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Antibacterial Potency Test of Suruhan Leaf Ethanol Extract (*Peperomia pellucida* (L.) Kunth) Against *Streptococcus mutans* Growth

Amalia[1], Trisnawaty K[1*], Anton[1]

[1]Dentistry Study Program, Faculty of Medicine, Sriwijaya University, Palembang, Indonesia
*Correspondence author email: trishfen@gmail.com

Abstract

Introduction: One of the pathogenic bacteria that causes dental caries was *Streptococcus mutans*. The use of antibacterial agents such as chlorhexidine was the gold standard for eliminating *Streptococcus mutans* but can cause side effects if used long term. Traditional medicine is still used as an alternative by Indonesian people to reduce the side effects of chemical drugs. Suruhan leaves were wild plants that contain antibacterial compounds in the form of alkaloids, flavonoids, saponins, tannins, and phenols which can inhibit bacterial growth. **Purpose:** This study aims to determine the antibacterial potency of suruhan leaves extract against the growth of *Streptococcus mutans* bacteria. **Methods:** This study was an in vitro laboratory experimental study. The treatment group consisted of ethanol extract of suruhan leaves in concentrations of 5%, 10%, 15%, 25%, and 50%. The control group consisted of 0.2% chlorhexidine as positive control and distilled water as negative control. Antibacterial activity test used the disc diffusion method. The inhibition zone values were measured then analyzed using statistical one-way ANOVA and post-hoc Tukey tests. **Results:** The largest mean inhibition zone of ethanol extract of suruhan leaves formed was 16.47 mm at a concentration of 50%, whereas at a concentration of 5%, it had no antibacterial activity. **Conclusion:** Ethanol extract of suruhan leaves (*Peperomia pellucida* (L.) Kunth) concentrations of 10%, 15%, 25%, and 50% had antibacterial activity against *Streptococcus mutans*.

Keywords: antibacterial, suruhan leaf, *Streptococcus mutans*.

Introduction

Most diseases in the oral cavity begin with the formation of dental plaque.[1] Dental plaque is an accumulation of bacteria in an organic matrix that adheres firmly to the surface of the teeth. It consists of microorganisms that multiply within the intercellular matrix in the form of sticky bacteria and their products.[2] Plaque formation is primarily initiated by *Streptococcus mutans*, which produces the enzyme glucosyl transferase (GTF). This enzyme converts sucrose into glucan, facilitationg the formation of dental plaque.[1]

Streptococcus mutans can adhere to the tooth surface and hydrolyze the remaining food particles between the teeth, leading to plaque accumulation on the tooth enamel. This is the initial stage of dental caries.[3] Dental plaque also serves as a risk factor for periodontal disease.[4] Its accumulation causing conditions such as gingivitis in soft tissue and dental caries in hard.[2,4] Dental caries result from several interacting factors, including the host (teeth and saliva),

microorganisms, substrate, and time.[5] *S. mutans* is also associated with other dental conditions like dry sockets and dentoalveolar abscess.[6,7] Dental caries involve the demineralization of hard tooth tissues due to carbohydrate fermentation by acid-producing bacteria.

Chlorhexidine is a commonly used antibacterial agent in dentistry. However, its long-term use can lead to side effects such as tooth discoloration, mouth and tongue irritation, xerostomia, and reduced taste sensation.[8] Efforts are made to reduce these side effects, including the use of natural or herbal alternatives. One promising herbal option is the suruhan plant, also known as Chinese betel leaf (*Peperomia pellucida* (L.) Kunth), to be used as an inhibitor of bacterial growth or antibacterial. Suruhan leaves contain important compounds such as tannins, flavonoids, glycosides, alkaloids, saponins, terpenoids, phenolic compounds, phytosterols, and other steroids.[9]

According to Harbone (1987) in Mappa et al. (2013) tannins and flavonoids exhibit antiseptic and antibacterial properties.[10] Research on the ethanol extract of suruhan leaves, which is believed to inhibit the growth of *S. mutans* bacteria has not been conducted previously. Therefore, this study aims to investigate the antibacterial efficacy of suruhan leaves extract (*Peperomia pellucida* (L.) Kunth) against *S. mutans*.

