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The Antibacterial Efficacy of Miana Leaves Extract against *Streptococcus Mutans*

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Abstract

Introduction: *Streptococcus mutans* is the main bacterium that causes dental caries. Miana leaves antimicrobial compounds such as flavonoids, alkaloids, tannins, and saponins are expected to have antibacterial properties against the growth of *Streptococcus mutans*. **Purpose:** To determine the antibacterial effect of miana leaves extract on *Streptococcus mutans* growth. **Methods**: This study was an in vitro laboratory experimental study with a post-test only control group design. The treatment groups consisted of Miana leaves extract using concentrations of 3,125%, 6,25%, 12,5%, and 25%, positive control (0.2% chlorhexidine) and negative control (dimethyl sulfoxide/DMSO). Inhibition zone test was done using disc diffusion method. **Results**: The results showed that each concentration of Miana leaves extract had antibacterial properties. The largest diameter of the inhibitory zone was at 25% concentration, but significant smaller than 0.2% chlorhexidine. **Conclusion**: Miana leaves extract had an antibacterial effect against *Streptococcus mutans*.
Keywords: Antibacterial; Miana leaves; *Streptococcus mutans*

Introduction

Dental caries is a chronic infectious disease that involves destruction of tooth hard tissues structure.[1] According to the results of the 2018 National Basic Health Research (RISKESDAS), the rate of dental caries occurrence in Indonesia is 88.8%. The data shows that dental caries have the highest incidence compared to other dental and oral health problems.[2] The etiology of dental caries is multifaceted, with bacterial involvement being one significant factor. Among these bacteria, *Streptococcus mutans* stands out as the primary culprit responsible for dental caries.

S. mutans, the predominant gram-positive bacterium in the oral cavity, is pathogenic and adept at adhering to tooth surfaces. It thrives in acidic conditions and produces acid through

carbohydrate fermentation, leading to tooth demineralization.[3,4] In dentistry, antibacterial agents are essential for managing oral health issues like dental caries by controlling bacterial growth.[5,6]

The efficacy of these agents in suppressing bacterial growth is termed antibacterial effectiveness. Natural antibacterials present a promising alternative due to their safety profile, minimal side effects, accessibility, and cost-effectiveness compared to synthetic one. They are derived from active components of herbal ingredients, available in extracts or as individual compounds.[7]

One of the utilization of active plant components found in Indonesia is miana leaves (*Coleus scutellarioides*) from the *Lamiaceace* family.[8] The active components of this plant are reported to have various pharmacological activities such as antibacterial, antioxidant, antihistamine, anti-diabetic which support medicinal functions. The phytochemical content of *C. scutellarioides* leaves includes alkaloids, flavonoid, terpenoids, tannin, steroids, and saponin, some of which have antibacterial effects.[9]

C. scutellarioides leaves extract as an antibacterial is known to inhibit gram-positive and gram-negative bacteria with a minimum inhibitory content (MIC) of 2.5% against *Staphylococcus aureus* and *Eschericia coli*.[10] Recent study by Anita et al. (2019) shows that the concentration of miana leaf extract (250; 125; 62.5; 31.25; 15.62 mg/ml) has an inhibitory effect on the growth of *E. Coli* with an inhibition zone of 18 mm; 15 mm; 11.03 mm, 9 mm; and 7 mm.[11] This study was aimed to investigate the antibacterial effect of miana leaves extract on the growth of oral pathogenic bacteria *Streptococcus mutans*.

Methods

This experimental study aimed to assess the antibacterial efficacy of *C. scutellarioides* leaf extract against *Streptococcus mutans* through an in vitro investigation employing a post-test only control group design. The ethanol extraction of *C. scutellarioides* leaves was conducted using the maceration technique. The *S. mutans* bacteria strain ATCC® 25175TM obtained from the Clinical Microbiology Laboratory, University of North Sumatra, was utilized. Treatment groups comprised four groups of *C. scutellarioides* leaf ethanol extract with concentrations of 3.125%,

6.25%, 12.5%, and 25%. The positive control group was treated with 0.2% chlorhexidine, while the negative control group received a DMSO solution. The repetition count in each group was determined using the Federer formula, and the results were replicated five times for each group. All equipment utilized in this research was washed and sterilized for 15 minutes at a temperature of 121℃ using an autoclave.[11]

Sample and extract preparation

The leaves of *C. scutellarioides* undergo a cleaning process under running water until free of impurities, followed by thin slicing and air-drying. Subsequently, the dried miana leaves are pulverized using a blender and sifted through a fine sieve to obtain a powdered form. Sixty grams of this powder are precisely measured using an analytical balance and transferred into an Erlenmeyer flask, followed by the addition of 225 ml of 95% ethanol solvent until the simplicia is completely immersed. The solution is then allowed to stand for 5 days, covered with aluminum foil, and occasionally stirred. Subsequently, the solution is filtered through filter paper to obtain a liquid filtrate, which is then evaporated using a rotary evaporator to yield a concentrated extract of miana leaves. This thick extract is left at room temperature until all traces of ethanol solvent evaporate. The resulting dense extract is then diluted with DMSO solvent to achieve desired concentrations (3.125%, 6.25%, 12.5%, and 25%) using the dilution formula M1.V1=M2.V2.

