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ORIGINAL RESEARCH

Antibacterial Effect of Coleus scutellarioides Leaf Extract Against the Growth of Streptococcus mutans

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KEYWORDS

Antibacterial agents; Coleus scutellarioides; Dental caries; Phytotherapy; Streptococcus mutans.

ABSTRACT

Natural antibacterial agents have attracted considerable attention as safer and more affordable alternatives to synthetic antimicrobials. Coleus scutellarioides (miana) leaves contain flavonoids, alkaloids, tannins, and saponins with potential antibacterial properties. This study evaluated the antibacterial activity of C. scutellarioides leaf extract against Streptococcus mutans, the primary causative bacterium of dental caries. An in vitro laboratory experiment with a post-test-only control group design was conducted. Leaf extract was tested at concentrations of 15.62, 31.25, 62.5, 125, and 250 mg/mL. Chlorhexidine 0.2% and dimethyl sulfoxide (DMSO) served as positive and negative controls, respectively. Antibacterial activity was assessed using the disk diffusion method, while the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by broth dilution. The extract showed concentration-dependent antibacterial activity. Mean inhibition zones were 3.80 ± 0.20, 5.25 ± 0.25, 7.50 ± 0.30, and 9.80 ± 0.20 mm at concentrations of 31.25, 62.5, 125, and 250 mg/mL, respectively. No inhibition zone was observed at 15.62 mg/mL or in the negative control. The MIC and MBC were 62.5 mg/mL and 125 mg/mL, respectively. It can be concluded that Coleus scutellarioides leaf extract demonstrated antibacterial activity against Streptococcus mutans and shows potential as a natural antibacterial agent for caries prevention.

1. INTRODUCTION

Natural antibacterial agents have attracted increasing interest as alternatives to synthetic antimicrobials because they are considered safer, more affordable, and associated with fewer adverse effects.[1] Their antibacterial efficacy is commonly evaluated by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).[2] Medicinal plants represent an important source of bioactive compounds with antimicrobial potential and have been widely investigated for the prevention and treatment of infectious diseases.[3] Coleus scutellarioides (miana), a medicinal plant belonging to the Lamiaceae family, contains several phytochemicals, including flavonoids, alkaloids, tannins, saponins, terpenoids, and steroids, which have demonstrated antibacterial activity.[4] Previous studies have reported that C. scutellarioides leaf extract inhibits both Gram-positive and Gram-negative bacteria, including Staphylococcus aureus and Escherichia coli, with antibacterial activity increasing in a concentration-dependent manner.[5] In dentistry, antibacterial agents play an important role in preventing dental caries by suppressing the growth of cariogenic bacteria.[6] Dental caries remains one of the most prevalent oral diseases worldwide and is a major public health concern in Indonesia. Streptococcus mutans is recognized as the primary etiological agent of dental caries because of its ability to adhere to tooth surfaces, metabolize dietary carbohydrates, produce organic acids, and survive in acidic environments, leading to enamel demineralization.[7] Although the antibacterial properties of C. scutellarioides have been demonstrated against several bacterial species, evidence regarding its activity against S. mutans remains limited. Therefore, this study aimed to evaluate the antibacterial effectiveness of C. scutellarioides leaf extract against Streptococcus mutans by determining its inhibition zone, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC).

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2. METHODOLOGY

This in vitro laboratory experimental study employed a post-test-only control group design to evaluate the antibacterial activity of Coleus scutellarioides (L.) Benth leaf extract against Streptococcus mutans ATCC® 25175. Fresh reddish-brown C. scutellarioides leaves were collected from Indralaya, Ogan Ilir, Indonesia. The leaves were washed, air-dried, powdered, and extracted by maceration in 95% ethanol for five days. The filtrate was concentrated using a rotary evaporator to obtain a crude extract, which was stored in a sealed container until use. The extract was tested at concentrations of 15.62, 31.25, 62.5, 125, and 250 mg/mL. Chlorhexidine 0.2% and dimethyl sulfoxide (DMSO) served as the positive and negative controls, respectively. Each group consisted of five independent replicates, giving a total of 35 samples. S. mutans ATCC® 25175™ was cultured and adjusted to a 0.5 McFarland standard before inoculation. Antibacterial activity was evaluated using the disk diffusion method on Mueller–Hinton agar. Sterile 6-mm paper disks were impregnated with 20 µL of each test solution and incubated at 37°C for 18–24 h. Inhibition zone diameters were measured using a digital caliper. The minimum inhibitory concentration (MIC) was determined using the broth dilution method in Mueller–Hinton broth, while the minimum bactericidal concentration (MBC) was determined by subculturing MIC-negative tubes onto Mueller–Hinton agar and identifying the lowest concentration showing no bacterial growth after incubation. Data were analyzed using SPSS software version 26.0; IBM Corp., Armonk, NY, USA. Normality and homogeneity were assessed using the Shapiro–Wilk and Levene's tests, respectively. Differences among groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post hoc test. Statistical significance was set at $p < 0.05$.