Methods

This research is an in vitro laboratory experimental study with a post-test only control group design, conducted in July 2023 at the Laboratory Chemical Engineering of the Sriwijaya Polytechnic, Palembang, to create suruhan leaf ethanol extract. In August 2023, antibacterial tests were conducted at the Research Center Laboratory of the Faculty of Dentistry, Airlangga University, Surabaya. The research subjects were colonies of *Streptococcus mutans* obtained from Research Center Laboratory of the Faculty of Dentistry, Airlangga University.

Fresh, green, non-perforated suruhan leaves with a height of ± 15-30 cm and diameter of ± 2.5 x 2 cm, aged from 4 months, were used. The leaves were sourced from the UPTD Center for Development and Production of Food Plant and Horticulture Seeds, South Sumatra Agricultural Service. A hundred grams of suruhan leaves were weighed, placed in a maceration container, and soaked in 1 liter of 96% ethanol. The container was covered with aluminum foil and left for 3 x 24 hours at room temperature with occasional stirring. The mixture was then filtered to obtain a liquid extract, which was concentrated using a rotary evaporator and further evaporated into a thick extract on a hotplate.[11]

For the antibacterial test, empty discs were used for each test material, and 0.01 ml of the test material was applied using a micropipette and left for 60 minutes. The discs were divided into 7 groups for each concentration.[1] Next, the discs with the test material were placed on the MHA media, incubated at 37°C, and observed after 48 hours. The clear zone formed around the disc was measured to determine antibacterial activity.[1]

After 48 hours of incubation, the diameter of the inhibition zone around each paper disc was measured using a ratio between the outer diameter of the inhibition zone and the diameter of the paper disc using a caliper. Measurements are carried out Fig.1.[12]

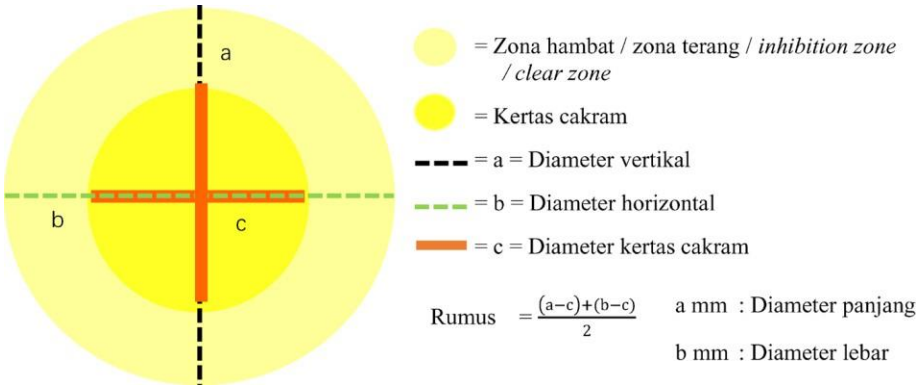

Figure 1. Measurement of the bacterial inhibition zone[12]

Data analysis and processing was carried out using SPSS. The normality test uses the Shapiro-Wilk test to determine whether the data distribution is normal or not. Homogeneity test using *Levene's* test. Then the One Way ANOVA statistical test was used to examine the two variables studied and continued with the Post-Hoc Tukey test to determine the significance of the inhibitory power value of the ethanol extract of suruhan leaves *(Peperomia pellucida* (L.) Kunth).

Results

The phytochemical test used in this research is qualitative where the test is carried out by looking at changes in color reactions using certain reagents. Factors that can influence phytochemical tests include the choice of solvent and extraction method. The solvent used is ethanol and the extraction method is maceration. The results of the phytochemical test for the ethanol extract of the suruhan leaves can be seen in Figure 2. The extract of the suruhan leaves positively contains active compounds in the form of alkaloids, flavonoids, tannins and

phenols from the qualitative phytochemical test results.