The Mueller Hinton Agar (MHA) was weighed as much as 3.8 g and then dissolved in 100 ml of distilled water, then the solution was transferred into an Erlenmeyer. The solution is heated using a hot plate and stirred. The media was sterilized in an autoclave at 121°C for 15 minutes, poured into a petri dish, and ready to use after hardening.[12] Next, the tested bacteria (*S. mutans*) taken with 10 µL inoculating loop blue and then suspended in a tube containing 0.45% NaCl solution until turbidity obtained was the same as the standard *McFarland* 0.5 turbidity, 0.5-0.63 McF as measured using a density check.[13]

The antibacterial inhibition test was carried out by the disc diffusion method. Paper discs with a diameter of 6 mm were taken using a lancet and then placed on MHA media inoculated with bacterial suspension. Each solution of tested and control groups were taken as much as 20

µl using a micropipette and then dripped onto each disc paper. The petri dish incubated for 18-24 hours at 37ºC.

Results

The assessment of *C. scutellarioides* leaf extract's antibacterial effectiveness against *Streptococcus mutans* was based on the presence of inhibition zones, which were statistically analyzed using the parametric One-Way ANOVA test. Inhibition zones are depicted as clear or translucent areas surrounding the disc where the antibacterial agent diffuses. These clear zones were measured in millimeters (mm) using a vernier caliper. The diameters of the inhibition zones are presented in Table 1.

Table 1. Inhibition zone diameter

No	Groups	Inhibition zone (mm)					Mean ±SD
		I	II	III	IV	V	
1	Miana leaf extract 3,125%	4,00	3,75	3,50	3,75	4,00	3,80±0,20
2	Miana leaf extract 6,25%	5,50	5,50	5,00	5,00	5,25	5,25±0,25
3	Miana leaf extract 12,5%	7,75	7,50	7,00	7,75	7,50	7,50±0,30
4	Miana leaf extract 25%	9,50	9,75	9,75	10,00	10,00	9,80±0,20
5	Control + (CHX 0,2%)	20,50	21,00	20,50	20,25	20,75	20,60±0,28
6	Control - (DMSO)	0,00	0,00	0,00	0,00	0,00	0,00±0,00

The measuring results of inhibition zone with disc diffusion method showed that all groups of miana leaf extract had an inhibition zone against the growth of *S. mutans* bacteria. The 25% concentration of miana leaf extract group had the largest average inhibition zone diameter compared to other concentrations with an average of 9.80 ± 0.20 mm. The positive control group showed the largest average inhibition zone diameter among all sample groups at 20.60 ± 0.28 mm. Data analysis was performed using the parametric *One-Way ANOVA* test to see the significance value of the difference in the average inhibition zone between test groups. The results of the *Saphiro-Wilk* normality test and *Levene's* test homogeneity test show that the significance value is greater than 0.05 (p>0.05) which means that the data is normally distributed and homogeneous. Furthermore, parametric tests were carried out in the form of the *One-Way ANOVA* test and the results can be seen in table 2.

Table 2. *One-Way ANOVA* test

No	Groups	n	Inhibition zones	*p*
1	Miana leaf extract 3,125%	5	3,80±0,20	0,00*
2	Miana leaf extract 6,25%	5	5,25±0,25	
3	Miana leaf extract 12,5%	5	7,50±0,30	
4	Miana leaf extract 25%	5	9,80±0,20	
5	Control + (CHX 0,2%)	5	20,60±0,28	
6	Control - (DMSO)	5	0,00±0,00	

**One way ANOVA*, *p*=0,05

Table 2 shows the significance value of the *One-Way ANOVA* test is 0.000 or $p<0.05$, which means that there is a significant difference in the inhibition zone between test groups. For the significance value of each test group, *Post-hoc* analysis (*Tukey* HSD) was conducted and the results can be seen in table 3.

