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Effectiveness of Butterfly Pea (Clitoria Ternatea) Extract Against Streptococcus Mutans Bacterial Growth In Vitro

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ABSTRACT

Dental caries is still a significant dental problem and is children's most common infectious disease. Caries is a microbiological infectious disease that can damage the hard tissue of the teeth. Several factors, including the microorganism Streptococcus mutans, cause caries. This bacterium is the main species in dental plaque that plays a role in the etiology of caries. One way to prevent caries is cleaning plaque regularly, one of which is mouthwash. This study aims to determine the effectiveness of butterfly pea flower extract (Clitoria ternatea) on the growth of Streptococcus mutans in vitro. This type of research is laboratory experimental research using a post-test-only control group design approach. The samples used were butterfly pea flower extract (Clitoria ternatea) diluted with DMSO to a concentration of 100%, 50%, 25%, 12.5%, 3.125%, 6.125% and Streptococcus mutans ATCC 25175. This research was conducted using the MIC test dilution method and KBM to see the effectiveness of the antibacterial extract against the test bacteria. Analysis of research data using the one-way ANOVA test ($p < 0.05$) to see significant differences between study groups with the number of test bacteria, followed by a double comparison test with the Post Hoc Least Significance Different (LSD) method to determine the average difference between treatment groups. Antibacterial effectiveness test results showed that butterfly pea flower extract (Clitoria ternatea) was effective in inhibiting Streptococcus mutans bacteria ($p<0.05$), at a concentration of 25% was the minimum inhibitory level (MIC) and 50% concentration was the minimum killing rate (MBC). This study concludes that the extract of butterfly pea flower (Clitoria ternatea) effectively inhibits and kills Streptococcus mutans bacteria.

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Introduction

Dental and oral health problems are significant in health development, especially in elementary school-age children (Sherlyta et al., 2017). Dental caries is still a significant dental problem and is the most common infectious disease in children (Fajriani & Andriani, 2015). Caries is a microbiological infectious disease of the teeth that can damage the hard tissue of the teeth. A cavity in the tooth is a sign of a bacterial infection (Fajriani & Andriani, 2015). The 2018 Basic Health Research results show that dental and oral problems have reached 57.6% in Indonesia (Kementerian Kesehatan RI, 2018).

Bagramian et al (2019) said that nearly 90% of school-age children worldwide suffer from dental caries. Meanwhile, according to the 2013 Centers for Disease Control and Prevention (CDC), dental caries is a chronic disease that often occurs in children aged 6-11 years (25%) and adolescents aged 12-19 years (59%). This problem also occurs in Indonesia. In 2014, the Indonesian Ministry of Health said that 89% of children under the age of 12 suffer from dental caries (Gayatri, 2017). Caries is a multifactorial disease, namely the presence of several primary factors, namely host factors, microorganisms, substrate and time. One of them is the role of Streptococcus mutans, which has acidogenic (acid-producing) and aciduric (acid-resistant) properties (Gayatri, 2017; Pintauli & Hamada, 2008).

Streptococcus mutans is the main species in dental plaque, which plays an important role in the etiology of caries. Streptococcus mutans has

glucosyltransferase (GTF) and fructosyltransferase (FTF) enzymes, which convert sucrose into glucans and fructans, which help the attachment of Streptococcus mutans bacteria and the formation of plaque on the tooth surface. It is essential to prevent caries from an early age; one way to prevent it is by cleaning plaque regularly.

Plaque cleaning can be done mechanically and chemically. Mechanically, it can be done by brushing your teeth ad flossing, while chemically, you can use toothpaste and mouthwash (Riwandy et al., 2014).

According to Fajrian and Andriani (2015), using 0.2% chlorhexidine daily can reduce Streptococcus mutans bacteria by 30-50%. However, chlorhexidine has a drawback, as it is a chemical that can give off an unpleasant taste and cause teeth staining. The return of attention to natural materials (back to nature) has long been considered helpful because it has been believed to prevent various diseases. Many studies have been carried out using natural materials to produce medicines to support dental health service programs, especially in preventing caries (Riwandy et al., 2014).

One plant with the potential as an herbal ingredient is the butterfly pea flower (Clitoria ternatea). This flower is increasingly popular in Indonesia and provides many health benefits. Butterfly pea flowers extracted using various solvents showed broad antimicrobial activity, including gram-positive, gram-negative, and fungi (Marpaung, 2020). Butterfly pea flower is an herbal plant in Indonesia that provides many health benefits. The ingredients in this flower contain phytochemical compounds, including flavonoids and their derivatives flavonol glycosides, kaempferol glycosides, quercetin glycosides, myricetin glycosides, and anthocyanins. This compound is present in all parts of the plant, including leaves, roots, wood, flowers, fruit, and seeds, which are identified as antibacterial (Angriani, 2019).