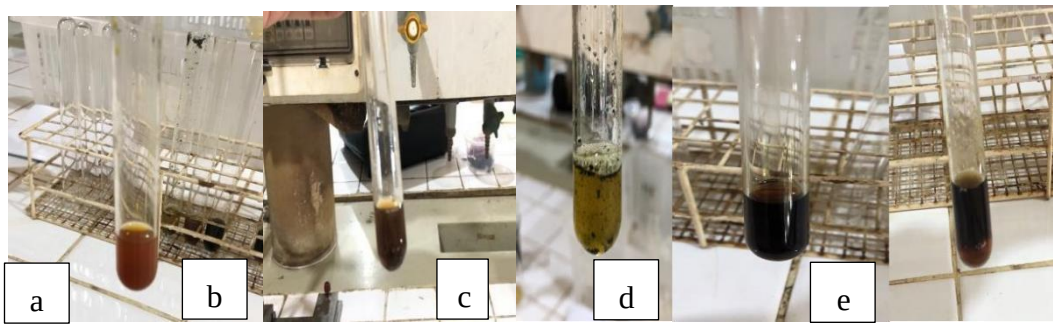

Figure 2. Phytochemical test results of ethanol extract of suruhan leaves: (a) Alkaloids, (b) Flavonoids, (c) Saponins, (d) Tannins, (e) Phenols.

Inhibition Zone of Suruhan Leaf Ethanol Extract

There were 7 test groups in this study which is the suruhan leaf extract group with concentrations of 5%, 10%, 15%, 20%, 50%, the positive control group in the form of 0.2% chlorhexidine, and the negative control group in the form of distilled water. This research used the disc paper diffusion method to see the antibacterial power so that the diameter of the inhibition zone was obtained in the form of a clear zone around the disc paper. This research was carried out four times and the results of the inhibitory power test for suruhan leaf extract including measuring the diameter of the inhibitory zone can be seen in Fig. 3 and Table 1.

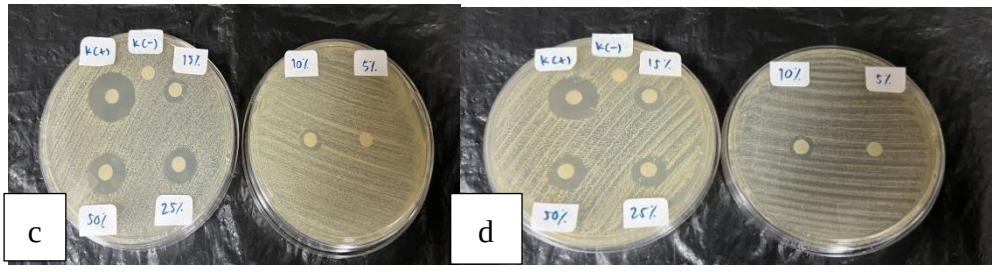

Figure 3. Inhibition Zone Test Results of Ethanol Extract of Suruhan Leaves (*Peperomia pellucida* (L.) Kunth) on the Growth of *Streptococcus mutans*. (a) Repetition 1, (b) Repetition 2, (c) Repetition 3, (d) Repetition 4.

Table 1 shows that the 0.2% chlorhexidine group as a positive control had the highest average diameter of the inhibition zone is 21.63 mm against *S. mutans* bacteria, followed by the ethanol extract treatment group of suruhan leaves with concentrations of 50%, 25%, 15 %, and 10%. The lowest average diameter of the inhibition zone was 0 mm in the 5% concentration of the 5% ethanol extract treatment group and in the negative control group of distilled water.

