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(RESEARCH ARTICLE)

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Inhibitory potency of butterfly pea (*Clitoria ternatea Linn.*) extract against the growth of *Streptococcus mutans*

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Abstract

Introduction: Dental caries is a multifactorial disease that causes demineralization of the teeth. One of the main bacteria causing dental caries is *Streptococcus mutans*. Various natural antibacterial materials from plant extract can be used as an attempt to inhibit the growth of *Streptococcus mutans* and it has minimal side effects, one of which is butterfly pea extract. From the research that has been done, butterfly pea extract contains phytochemical compounds such as alkaloid, flavonoid, tannin, saponin, and ternatin which act as antibacterial agents against the growth of microorganisms.

Objective: To prove that butterfly pea extract has potential as an antibacterial agent that can be observed from the formation of the inhibition zone of *Streptococcus mutans*.

Methods: The *in vitro* study was conducted by diffusion method using 12 test groups with 4 times repetitions. The test group consisted of a positive control group with chlorhexidine 0.2%, a treatment group with 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% concentration of butterfly pea extract, and a negative control group with sterile distilled water. The samples were then incubated at 37ºC under anaerobic conditions for 24 hours. The inhibition zone formed was then measured using a caliper in millimeters (mm).

Results: Butterfly pea extract can inhibit the growth of *Streptococcus mutans* starting at a concentration of 50%. One Way ANOVA test results P=0.001 (P<0.05), means that there are differences in the inhibition zone of *Streptococcus mutans* in each group.

Conclusion: Butterfly pea extract can inhibit the growth of *Streptococcus mutans*.

Keywords: Butterfly Pea; *Streptococcus mutans*; Dental Caries; Antibacterial

1. Introduction

Dental caries is the most common dental problem complained about by Indonesian people. Based on the results of the Riset Kesehatan Dasar (Riskesdas) in 2018, dental caries is a dental problem with the largest proportion in Indonesia, which is 45.3% with a prevalence of 88.8%. The prevalence of dental caries tends to be high (more than 70%) in all age groups [1]. Dental caries is a multifactorial disease that causes demineralization of the teeth. Dental caries is called a multifactorial disease because the occurrence of dental caries can't be attributed to just one cause. There are three main factors related to the occurrence of dental caries, i.e. tooth surface which is covered by the pellicle, cariogenic bacteria,

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and the presence of fermentable carbohydrates. The two main bacteria that cause dental caries are *Streptococcus mutans* and *Lactobacillus acidophilus*, which are acid-producing bacteria [2].

Streptococcus mutans is a gram-positive bacteria that is the main cause of dental caries. *Streptococcus mutans* has a habitat in the human oral cavity, especially dental plaque. Since it is known that *Streptococcus mutans* is the main bacteria that cause dental caries, prevention and control measures are taken to eliminate or reduce its presence in the oral cavity [3, 4]. Dental caries can be prevented by using topical antibiotics such as vancomycin and bisbiguanides such as chlorhexidine. However, the use of topical antibiotics is not good for long-term use because dysbiosis can occur. The use of chlorhexidine can also cause brownish stains on the tongue and teeth. Irritation of the oral mucosa may also occur. Therefore, the use of plant extracts as antibacterial agents is growing rapidly, since their side effects are minimal [5, 6]. One of the plant extracts that can be used is butterfly pea (*Clitoria ternatea Linn.*) extract. Butterfly pea is used worldwide as an ornamental plant and food coloring material. In addition, butterfly pea flowers are also used traditionally as a medicinal plant in Ayurvedic medicine. The chemical substances contained in the butterfly pea extract are flavonoid, anthocyanin, alkaloid, ternatin, saponin, tannin, taraxerol, and taraxerone [7, 8].

Previous research on the antibacterial activity of butterfly pea extract shows that butterfly pea extract could inhibit the growth of food spoilage bacteria, *Bacillus cereus* and *Pseudomonas aerugenosa*. Butterfly pea extract inhibits *Bacillus cereus* starting at a concentration of 30% with an average diameter of the inhibition zone of 5.92 mm and starting at a concentration of 10% with an average diameter of the inhibition zone of 9.87 mm for *Pseudomonas aerugenosa* [9]. Based on previous research, it can be concluded that butterfly pea extract can inhibit food spoilage bacteria such as *Bacillus cereus* and *Pseudomonas aerugenosa*. It encourages researchers to conduct a research to prove whether there is inhibition of butterfly pea extract against one of the cariogenic bacteria, *Streptococcus mutans*.