3. RESULTS

The mean inhibition zone diameters of Coleus scutellarioides leaf extract against Streptococcus mutans are presented in Table 1. The largest mean inhibition zone was observed in the group treated with Coleus scutellarioides leaf extract at 250 mg/mL. The lowest concentration that produced an inhibition zone was 31.25 mg/mL, indicating that this was the minimum concentration exhibiting antibacterial activity against Streptococcus mutans in the disk diffusion assay. As expected, the positive control (0.2% chlorhexidine) produced the largest inhibition zone among all groups. The Shapiro–Wilk normality test and Levene's test for homogeneity showed that the data were normally distributed and homogeneous ($p > 0.05$), supporting the use of parametric statistical analysis.

Group	Mean	S.D	One-way ANOVA
15.62 mg/ml C. Scutellarioides extract	0.00	0.00	
31.25 mg/ml C. Scutellarioides extract	3.80	0.20	
62.5 mg/ml C. Scutellarioides extract	5.25	0.25	
125 mg/ml C. Scutellarioides extract	7.50	0.30	0,00*
250 mg/ml C. Scutellarioides extract	9.80	0.20	
Chlorhexidine 0.2%	20.60	0.28	
DMSO	0.00	0.00	

Table 1. Mean Inhibition Zone Diameters of Coleus scutellarioides Leaf Extract Against Streptococcus mutans with One-Way ANOVA.

Based on Table 1, one-way ANOVA demonstrated a statistically significant difference in the inhibition zone diameters among the experimental groups ($p < 0.001$). Therefore, Tukey's honestly significant difference (HSD) post hoc test was performed to identify the specific groups that differed significantly.

Group	15.62 mg/ml	31.25 mg/ml	62.5 mg/ml	125 mg/ml	250 mg/ml	Chlorhxidine 0.2%	DMSO
15.62 mg/ml		0.00*	0,00*	0.00*	0.00*	0.00*	1.00
31.25 mg/ml			0.00*	0.00*	0.00*	0.00*	0.00*
62.5 mg/ml				0.00*	0.00*	0.00*	0.00*
125 mg/ml					0.00*	0.00*	0.00*
250 mg/ml						0.00*	0.00*
Chlorhxidine 0.2%							0.00*
DMSO							

Table 2. Tukey's HSD Post Hoc Analysis of Inhibition Zone Diameters.

The results of Tukey's HSD Post Hoc analysis (Table 2) showed statistically significant differences between all experimental groups ($p < 0.05$), except between the 15.62 mg/mL Coleus scutellarioides leaf extract group and the negative control (DMSO). These findings indicate that C. scutellarioides leaf extract exhibited concentration-dependent antibacterial activity against Streptococcus mutans. The antibacterial activity of Coleus scutellarioides leaf extract against Streptococcus mutans was further evaluated using the broth dilution method to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The results of the broth dilution assay are presented in Table 3.

Group	Visual Observation of Broth Dilution Tubes				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
15.62 mg/ml C. Scutellarioides extract	+	+	+	+	+
31.25 mg/ml C. Scutellarioides extract	+	+	+	+	+
62.5 mg/ml C. Scutellarioides extract	-	-	-	-	-
125 mg/ml C. Scutellarioides extract	-	-	-	-	-
250 mg/ml C. Scutellarioides extract	-	-	-	-	-
Chlorhexidine 0.2%	-	-	-	-	-
DMSO	+	+	+	+	+

Table 3. Visual Observation of Broth Dilution Tubes for MIC Determination.

Based on the broth dilution assay, turbidity was observed in the 15.62 and 31.25 mg/mL extract groups and the negative control (DMSO), indicating bacterial growth. In contrast, the 62.5, 125, and 250 mg/mL extract groups, as well as the positive control (0.2% chlorhexidine), remained clear, indicating inhibition of Streptococcus mutans growth. Therefore, the minimum inhibitory concentration (MIC) was determined to be 62.5 mg/mL. To confirm the MIC and determine the minimum bactericidal concentration (MBC), aliquots from each dilution tube were subcultured onto Mueller–Hinton agar and incubated at 37°C for 24 h. Bacterial colonies were counted manually, and the MBC was defined as the lowest concentration showing no visible bacterial growth. The colony count results used to determine the MBC are presented in Table 4.

Group	Colony Counts of Streptococcus mutans (CFU/ml)					Mean
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	
15.62 mg/ml C. Scutellarioides extract	>300	>300	>300	>300	>300	>300
31.25 mg/ml C. Scutellarioides extract	292	279	287	248	287	278,6
62.5 mg/ml C. Scutellarioides extract	167	158	179	188	196	177,6
125 mg/ml C. Scutellarioides extract	0	0	0	0	0	0
250 mg/ml C. Scutellarioides extract	0	0	0	0	0	0
Chlorhexidine 0.2%	0	0	0	0	0	0
DMSO	>300	>300	>300	>300	>300	>300

Table 4. Streptococcus mutans Colony Counts (CFU/mL) for MBC Determination.

