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Antibacterial effect of bitter melon extract (*Momordica charantia*) against *Staphylococcus aureus* and *Streptococcus pyogenes*

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Abstract
Bitter melon with phytochemical contents has been proven to contain anti-microbial activity. However, previous studies only used diffusion method with uncertain breakpoint for the inhibition zone diameter (IZD) produced. The aim of this research was to examine the effect of Bitter Melon Extract (BME) on *S. aureus* and *S. pyogenes* by the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) methods as well as IZD. The method of this research was experimental research with post-test only control group design was carried out using 6 groups consisted of 1 positive control (K+) as the indicator of visual clarity, 4 different concentrations of BME (100%, 50%, 25% and 12,5%) inoculated with bacteria and 1 negative control (K-). MIC was determined by comparing the turbidity of inoculated tubes with K+. MBC was evaluated by plating the culture on agar plate to examine whether sterile growth produced. This study showed that MIC of BME against *S. aureus* and *S. pyogenes* are 50% and 25%. MBC study showed bacterial growth. IZD of *S. aureus* and *S. pyogenes* for BME to be claimed as sensitive were 12 mm and 9 mm. The higher the concentration of BME, the wider the IZD.

Keywords: Bitter melon extract, antibacterial, *Staphylococcus aureus*, *Streptococcus pyogenes*

1. Introduction
Staphylococcus aureus and *Streptococcus pyogenes* are bacteria that cause various infections. One of the most common infections that occur in society is a skin infection disease [1]. In 2010, a Global Burden of Disease Study showed that impetigo caused by *S. pyogenes* was 140 million cases per year. Other skin diseases caused by *S. pyogenes* were cellulitis and erysipelas with an incidence value of 200 cases per 100,000 patients per year. *S. pyogenes* can also infect other organs, specifically pharynx. Pharyngitis can cause complications namely acute rheumatic fever and post-streptococcal glomerulonephritis [2-3]. Skin infections caused by *S. aureus* include Staphylococcal scalded skin syndrome (SSSS), furuncle and impetigo. Research in the United States shows that the annual SSSS rate in children is 7.67 cases per million, in infants under 2 years is 45 cases per million and in one-year infants is 20.9 per million [4-5].
The increasing problem of antimicrobial resistance encourages the use of herbal ingredients to be developed into antimicrobials. One of the choice plants for this is bitter melon or *Momordica charantia*. Phytochemicals including proteins, polysaccharides, flavonoids, triterpenes, saponins, ascorbic acid and steroids are found in this plant. Various biological activities of *M. charantia* have been reported, such as antihyperglycemic, antibacterial, antitumor, antioxidant, antidiabetic, anthelmintic and anti-inflammatory properties [6-7].
The antibacterial activity of bitter melon has been extensively studied and tested for its effect on bacterial growth, but previous studies have shown the antibacterial effect is only based on the inhibitory zone diameter (IZD). Even though it is unknown what is the minimum limit of inhibition zone diameter of *M. charantia* extract to be said to be effective as an antimicrobial. More precise microbiological examination methods are needed to conclude that bitter melon extract has antimicrobial properties, namely Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) [8].

2. Materials and Methods
2.1. Research design
Experimental research with post-test design only control group design was carried out using 6 groups consisted of 4 different concentrations bitter melon extract (12.5%, 25%, 50% and 100%) inoculated with bacteria, 1 negative control (K-) and 1 positive control (K+) as the indicator of expected visual clarity.

MIC was determined by comparing the turbidity of Inoculated tubes with K+. MBC was evaluated by plating the culture on agar plate to examine whether no bacterial growth produced.

2.2. Tools and Materials
Tools that were used in this research were knives, test tubes, analytical scales, spiritus burner, lighters, beaker glass, petri dishes, ose, incubator, micropipette, vortex, sterile cork borer. Materials that were used in this research were bitter melon, aquadest, Mueller Hinton Broth (MHB) and Mueller Hinton Agar (MHA).

2.3. Procedure
Extract was made by maceration method, then concentration was diluted to 100%, 50%, 25%, 12.5%. One ml of each concentration was pouredinto P1, P2, P3, P4 tubes. K+ tubes for gentamicin. K- tubes for aquadest.

Suspension of *Staphylococcus aureus* and *Streptococcus pyogenes* bacteria obtained from the Laboratory of Microbiology, Faculty of Medicine, Diponegoro University. Each bacterial culture that had been planted on Tryptic Soy Agar (TSA) was carried out by taking a colony from the media with sterile ose, inserted and homogenized into the Mueller Hinton Broth (MHB) using vortex and incubated at 37 °C for 24 hours. The bacterial suspension was ironed with McFarland 0.5 standard which is equivalent to 1.5 x 10^8 CFU/ml.

One ml of bacterial suspension was added into each of the K +, P1, P2, P3, P4 and K- tubes. The experiment was conducted 5 repetitions on each number of the tube. All tubes were incubated at 37 °C for 1 x 24 hours.

The clarity of the treatment tube after incubation was observed. The tube with the smallest concentration that produces a clear state is visually expressed as the MIC.

A tube that has a clarity in accordance with positive control was prepared. 1 ml of that tube was poured into the MHA then incubated at 37 °C for 24 hours. MBC determination was based on the smallest concentration that did not occur colony growth.

Media was made by pouring MHA into each petri dish. The suspension of the bacteria was applied to the entire surface of the petri dish until evenly let stand for 1 hour. A well containing BME was made with various concentrations at a distance on the agar surface. Petri dishes were incubated at 37 °C for 18-24 hours. IZD were measured using a calipers [9].