2. Material and methods

2.1. Materials and tools

The tools used in the research are a bunsen burner, petri dish, sterile cotton swab, cotton, test tube, ose, anaerobic jar, incubator, paper disc, micropipette, and caliper. The materials used are disinfectant, spiritus, *Streptococcus mutans*, BHIB, MHA, alcohol, butterfly pea extract, sterile distilled water, and chlorhexidine 0.2%. The butterfly pea flower used comes from Blora, Central Java, which was then determined and extracted by maceration technique with 70% ethanol solvent at the UPT Laboratorium Herbal Materia Medica Batu.

2.2. Preparation of butterfly pea extract

100 grams of dried butterfly pea flowers were mashed into powder. The powder was then put into the extractor and 1,000 ml of 70% ethanol solvent was added (powder: solvent = 1 : 10) and then soaked for 24 hours. The solution was filtered using sterile gauze to separate the filtrate and residue. The residue was remacerated once and then the extract filtrate was evaporated using a rotary evaporator. A viscous extract (extract with a concentration of 100%) was obtained. The extract with a concentration of 100% was diluted with distilled water to obtain concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 90% [9].

2.3. Preparation of *Streptococcus mutans*

Streptococcus mutans isolated by the Research Center of the Faculty of Dental Medicine, Universitas Airlangga, Surabaya was collected using an ose needle and then cultured on BHIB media in a test tube. *Streptococcus mutans* in BHIB media were incubated at 37ºC under anaerobic conditions using an anaerobic jar for 24 hours. After incubated, the bacteria were equalized to 0.5 McFarland or equivalent to 1.5 x 108 CFU/ml.

2.4. Antibacterial activity test

Streptococcus mutans were then grown in a petri dish containing MHA. The sample was then divided into the positive control group, treatment group, and negative control group. In the positive control group, *Streptococcus mutans* in MHA were given a paper disc containing 10 µl of chlorhexidine 0.2%. For the treatment group, *Streptococcus mutans* in MHA were given a paper disc which each contained 10 µl of butterfly pea extract with a concentration of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%. In the negative control group, *Streptococcus mutans* in MHA were given a paper disc containing 10 µl of sterile distilled water. MHA was then incubated at 37ºC under anaerobic conditions for 24 hours after the paper disc was placed on top of it.

After MHA was incubated, an inhibition zone formed. The inhibition zone formed appears as a clear zone around the paper disc. The diameter of the inhibition zone was measured using a caliper in millimeters. The test was repeated four times for accuracy.

2.5. Data Analysis

Data were analyzed using IBM SPSS Statistics 25.0 for windows. The normality of data distributions was assessed using the Shapiro-Wilk test because the number of samples is less than 50 (n<50). Levene test was used to assess whether each group has homogenous data. Comparative test using One Way ANOVA to compare the results between each group and to determine whether there is a difference in the inhibition of *Streptococcus mutans* growth.

3. Results

The results of the study were the calculation of the mean inhibition zone of *Streptococcus mutans* diameter and its standard deviation from each group.