Research conducted by Riyanto et al. (2019) said that 70% ethanol extract from butterfly pea flowers could inhibit the growth of Pseudomonas aeruginosa bacteria with a concentration of 10% and Bacillus cereus with a concentration of 30%. Previous research was also conducted by Widyarman et al. (2018) regarding the effectiveness of butterfly pea flower extract (Clitoria ternatea) in vitro to inhibit Porphyromonas gingivalis bacteria. The results of the study by Widyarman et al. said that the

concentration of butterfly pea flower extract that can inhibit Porphorymonas gingivalis bacteria is at a concentration of 50%.

Based on the background of the problems that have been described above, research on the effectiveness of butterfly pea flower extract (Clitoria ternatea) against Streptococcus mutans bacteria is still limited, so researchers are interested in researching butterfly pea flower extract (Clitoria ternatea) at concentrations of 100%, 50%, 25%, 125%, 6.25%, and 3.125% which were extracted with 70% alcohol using a positive control of chlorhexidine and negative control of Dimethylsulfoxide (DMSO) in inhibiting the growth of Streptococcus mutans so that later butterfly pea flowers can be used as an alternative to traditional caries prevention. This study aims to determine the antibacterial effectiveness of butterfly pea flower extract (Clitoria ternatea) with each concentration of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, and chlorhexidine on the growth of Streptococcus mutans bacteria.

Research Methods

The type of research is laboratory experimental research using a post-test-only control group design approach. The samples used were butterfly pea flower extract (Clitoria ternatea) diluted with DMSO to a concentration of 100%, 50%, 25%, 12.5%, 3.125%, 6.125% and Streptococcus mutans ATCC 25175. This research was conducted using the MIC test dilution method and KBM to see the effectiveness of the antibacterial extract against the test bacteria. Analysis of research data using the one-way ANOVA test ($p < 0.05$) to see significant differences between study groups with the number of test bacteria, followed by a double comparison test with the Post Hoc Least Significance Different (LSD) method to determine the average difference between treatment groups.

Results and Discussion

a post-test-only control group design research conducted in two laboratories, namely the Traditional Medicine Laboratory, USU Faculty of Pharmacy, to extract butterfly pea flower (Clitoria ternatea) and then test the effectiveness of the extraction results on the growth of Streptococcus mutans bacteria in vitro at the USU Hospital Microbiology Laboratory. This study used six treatment groups consisting of six different concentrations of butterfly pea flower extract (Clitoria ternatea), namely 100%, 50%, 25%, 12.5%, 6.25%, and 3.125%. Chlorhexidine 0.2% was used as a positive control, and DMSO as a negative control. This study used the dilution method to determine the Minimum Inhibitory Concentration (MIC) and Minimum Inhibitory Concentration (MBC) with four repetitions in each test tube.

The test tube, which already contains Nutrient Broth media, is added to the butterfly pea flower extract in various concentrations, and then Streptococcus mutans suspension with 0.5 McFarland turbidity is added. The tubes were vortexed and incubated in an incubator for 24 hours at 37 ℃. After that, observations were made for the presence or absence of sediment on the bottom of the tube. The tube that looks clear (bright purple) and has no precipitate with a minor concentration of butterfly pea extract is the temporary MIC value of the butterfly pea extract on the growth of Streptococcus mutans bacteria.

Observation of the dilution tube has been completed. To ensure that the tube has bacterial growth, it is continued to the subculture stage on TYCSB media and incubated for 24 hours at 37℃; after 24 hours, it will be observed whether or not bacterial colonies are growing on TYCSB media. The petri dish where there was no bacterial growth with a minor concentration of butterfly pea extract was the MBC value of the butterfly pea extract on the growth of Streptococcus mutans bacteria.

Determination of Minimum Inhibitory Content (MIC) and Minimum Killing Content (KBM) of Butterfly Pea Flower Extract (Clitoria ternatea) on the Growth of Streptococcus mutans Bacteria

In the dilution method, the MIC and MBC values were observed after incubation by observing each concentration tube's turbidity level. However, the turbidity level of the butterfly pea extract could not be determined because the concentration was too concentrated and dark. Therefore, it could only be seen by categorizing the color purple as dark or light purple. Observations were made on all repetitions of each concentration of butterfly pea extract at the same time.

Based on Figure 1, the Minimum Inhibitory Content (MIC) value is 25%, which at this concentration has a bright purple color. In comparison, the Minimum Inhibitory Concentration (KBM) value is found at a concentration of 50%, which has a dark purple color. Furthermore, to prove the MIC and MBC values with certainty, a subculture stage was carried out in each dilution tube using TYCSB (Tryptone-yeast-cysteinesucrose-bacitracin) media.

Subculture on TYCSB media was used to obtain Streptococcus mutans ATCC 25175's MIC and MBC values. The number of bacterial colonies on a petri dish was calculated using the TPC (Total Plate Count) method by visual observation and counting.