2.4. Data analysis
The data obtained were processed using a computer program and firstly analyzed with Saphiro-Wilk normality test. The hypothesis about differences MIC of intervention group and positive control was tested using the Kruskal Wallis test because data was not normally distributed. Data said to be significant if $p< 0.05$. Furthermore, the hypothesis about correlation between concentration and IZD was tested using Spearman. Data said to have strong correlation if r>0.51.

3. Result
The results showed the concentration of BME which began to show clarity in *S. aureus* was 50%. All intervention groups with concentrations of 12.5% and 25% showed turbidity. It can be determined the MIC of bitter melon extract against *S. aureus* is 50%. The concentration of bitter melon extract which began to show clarity in *S. pyogenes* was 25%. All intervention groups with concentrations of 12.5% showed turbidity. It can be determined the MIC of bitter melon extract against *S. pyogenes* is 25%.

Table 1: IZD study of BME against *S. aureus*

Group	Inhibition zone diameter (mm)						
	I	II	III	IV	V	Mean ± SD	r
Control +	22.12	22.21	23.11	23.18	22.13	22.55± 0,48	
100%	15.28	13.29	12.91	15.14	14.12	14.15 ± 0,95	
50%	11.54	11.23	10.41	11.44	12.23	11.37± 0,59	0.886
25%	0	0	0	0	0	0	
12,50%	0	0	0	0	0	0	
Control -	0	0	0	0	0	0	

From the table above, it can be seen that the IZD that corresponds to the MIC of BME for *S. aureus*, which is 50%, is between 10.41 –12.23 mm with an average of 11.37 mm. Thus it can be concluded that the minimum IZD for BME expressed as having an antimicrobial effect against *S. aureus* is 12.23 mm rounded to 12 mm.

The Spearman correlation test results showed r = 0.886 so that statistically with the addition of concentration the IZD also widened with a very strong correlation.

Table 2: IZD study BME against *S. pyogenes*

Group	Inhibition zone diameter (mm)						
	I	II	III	IV	V	Mean± SD	r
Control +	24.45	23.25	22.18	23.19	22.58	23.15 ± 0,77	
100%	15.57	16.42	15.98	16.11	16.12	16.04 ± 0,28	
50%	10.54	11.23	10.57	10.44	10,.3	10.61 ± 0,33	
25%	8.21	9.11	8.58	9.11	8.78	8.76 ± 0,34	0.985
12,50%	6.11	6.13	6.21	6.15	6.41	6.2 ± 0,11	
Control -	0	0	0	0	0	0 ± 0	

From the table above, it can be seen that the IZD that corresponds to the MIC of BME for *S. pyogenes*, which is 25%, is between 8.21 –9.11 mm with an average of 8.76 mm. Thus it can be concluded that the minimum IZD for BME expressed as having an antimicrobial effect against *S. pyogenes* is 9.11 mm rounded to 9 mm.

The Spearman correlation test results showed *p* values<0.01 and r = 0.985 so that statistically with the addition of concentration the IZD also widened with a very strong correlation.

4. Discussion
The results showed that the MIC for BME against *S. aureus* was 50% and the MIC for *S. pyogenes* was 25%. This shows that there are phytochemical compounds from BME which work to inhibit *S. aureus* and *S. pyogenes* growth. BME contains a variety of biologically active plant chemicals including flavonoids, tannins and saponins which in some studies have antibacterial effects [10-11].

Flavonoids as antibacterial have several cellular targets. One of their molecular actions is to inhibit DNA gyrase so that nucleic acid synthesis is disrupted [10, 11]. In addition, flavonoids can also form complexes with extracellular proteins that interfere with cell wall integrity. Tanin can bind peptidoglycans of *S. aureus* and *S. pyogenes* cell walls so that they interfere with cell wall integrity, inhibit biofilm formation and lysis occurs [12]. Saponins work by interfering with cell membrane permeability bacteria that result in cell leakage [13].

To determine which is the most dominant antibacterial work of BME, it is necessary to conduct a self-study by separating the molecules that have the antibacterial properties above and testing them separately.

Thus, BME cannot be used for conditions that require rapid antibacterial effects such as antiseptics and disinfectants. Its use may be more appropriate for conditions where the slow antibacterial effect remains beneficial, for example, as an ointment, or as an oral medication in patients with a normal immune system.

This study found that minimal IZD for BME could be claimed to have an antibacterial effect against *S. aureus* was 12 mm and against *S. pyogenes* was 9 mm.This study also showed a very strong correlation between BME concentrations with DDH both against *S. aureus* (r = 0.886) and *S. pyogenes* (r = 0.985). This is due to the higher concentration, the greater the antibacterial compounds contained. However, it still needs to be analyzed the relationship between concentration and IZD over a wider range of EBP concentrations so that it can be extrapolated to wider use.

This research has a weakness because the concentrations tested were only 12.5%, 25%, 50% and 100%, so there is a possibility of a lack of precision in setting the MIC. It is necessary to test at concentrations between 25-50% in *S. aureus* and 12.5%-25% in *S. pyogenes* so that the MIC is known to be more precise so that subsequent BME production is more efficient.

The strength of this research is that it uses two methods for the MIC test, namely dilution and diffusion so that IZD can be determined which is sensitive to bitter melon extract based on the results of the MIC dilution test that shows clarity. Other studies that only measure IZD may need to be reinterpreted because the determination of whether or not antibacterial substances are sensitive is not based on appropriate methods [8].

5. Conclusion

The results of the study concluded that the BME was bacteriostatic against *S. aureus* with a MIC of 50% and IZD 12 mm and *S. pyogenes* with a MIC of 25% and IZD 9 mm.

Further research needs to be done on the antibacterial effectiveness of BME against *S. aureus* and *S. pyogenes* using different extraction method, using multilevel concentrations with smaller interval and synergistic effect of a combination of BME with other antibacterial compounds

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