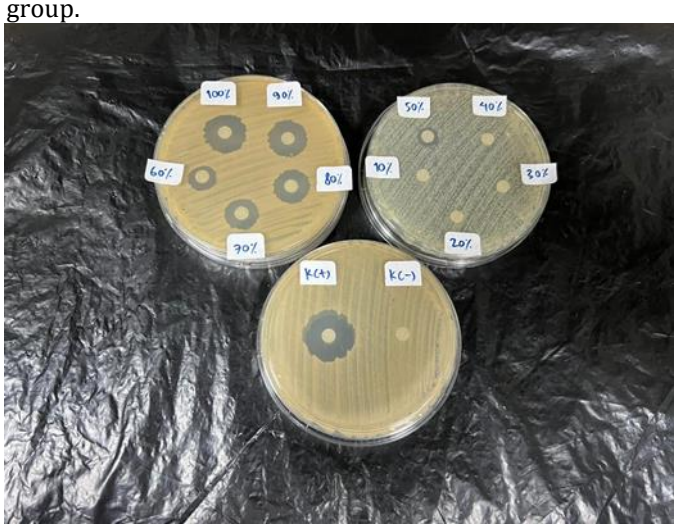

Figure 1 Inhibition Zone Results from the First Repetition

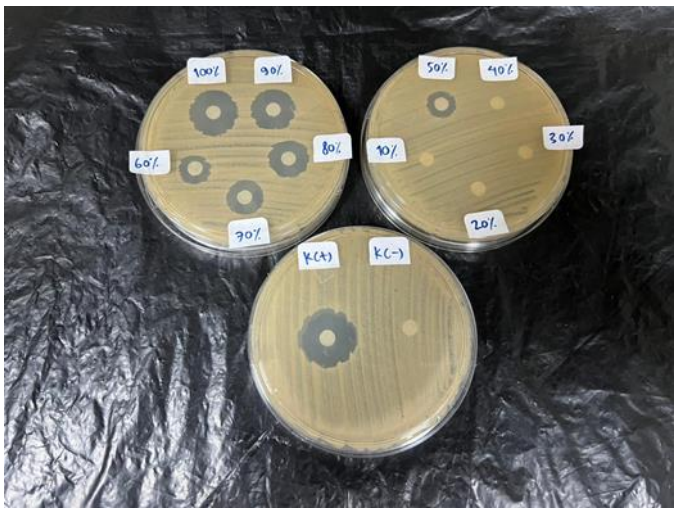

Figure 2 Inhibition Zone Results from the Second Repetition

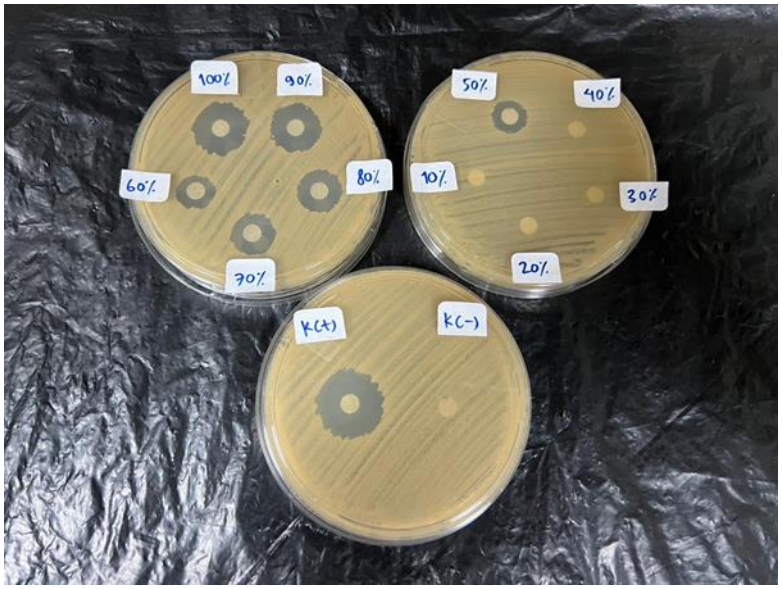

Figure 3 Inhibition Zone Results from the Third Repetition

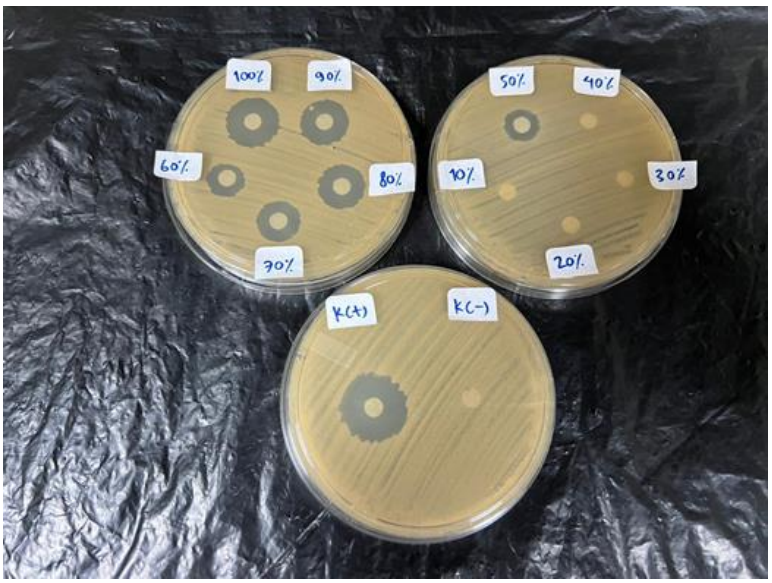

Figure 4 Inhibition Zone Results from the Fourth Repetition

Based on the research data (table 1), there was no inhibition zone formed from 40%, 30%, 20%, 10% butterfly pea extract and sterile distilled water as a negative control group. Butterfly pea extract can inhibit the growth of *Streptococcus mutans* starting at a concentration of 50% with an average inhibition zone diameter of 10.837 mm. The largest inhibition zone diameter was from 100% butterfly pea extract with an average inhibition zone diameter of 22.437mm, although it was still lower than chlorhexidine 0.2% as a positive control group with an average inhibition zone diameter of 24.837 mm.