In Table 1, it can be seen that the results of dilution tube culture at the lowest concentration that can inhibit bacterial growth and show a bacteriostatic effect is a concentration of 25% with an average value of 37 CFU/ml, then this concentration is determined as the MIC value. The concentration of 50% is the lowest concentration where there is no bacterial growth and shows a bacteriocidal effect. A concentration of 50% with an average value of the number of bacterial colonies of 0 CFU/ml is determined as the MBC value.

Antibacterial Effectiveness Test of Each Concentration of Butterfly Pea Flower (Clitoria ternatea) Extract on the Growth of Streptococcus mutans Bacteria

The antibacterial effectiveness of each concentration of butterfly pea flower extract (Clitoria ternatea) against Streptococcus mutans was analyzed using the Way ANOVA test. Before the One-Way ANOVA test was carried out, the data normality test used the Shapiro-Wilk test to determine whether the data was normally distributed. If the data is normally distributed, the test is continued using the One-Way ANOVA and LSD (Least Significant Difference) tests.

The results of the Shapiro-Wilk normality test obtained data at a concentration of 25% with a value of $p = 0.319$, a concentration of 12.5% with a value of $p = 0.934$, a concentration of 6.25% with a value of $p = 0.636$, and a concentration of 3.125% with a value of $p = 0.448$ ($p>0.05$). This shows that the data at each concentration is usually distributed because all p values are> 0.05, so the Way ANOVA test can be carried out. In Table 3, a significance of $p = 0.000$ ($p <0.05$) is obtained, which means that there is

antibacterial effectiveness of butterfly pea flower extract (Clitoria ternatea) on the growth of Streptococcus mutans bacteria.
After the One-Way ANOVA test was carried out, the LSD (Least Significant Difference) test was performed to find out which treatment group pairs were significant. The LSD (Least Significant Difference) test results showed a statistically significant difference between each concentration group.

Discussion

Streptococcus mutans bacteria was chosen as the research sample because Streptococcus mutans is one of the most caries-causing microbial pathogens found in plaque. In children with high levels of caries, there will be an increase in the number of Streptococcus mutans colonies in the oral cavity (Fajriani & Andriani, 2015). Researchers used butterfly pea extract in this study because the butterfly pea flower can inhibit the growth of several types of bacteria.[8] This study used butterfly pea flower (Clitoria ternatea) extract with 70% ethanol at concentrations of 100%, 50%, 25%, 12.5%, 6.25%, and 3.125% and used pure Streptococcus mutans isolates. The isolate was obtained from the American Type Culture Collection (ATCC), numbered 25175.
The one-way ANOVA statistical test, namely the effectiveness test of butterfly pea flower extract (Clitoria ternatea) with concentrations of 100%, 50%, 25%, 12.5%, 6.25%, and 3.125% on the growth of Streptococcus mutans ATCC 25175 in a vitro has a significance value of $p=0.000$ ($p<0.05$). This value indicates the inhibition of butterfly pea flower extract, which has an antibacterial effect on the growth of Streptococcus mutans ATCC 25715 in vitro. To see significant differences between treatment groups, a post hoc Least Significant Difference (LSD) statistical test was performed.

The results of this study prove that butterfly pea flower extract (Clitoria ternatea) has antibacterial activity. This effect can occur because the butterfly pea flower (Clitoria ternatea) has a compound that has the potential as an antibacterial, namely flavonoids. According to research by Sankari et al. (2014), the tested flavonoids have antibacterial activity, especially against Streptococcus mutans. Flavonoids cause damage to the permeability of bacterial cell walls, microsomes, and lysosomes due to interactions between flavonoids and bacterial DNA (Nomer et al., 2019). Flavonoids in the butterfly pea flower are 20.07 ± 0.55 mmol/mg. The purple color of the butterfly pea flower indicates the presence of anthocyanin compounds, so in some countries, the butterfly pea flower is used as a natural food coloring. Nigam and Shrivastava in Riyanto EF et al. in 2019 stated that phytochemical compounds such as alkaloids, flavonoids, and others could be helpful as antibacterials against the growth of many microorganisms.[10] antibacterial Phytochemical compounds are effective against the growth of gram-positive and gram-negative bacteria. This can be seen from previous studies regarding the effectiveness of butterfly pea flower extract as an antibacterial.

This research is in line with the research of Pratap Gowd et al., (2012), which stated that butterfly pea flower extract (Clitoria ternatea) used Distilled water as a solvent against Streptococcus mutans, Lactobacillus casei, Staphylococcus aureus the most effective concentration was 50% where the inhibition zone formed from Streptococcus mutans 7 mm, Lactobacillus casei 8 mm, Staphylococcus aureus 10 mm, so it was found that Staphylococcus aureus had the largest inhibition zone.