Table 1. Diameter of Inhibitory Zone of Ethanol Extract of Suruhan Leaves (*Peperomia pellucida* (L.) Kunth) against *Streptococcus mutans*

No.	Treatment	Inhibition Zone Diameter (mm)				Mean (mm) ± SD
		1	2	3	4	
1.	**Suruhan Leaves Extract 5%**	0	0	0	0	0±0.000
2.	**Suruhan Leaves Extract 10%**	10.20	10.05	10.15	10.05	10.11 ± 0.075
3.	**Suruhan Leaves Extract 15%**	11.40	11.60	11.75	11.55	11.58 ± 0.144
4.	**Suruhan Leaves Extract 25%**	13.60	13.40	13.80	13.55	13.59 ± 0.165
5.	**Suruhan Leaves Extract 50%**	16.20	16.40	16.55	16.75	16.47 ± 0.233
6.	**Positive Control (Chlorhexidine 0.2%)**	21.80	21.60	21.35	21.75	21.63 ± 0.202
7.	**Negative Control (Aquades)**	0	0	0	0	0±0.000

Discussion

The results of this study indicate that the ethanol extract of suruhan leaves has antibacterial power against *Streptococcus mutans* at concentrations of 10%, 15%, 25%, and 50% with an average zone of inhibition of 10.11 mm respectively; 11.58mm; 13.59mm; and 16.47 mm, while at an extract concentration of 5% no clear zone was formed, which means it was not able to inhibit *Streptococcus mutans* bacteria in four repetitions. This is not in line

with research by Imansyah et al. (2022) where a concentration of 5% has formed a clear zone of 6 mm, which means it has antibacterial activity. This difference lies in the type of bacteria used, namely Propionibacterium acnes.[11] The lowest concentration has a difference in inhibiting these bacteria due to the response of bacterial cells and the sensitivity factor of the test to the antibacterial compounds in the extract of suruhan leaves.[13] This difference in results is because each bacteria has different properties and resistance to an antibacterial even though the bacteria belong to the same group, namely the Gram-positive bacteria group.[14]

According to Jawetz et al (2005), there are 4 factors that influence antibacterial activity: extract concentration, metabolite compound content, extract diffusion power, and the type of bacteria being inhibited. Fraction concentration and type of bacteria can influence diffusion.[15] The antibacterial activity will increase as the solution concentration increases. The concentration of the antibacterial compound is one of the determining factors that determines the ability of the compound to inhibit the growth of the test bacteria. However, at a certain concentration, an increase in concentration is not always accompanied by an increase in the diameter of the inhibition zone.[16] It is possible that this occurs because the thickness of the media and the diameter of the disc can influence differences in the diffusion speed of antibacterial compounds in the disc media.[15,16]

The antibacterial activity in inhibiting the growth of *S. mutans* can be caused by the role of the bioactive compounds contained in the suruhan leaf extract. The content of bioactive compounds in the suruhan leaf extract has been tested through qualitative phytochemical tests before carrying out the antibacterial inhibition zone test. The test results showed that the extract of suruhan leaves positively contained active compounds in the form of alkaloids, flavonoids, saponins, tannins, and phenols which was in line with the research of Igwe et al.

and Idris et al. Quantitatively in their research, the extract of suruhan leaf contained alkaloids, flavonoids, saponins, tannins, phenols, steroids, glycosides, and anthraquinones.[17,18]

The average diameter of the inhibitory zone of 0.2% chlorhexidine was 21.63 mm, which based on the classification of antibacterial activity categories proposed by Morales, 0.2% chlorhexidine was included in the very strong inhibitory activity group, while the average diameter of the inhibitory zone of the ethanol extract suruhan leaves of 16.47 mm are included in the strong inhibitory activity group at a concentration of 50%.[19] Meanwhile, distilled water was used in the negative control group because it is a compound that cannot affect growthbacteria.43 This is shown by the fact that there is no zone of inhibition against the growth of *S. mutans* with distilled water dripped on the media. Thus, distilled water is declared safe as a solvent by relying on it as a concentrated solvent for the ethanol extract of suruhan leaves.[16,20]

The weakness of this research is that the phytochemical test was not carried out quantitatively. Quantitative phytochemical tests can be used to determine the total levels of active compounds contained in suruhan leaves. It is hoped that the results of quantitative phytochemical tests can be used as a reference to support further research so that their use is maximized.

Conclusion

The ethanol extract of suruhan leaves (*Peperomia pellucida* (L.) Kunth) has antibacterial power against the growth of *Streptococcus mutans* bacteria. The concentration of 50% ethanol extract of suruhan leaves had the largest zone of inhibition in this study is 16.47 mm in average.

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