory power test of telang leaf extract against *S. Typhi*

Sample (Extract telang leaf)	Diameter of the Inhibitory Zone (mm)		
	U 1	U2	U3
10%	9.6	9.2	9.4
20%	9.2	9.1	10.6
30%	11.8	11.3	12.0
40%	12.2	13.7	15.5
50%	9.2	7.8	8.9
K+	18.9	15.6	19.3
K-	0	0	0

Based on Figure 1. and Table 1., the extract that gives the highest inhibition zone is telang leaf extract which is effective in inhibiting the growth of *Salmonella Typhi* bacteria with an inhibition diameter of 15.5 mm in the medium category. This result is not much different compared to the positive control of pure amoxicillin with an inhibition zone of 19.3 mm. While the fractions obtained from telang leaf extract can be done variation of concentration 0.25%, 0.50%, 0.75%, 1% of each fraction. The results of the inhibition zone of the fractions of telang leaves against *Salmonella Typhi* are shown at Tabel 2.

Based on Table 2, the fraction that gives the highest inhibition zone is the ethyl acetate fraction of telang leaves which is effective in inhibiting the growth of *Salmonella typhi* bacteria with an inhibition diameter of 18.4 mm at a concentration of 1% showing the highest antibacterial activity against *S. typhi* when compared to all test fractions which are sequentially followed by chloroform, hexane, and water fractions. these results are also not much different compared to the positive control of pure amoxicillin with an inhibition zone of 19.3 mm. The ethyl acetate

fraction sample of telang leaves has a greater average inhibition zone than the ethanol extract of telang leaves from the concentration series made. This is possible because the test fraction used contains active compounds that act as antibacterials, so as to inhibit the growth of *salmonella typhi* bacteria. Antibacterial activity in the sample can be influenced by the class of secondary metabolites that can inhibit bacterial growth. This activity can come from the mechanism of action of a compound individually or a combination of the mechanisms of the compounds contained therein [14]. There is a possibility that the magnitude of the inhibition zone produced by the fraction of telang leaves occurs due to the nature of synergy between the mechanism of the compounds in the fraction, namely the perfect separation occurs in the fractionation process so that the withdrawal of compounds by the appropriate solvent is perfectly perfect so as to produce pure compounds, so that the antibacterial activity is stronger than the ethanol extract of telang leaves even at small concentrations [15]. This is because the ethyl acetate fraction is able to attract semi-polar telang leaf chemical compounds such as flavonoids with higher levels compared to ethanol extract samples.

Table 2. Results of the inhibitory power test of telang leaf Fraction against *S. Typh*

Sample (Fraction telang leaf)	Concentration (%)	Diameter of the Inhibitory Zone (mm)		
		U1	U2	U3
Water fraction	0.25	7.8	7.1	9.1
	0.5	9.7	8.2	8.0
	0.75	8.8	8.4	7.6
	1	9.7	11.7	11.1
N-Hexana fraction	0.25	8.5	9.7	8.6
	0.5	9.1	10.2	8.2
	0.75	9.2	10.5	11.4
	1	12.4	11.0	12.0
Chloroform fraction	0.25	7.0	7.2	6.2
	0.5	9.0	8.4	9.7
	0.75	8.7	11.7	7.4
	1	13.0	12.1	12.4
Ethyl Acetate fraction	0.25	10.0	9.5	8.5
	1	12.4	18.4	13.2
Amoxilin	K+	18.9	15.6	19.3
DMSO	K-	0	0	0

Conclusion

The ethyl acetate fraction of Telang leaves has the highest antibacterial activity against *S. typhi* compared fractions of hexane, chloroform, water and ethanol extract. The existence of synergistic properties between metabolites Secondary to the ethylacetate fraction causes the fraction to have activity highest antibacterial in *S. typhi*. Therefore, phytochemical screening should be carried out using thin layer chromatography (TLC) method as a further test for Ensure the presence of compound classes in the extract and fraction of telang leaves.

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