Table 1 Inhibition zone diameter of *Streptococcus mutans*

Group	N	Average inhibition zone diameter (mm) ± SD
Positive control (Chlorhexidine 0.2%)	4	24.837 ± 0.179
100% butterfly pea extract	4	22.437 ± 0.828
90% butterfly pea extract	4	19.90 ± 0.339
80% butterfly pea extract	4	17.912 ± 0.265
70% butterfly pea extract	4	14.975 ± 0.409
60% butterfly pea extract	4	12.662 ± 0.325
50% butterfly pea extract	4	10.837 ± 0.962
40% butterfly pea extract	4	0
30% butterfly pea extract	4	0
20% butterfly pea extract	4	0
10% butterfly pea extract	4	0
Negative control (Sterile distilled water)	4	0

Description: 6 mm paper disc diameter

Table 2 One Way ANOVA test results

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	632.611	6	105.435	351.067	.001
Within Groups	6.307	21	.300		
Total	638.917	27			

The One Way ANOVA test results found that P = 0.001 (P<0.05). It can be interpreted that there are differences in the inhibition of *Streptococcus mutans* growth in each group.

4. Discussion

Plant extracts are studied for their antibacterial activities to be used as a therapy. One of the plant extracts studied is butterfly pea extract. The butterfly pea flower has been widely used by the public as a food coloring agent and medicinal plant in Ayurvedic medicine. In previous studies, butterfly pea extract has been proven to inhibit the growth of various bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus subtilis*, *Pseudomonas aerugenosa*, and *Streptococcus faecalis* [7, 8, 9, 10, 11, 12].

In this study, the butterfly pea extract used was a butterfly pea extract with 70% ethanol as a solvent. 70% ethanol was chosen as a solvent because it can produce extracts with the highest content of bioactive compounds [13]. After *Streptococcus mutans* were treated with butterfly pea extract, an inhibition zone formed which is indicated by the formation of a clear zone around the paper disc. From the results, it can be seen that the highest average diameter of the inhibition zone was found in the 100% butterfly pea extract group, although it was still lower compared to the 0.2% chlorhexidine group as a positive control. The lowest average diameter of the inhibition zone was found in the 40%, 30%, 20%, and 10% butterfly pea extract groups because there was no inhibition zone formed, the same as the sterile distilled water group as a negative control.

The inhibition zones formed around the paper disc in 50%, 60%, 70%, 80%, 90%, and 100% butterfly pea extract showed that the butterfly pea extract had antibacterial substances that could inhibit the growth of *Streptococcus mutans* . In a previous study, the phytochemical compounds that play a role in inhibiting bacterial activities in butterfly pea

extract are alkaloid, saponin, tannin, and flavonoid [9]. Butterfly pea extract also has a special polyacylated anthocyanin compound, ternatin [14]. All of these active compounds play a role in inhibiting bacterial growth with various specific mechanisms.

Alkaloids inhibit bacterial growth by disrupting the constituent components of peptidoglycan which then cause bacterial cells susceptible to lysis. Alkaloids also inhibit bacterial growth through the process of DNA interaction [15]. Saponin inhibits bacterial growth by acting as a 'detergent' agent. It can cause a decrease in the surface tension of bacterial cells which then leads to a decrease in the bacterial cell wall permeability [16]. Tannin inhibits bacterial growth by inhibiting the bacterial absorption of glucose and amino acids [17]. The mechanism of flavonoids in inhibiting bacterial growth is by acting as DNA intercalating agents [18]. Ternatin inhibits bacterial growth by disrupting the integrity of bacterial cell membranes which has a function as a barrier between the cytoplasm and extracellular [19].

From the results of this study, it was found that the higher the concentration of butterfly pea extract, the greater the average diameter of the inhibition zone formed. This can happen because the higher concentration of the extract, the higher the phytochemical compounds contained in it so that the inhibition zone formed around the paper disc will also be greater [20].

This study showed that butterfly pea extract has an inhibitory power against *Streptococcus mutans* . This is because the butterfly pea extract contains various phytochemical compounds as antibacterial agents against *Streptococcus mutans* . In future research, this butterfly pea extract can be developed into an alternative topical drug or mixed ingredients in mouthwash for the preventive treatment of dental caries and other oral infections.

5. Conclusion

Butterfly pea extract can inhibit the growth of *Streptococcus mutans*.

Compliance with ethical standards

Acknowledgements

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Disclosure of conflict of interest

There is no conflict of interest between the authors.

Statement of ethical approval

An ethical clearance certificate which is a requirement for conducting this research has been obtained from the Health Research Ethical Clearance Commission of the Faculty of Dental Medicine, Universitas Airlangga (No. 561/HRECC.FODM/VIII/2022).

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