Table 3. *Post-hoc* (*Tukey* HSD) test

Groups	Tukey HSD test					
	3,125%	6,25%	12,5%	25%	CHX 0,2%	DMSO
3,125%		0,000*	0,000*	0,000*	0,000*	0,000*
6,25%	0,000*		0,000*	0,000*	0,000*	0,000*
12,5%	0,000*	0,000*		0,000*	0,000*	0,000*
25%	0,000*	0,000*	0,000*		0,000*	0,000*
CHX 0,2%	0,000*	0,000*	0,000*	0,000*		0,000*
DMSO	0,000*	0,000*	0,000*	0,000*	0,000*	

**Post-hoc*, Tukey HSD test, *p*=0,05

The results of *Post-hoc* analysis (*Tukey* HSD) in Table 3 show that there is a significant difference in the average diameter of the inhibition zone ($p<0.05$) between all test groups. *C. scutellarioides* leaves extract with the smallest concentration (3.125%) has a significant difference compared to the negative control, so the result shows that *C. scutellarioides* leaves extract at a concentration of 3.125% has an antibacterial effect on the growth of *Streptococcus mutans*. The highest concentration (25%) has a significant difference compared to all other extract groups and the control group, but the antibacterial effect was not as good as chlorhexidine 0.2%.

Discussion

The antibacterial effect of miana leaf extract on the growth of *Streptococcus mutans* bacteria in this study uses a disc diffusion test to see the presence of an inhibition zone. Based on the results of the disc diffusion test, all concentrations of miana leaf extract had antibacterial power against *S. mutans*, as seen from the formation of inhibition zones at all concentrations with significant differences from the negative control. This is the same as the results of research by Muljono et al. (2016) who found that there was antibacterial activity of miana leaf extract against *Streptococcus sp.* and *Pseudomonas sp*. The average inhibition zone of miana leaf extract in this study increased as the concentration of miana leaf extract increased.[13] This is in line with the research of Anita et al. (2019) state that the concentration of miana leaf extracts 15.62 mg/ml, 31.25 mg/ml, 62.5 mg/ml, 125 mg/ml, 250 mg/ml has inhibition against *E. coli* growth with inhibition zones of 7 mm, 9 mm, 11.03 mm, 15 mm, and 18 mm, respectively.[11] The bactericidal effect of miana leaf extract influenced by the extract concentration. The higher the concentration of miana leaf extract, the higher the active compounds contained in the extract, the antibacterial activity is also greater.[14] As in the research of Kawengian et al. (2017) on the antibacterial ability of lemongrass leaf extract, tannins at low extract concentrations have a bacteriostatic effect, and at higher extract concentrations have the ability to kill bacteria or bactericidal.[15]

The antibacterial activity of miana leaf extract is derived from its secondary metabolite compounds, namely flavonoids, alkaloids, tannins, and saponins.[9] The results of phytochemical tests by Novalia et al. (2018) on miana leaves obtained flavonoid, steroid, saponin, and terpenoid compounds. Flavonoids work as antibacterials with their ability to form complex compounds with soluble extracellular proteins, affecting bacterial cell integrity and disrupting intracellular compounds. Flavonoids can also inhibit bacterial cell metabolism and nucleic acid synthesis due to the interaction with bacterial DNA.[16] Saponins can reduce the surface tension of the cell wall, causing bacterial cell leakage.[11] Alkaloids and tannins as antibacterials can inhibit cell wall synthesis and lead to bacterial cell death by disrupting peptidoglycan, a component of the cell wall of bacterial cells so that the cell wall layer formed imperfectly. Tannin can also inhibit the formation of bacterial cell proteins.[17]

The positive control group (chlorhexidine 0.2%) produced a larger average inhibition zone than the miana leaf extract test group. There was a statistically significant difference in the average inhibition zone between chlorhexidine 0.2% and 25% concentration extract. Chlorhexidine 0.2% is a gold standard antibacterial ingredient known to be effective as an antibacterial. The mechanism of chlorhexidine as an antibacterial is by forming a bond between positive molecules in chlorhexidine to negative molecules in the bacterial cell wall.[17] The occurrence of these bonds will damage the bacterial cell wall so that chlorhexidine can diffuse into bacterial cells and attack the cytoplasmic membrane causing leakage of bacterial cell components. In the negative control group, all repetitions of treatment did not produce an inhibition zone, which means that DMSO has no antibacterial activity. This is in line with the research of Djuanda et al (2019) who used DMSO as a negative control with the results of DMSO having no antibacterial power.[18]

Conclusion

Miana leaf extract has an antibacterial effect on the growth of *Streptococcus mutans*, but at a 25% concentration its effectiveness is not as good as chlorhexidine 0.2%.

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