Based on Table 4, Coleus scutellarioides leaf extract completely inhibited the growth of Streptococcus mutans at a concentration of 125 mg/mL, which was identified as the minimum bactericidal concentration (MBC). At 31.25 mg/mL, bacterial growth was still observed, with colony counts comparable to those of the negative control. A marked reduction in bacterial colony counts was observed at 62.5 mg/mL, indicating the minimum inhibitory concentration (MIC). These findings demonstrate that the MIC and MBC of C. scutellarioides leaf extract against S. mutans were 62.5 mg/mL and 125 mg/mL, respectively.

4. DISCUSSION

The antibacterial activity of Coleus scutellarioides leaf extract against Streptococcus mutans was evaluated using the disk diffusion method to determine the inhibition zone and the broth dilution method to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The disk diffusion assay demonstrated that inhibition zones first appeared at a concentration of 31.25 mg/mL and increased with increasing extract concentration. These findings are consistent with those reported by Aprilia et al., who observed that C. scutellarioides extract inhibits the growth of both S. pyogenes and S. mutans.[4] A larger inhibition zone indicates greater antibacterial activity, which is likely associated with the increased concentration of bioactive compounds present in the extract. The antibacterial activity of C. scutellarioides is attributed to its secondary metabolites, including flavonoids, alkaloids, tannins, and saponins.[8] Flavonoids exert antibacterial effects by forming soluble complexes with extracellular proteins, disrupting bacterial cell integrity, inhibiting intracellular metabolic processes, and interfering with nucleic acid synthesis through interactions with bacterial DNA.[9] Saponins increase cell membrane permeability, leading to leakage of intracellular components.[10] Alkaloids and tannins inhibit bacterial cell wall synthesis by disrupting peptidoglycan formation, while tannins also interfere with bacterial protein synthesis.[11] The combined action of these phytochemicals likely contributes to the antibacterial activity observed in the present study. As expected, the positive control (0.2% chlorhexidine) produced a larger inhibition zone than all extract-treated groups, whereas the negative control (DMSO) showed no antibacterial activity. Chlorhexidine is considered the gold-standard antimicrobial agent in dentistry because it binds to negatively charged bacterial cell walls, disrupts membrane integrity, and causes leakage of intracellular components, ultimately leading to bacterial cell death.[12] The broth dilution assay showed that the extract solutions at concentrations of 62.5, 125, and 250 mg/mL remained clear, whereas turbidity was observed at 15.62 and 31.25 mg/mL, indicating bacterial growth at the lower concentrations. Accordingly, the MIC was determined to be 62.5 mg/mL. Subculture onto Mueller–Hinton agar demonstrated a marked reduction in bacterial colonies at 62.5 mg/mL and complete inhibition of bacterial growth at 125 mg/mL, establishing the MBC at 125 mg/mL. The concentration-dependent antibacterial effect observed in this study is consistent with previous reports demonstrating that increasing extract concentrations enhance bacteriostatic and bactericidal activity because of the higher content of active phytochemicals.[13] Similar findings have been reported for other medicinal plants, where low extract concentrations primarily inhibit bacterial growth, while higher concentrations exert bactericidal effects.[14] The absence of bacterial growth in the chlorhexidine group is attributable to the bactericidal activity of 0.2% chlorhexidine, whereas lower concentrations are generally considered bacteriostatic. This study has several limitations. First, bacterial colonies were counted manually, which may introduce observer-related variability. In addition, environmental factors, including culture medium, incubation conditions, pH, and oxygen availability, may influence colony formation and affect colony counts. Future studies should employ automated colony-counting systems to improve the accuracy, reproducibility, and efficiency of bacterial quantification.

Future studies should investigate the antibacterial activity of Coleus scutellarioides leaf extract against multispecies oral biofilms, as dental caries is associated with complex microbial communities rather than a single bacterial species. Further research is also needed to isolate and characterize the active phytochemical compounds responsible for its antibacterial activity and to elucidate their molecular mechanisms of action. In addition, in vivo studies and clinical trials are warranted to evaluate

the safety, efficacy, optimal dosage, and potential application of C. scutellarioides leaf extract as a natural antibacterial agent in oral healthcare products, such as mouthwashes, toothpaste, or oral gels.

5. CONCLUSION

Coleus scutellarioides leaf extract demonstrated antibacterial activity against Streptococcus mutans in vitro. The extract produced measurable inhibition zones at concentrations of 31.25 mg/mL and above, with a mean inhibition zone of 3.80 mm at 31.25 mg/mL. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined to be 62.5 mg/mL and 125 mg/mL, respectively. These findings suggest that C. scutellarioides leaf extract has potential as a natural antibacterial agent for the prevention and management of dental caries. Further in vivo and clinical studies are required to confirm its safety and therapeutic efficacy.

Conflict of Interest

The authors declare no conflicts of interest in this study.

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Authors Contribution

Conceptualization: KAAP, IA, Methodology: KAAP,NI, Formal Analysis: SRPD, Funding Acquisition: KAAP, Data Curation: KAAP, IA, NI, Original Draft Writing: SRPD, Supervision and Editing: SRPD, and Administration: SRPD.

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