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Antibacterial activity of miana (*Coleus scutellarioides*) against *Streptococcus pyogenes* and *Streptococcus mutans*

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Abstract

Miana leaves (*Coleus scutellarioides*) have compounds that have the potential to be antibacterial. The bacteria *Streptococcus pyogenes* and *Streptococcus mutans* are a group of positive bacteria containing a lipid layer that is low in peptidoglycan and teichoic acid. This study aims to determine the antibacterial activity of Miana leaves against *Streptococcus pyogenes* and *Streptococcus mutans* because both types of infectious bacteria. This research used the disc diffusion method. The preparation involved the maceration method using 96% ethanol as a solvent for five days, followed by concentration using a water bath at 70°C to obtain a thick extract. Observations were made on the diameter of the inhibition zone visible in the clear zone around the disc. The average inhibition diameter by ethanol extract against *S. pyogenes* is 9.83 mm, 10.76 mm, 12.42 mm, 15.66 mm, and 28.56 mm, respectively at extract concentrations of 5, 10, 20, 40, and amoxicillin (positive control). The negative control 1% DMSO did not form an inhibition zone. The statistical analysis using One-Way ANOVA (SPSS 27) yielded a p-value of < 0.05, confirming a significant difference in antibacterial activity across all tested groups. This result demonstrates that Miana leaf ethanol extract at concentrations of 5%, 10%, 20%, and 40% effectively inhibits the growth of both *S. pyogenes* and *S. mutans*. Furthermore, the inhibition zones varied significantly between the extract concentrations and the control groups, proving that the extract's efficacy is concentration dependent. These findings suggest that Miana leaf ethanol extract has potential as a natural antibacterial agent for developing alternative phytotherapeutic treatments against *S. pyogenes* and *S. mutans* infections.

Introduction

Indonesia has many types of plants that can be cultivated due to their benefits and significant uses for humans, especially in medicine. Currently, plants containing chemical components can be used as medicine. Many people prefer natural ingredients in their daily lives, avoiding synthetic chemicals and relying more on natural substances. One of the medicinal plants is Miana (Anita *et al.*, 2019).

Streptococcus pyogenes (Group A *Streptococcus*) is a clinically important pathogenic bacterium that is exclusively adapted to human hosts. It is responsible for a wide range of clinical manifestations, from mild skin/soft tissue infections and pharyngitis to more serious diseases. This bacterium commonly causes respiratory tract infections with mild to moderate symptoms, such as tonsillitis and pharyngitis (strep throat). *S.pyogenes* can colonize the skin and lead to infections (Brouwer *et al.*, 2016).

S.mutans is a facultative anaerobic, Gram-positive coccus bacterium that belongs to the *Streptococcus viridans* group. It is a normal flora of the oral cavity with α-hemolytic and opportunistic commensal properties. *S.mutans* is considered the primary pathogen in the development of dental caries, particularly

early childhood dental caries. It possesses several virulence factors, one of which is a sucrose-dependent adhesion mechanism responsible for colonizing tooth surfaces (Melani *et al.*, 2018).

Miana leaves (*Coleus scutellarioides*), a species from the *Lamiaceae* family, have long been used empirically to treat various diseases. The plant has a dark reddish color and is highly beneficial. The leaves contain essential oils, saponins, flavonoids, polyphenols, alkaloids, and minerals. The secondary metabolites in miana leaf extract contain active compounds with antibacterial properties, including saponins, flavonoids, and tannins. Saponins can inhibit bacterial cell growth by reducing the surface tension of the cell wall, causing cell wall leakage (Zhang *et al.*, 2021).

The phytochemical compounds in miana have been supported by previous research as potential medicinal and antibacterial agents. A study by Kusumawati et al. (2014) demonstrated that miana has antibacterial activity against two pathogenic bacteria, *Escherichia coli* and *Staphylococcus aureus*. Research by Basir et al. (2023) found that ethanol, n-hexane, and chloroform-ethanol extracts exhibited strong inhibitory effects against *Vibrio* sp. Additionally, according to a study by Anjely J. Makatempuge et al. (2023), miana leaf extract at concentrations of 5%, 10%, 20%, 40%, and 80% effectively inhibited *Streptococcus mutans* (Pakadang *et al.*, 2023).

This study investigated the inhibition diameter and minimum inhibitory concentration of ethanol extract from miana leaves at concentrations of 5%, 10%, 20%, and 40%. The aim is to compare the antibacterial activity of miana ethanol extract against *S.pyogenes* and *S.mutans* using the disk diffusion method. One approach to measuring bacterial activity is the disk diffusion method, in which a paper disk soaked with an antimicrobial agent is placed on a test medium. The clear zone surrounding the paper disk is observed to determine microbial growth inhibition (Fati *et al.*, 2020).

Materials and methods

This study include bacterial cultures of *S.pyogenes* (ATCC19615) and *S.mutans* (ATCC19615) obtained from Thermo Fisher Scientific, *Miana* leaves (*Coleus scutellarioides*), 500 mg amoxicillin tablets (Trihydrate), 96% ethanol, Nutrient Agar (NA), Mueller-Hinton Agar (MHA), Dimethyl Sulfoxide (DMSO), distilled water, 0.9% physiological NaCl, 2N HCl, 1% H_2SO_4, 1% $BaCl_2$, magnesium powder, $FeCl_3$, Mayer's reagent, Dragendorff's reagent, Wagner's reagent, chloroform, filter paper, kraft paper, aluminum foil, and disc paper (Macherey-Nagel®).