Another previous study was also conducted by Widyarman et al. (2018), stating that the extract of butterfly pea flower juice (Clitoria ternatea) was effective in inhibiting the growth of Porphyromonas gingivalis bacteria at a concentration of 50%. Another study

was also conducted by Riyanto et al. (2019) said that 70% ethanol extract of butterfly pea flowers could inhibit the growth of Pseudomonas aeruginosa bacteria with a minimum inhibitory concentration of 10%, and can inhibit the growth of Bacillus cereus bacteria with a minimum inhibitory concentration of 30%.

Differences in the antibacterial effect result from previous studies that used natural ingredients against Streptococcus mutans bacteria could have been caused by differences in methods, materials, solvents, extraction methods, and the types of active compounds of each of these natural ingredients (Kamilla et al., 2009; Pratap Gowd et al., 2012).

The results of this study also found that the MIC value of the butterfly pea flower extract test was not able to exceed the value of the positive control test (0.2% chlorhexidine) because 0.2% chlorhexidine contained a stable bisbiguanide compound (Pratap Gowd et al., 2012). This is in contrast to the flavonoid compounds

found in butterfly pea flower (Clitoria ternatea), which are unstable. Unstable flavonoid compounds can occur because the solvent or temperature can influence them during extraction (Pratap Gowd et al., 2012).

Plaque inhibition can occur due to flavonoid compounds in butterfly pea flowers. These compounds can inactivate the glucosyltransferase enzyme, which plays an important role in plaque formation (Sajjan et al., 2016; Sankari et al., 2014). Caries will quickly occur if plaque formation is not inhibited because Streptococcus mutans also produce extracellular polysaccharides, which can facilitate plaque attachment to the tooth surface. Bacteria will produce acid, which can affect demineralization (Matsumoto-Nakano, 2018).

This study also proves that the hypothesis proposed by the researcher is acceptable, namely that the butterfly pea flower extract with 70% ethanol solvent can inhibit plaque formation and inhibit the Streptococcus mutans bacteria in the oral cavity because it contains flavonoids, alkaloids, and anthocyanins, so research on butterfly pea extract (Clitoria ternatea) Later it can be used as a reference for research on butterfly pea flower (Clitoria ternatea) with mouthwash preparations or topical preparations for young children because it can inhibit Streptococcus mutans bacteria in the oral cavity at a concentration of 25% and kill Streptococcus mutans bacteria at a concentration of 50%.

Table 1
Streptococcus mutans ATCC 25175 Colonies from Dilution Tube Culture

	Number of Bacterial Colonies
No. Concentration of Butterfly Pea Flower Extract (CFU/ml) □□ ± □□□□ P- value **Replication I II III IV**	44 25 37±8.37 4 12.5% 169 143 122 156 147.50±20.04 5 6.25% 215 253 228 247 235.75±17.50 6 3.125% 289 296 277 275 284.25±9.98 7 K+ 0 0 0 0 0 8 K- 301 338 379 356 343.5±32.92 *p- value <0.05 (significant) 0.000*
1 100% 0 0 0 0 0 2 50% 0 0 0 0 0 3 25% 41 38	

Table 2. Differences in Antibacterial Effectiveness of Each Concentration of Butterfly Pea Flower Extract (Clitoria ternatea) against Streptococcus mutans ATCC 25175

Concentration	N	p-values	
100% 50%	4	1,000	
	25%	4	0.003*
	12.5%	4	0.000*
	6.25%	4	0.000*
	3.125%	K+	
	K	0.000*	0.000*
	4	4	4
		1,000	
50% 25%	4	0.003*	
12.5%	4	0.000*	
	6.25%	4	0.000*
	3.125%	K+	
	K	444	1,000
		0.000*	0.000*
25% 12.5%	4	0.000*	
	6.25%	4	0.000*
	3.125%	K+	
		K	0.000* 0.003*
		4 4 4	0.000*
12.5% 6.25%	4	0.000*	
	3.125%	K+	
		K	0.000*
	K	4 4 4 4 4 4 4 4	0.000*
	3.125%	K+	0.000*
		0.000*	0.000*
6.25% 3.125%	K	0.000*	0.000*
	K+	0.000*	0.000*y6

Conclusion

This study confirms that butterfly pea flower (Clitoria ternatea) extract effectively inhibits the growth of Streptococcus mutans, a key bacterium in the etiology of dental caries. The Minimum Inhibitory Concentration (MIC) was determined to be 25%, while the Minimum Bactericidal Concentration (MBC) was 50%. These findings highlight the potential of C. ternatea extract as a natural antibacterial agent.

The research achieves its objective by demonstrating the extract's antibacterial effectiveness and its applicability as an alternative to synthetic chemicals like chlorhexidine. This study contributes to developing herbal-based solutions for dental caries prevention, providing a safer and more effective approach to oral health management.

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