The equipment used includes an autoclave (Webeco), stirring rods, petri dishes (Pyrex®), funnels (Pyrex®), Erlenmeyer flasks (Pyrex®), measuring cylinders (Pyrex®), an incubator (Heraeus®), a blender (Panasonic®), calipers, inoculating loops, cotton swabs, a spirit lamp, mesh 40 sieves (Sieve Stainless®), pH paper, Laminar Air Flow (LAF®), micropipettes, an oven (Memmert®), tweezers, an analytical balance (Fujitsu®), graduated pipettes, a horn spoon, syringes, a support stand, test tubes (Pyrex®), a refrigerator, a test tube rack, and calipers.

~~Work~~ Procedure

The first stage involved collecting raw materials from Laang Tanduk Village, Rantepao District, North Toraja Regency. The collected *Miana* leaves underwent a wet sorting process, washing, drying, and dry sorting. Once dried, the leaves were weighed, ground into powder using a blender, and sieved with a mesh 40 sieve to obtain a uniform fine powder. The powder was then weighed and stored in a closed container. A total of 200 g of *Miana* leaf powder was weighed and placed in a closed glass container, then macerated with 2000 mL of 96% ethanol for five days. After five days, the maceration process was repeated once more for three days with an additional 600 mL of 96% ethanol. The solution was stored in a dark place to prevent hydrolysis reactions caused by light exposure. The obtained filtrate was collected and concentrated using a water bath at 70°C until a thick extract was obtained. The extract was left at 25°C until all the ethanol evaporated (Imansyah, 2021).

1. Phytochemical Screening

Alkaloid Identification

A total of 0.5 g of *Miana* leaf extract was placed into a test tube, followed by the addition of 1 mL of 2% HCl. Then, 9 mL of distilled water was added, and the solution was heated for 2 minutes. After cooling, it was filtered. The filtrate was divided into three portions, and each portion was treated with Mayer's, Wagner's, and Dragendorff's reagents (Setyowati, 2014).

Flavonoid, Tannin, and Saponin Identifications

Flavonoid, the ethanol extract of *Miana* leaves was dissolved in hot methanol, followed by the addition of 0.1 g of magnesium powder and 1 mL of concentrated HCl (Setyowati, 2014). **Tannin,** the ethanol extract of *Miana* leaves was dissolved in 10 mL of distilled water and filtered. The filtrate was then treated with 1% $FeCl_3$ (Setyowati, 2014). **Saponin,** the ethanol extract of *Miana* leaves was dissolved in 10 mL of hot water and shaken for 10 seconds (Setyowati, 2014).

Bacterial Culture Preparation

Pure cultures of *S.pyogenes* and *S.mutans* were taken using a sterile inoculating loop from the bacterial stock. The bacteria were inoculated into sterile Nutrient Agar (NA) plates under aseptic conditions inside a Laminar Air Flow (LAF) cabinet. The inoculated bacteria were then incubated at 37°C for 24 hours (Mulyadi *et al.*, 2017).

Bacterial Suspension Preparation

A single colony of *S. pyogenes* and *S. mutans* (24-hour-old culture) was suspended in 5 mL of sterile 0.9% physiological NaCl. The suspension was homogenized using a vortex mixer, and its turbidity was compared to the 0.5% McFarland standard, equivalent to a bacterial concentration of 9×10^8 CFU/ml (Syafriana *et al.*, 2020).

Antibacterial Activity Testing

Test solutions were prepared at concentrations of 5%, 10%, 20%, and 40%. This was done by weighing 0.05 g, 0.1 g, 0.2 g, and 0.4 g of *Miana* leaf ethanol extract, which were then dissolved in DMSO to a final volume of 1 mL. Positive control (amoxicillin) and negative control (DMSO) were also prepared (Wangkanusa, 2016). Mueller-Hinton Agar (MHA) medium was sterilized using an autoclave at 121°C for 20 minutes. After cooling, the agar was poured into sterilized petri dishes (Sidoretno, 2022). The bacterial suspensions of *S.pyogenes* and *S.mutans* were inoculated onto the solidified Mueller-Hinton Agar medium by swabbing the surface with a sterile cotton swab. Sterile filter paper discs were soaked for 15 minutes in the *Miana* leaf extract solutions at different concentrations to ensure complete absorption of the extract. The discs were then placed onto the bacterial-inoculated MHA plates. The plates were incubated at 37°C for 24-48 hours (Intan *et al.*, 2021).

2. Data Analysis

Data analysis was performed using SPSS IBM version 27**.** The data analysis aimed to assess the sensitivity of *S.pyogenes* and *S.mutans* to different concentrations of *Miana* leaf extract The statistical tests performed included One-Way ANOVA**,** Post-Hoc LSD**,** and Shapiro-Wilk tests to determine whether there was any antibacterial activity of *Miana* leaf extract against *S.pyogenes* and *S.mutans.*

Results and discussion

The study began with phytochemical screening. Based on the phytochemical test screening, specific results were obtained for each test using qualitative analysis techniques related to color change reactions and precipitation (Table 1).

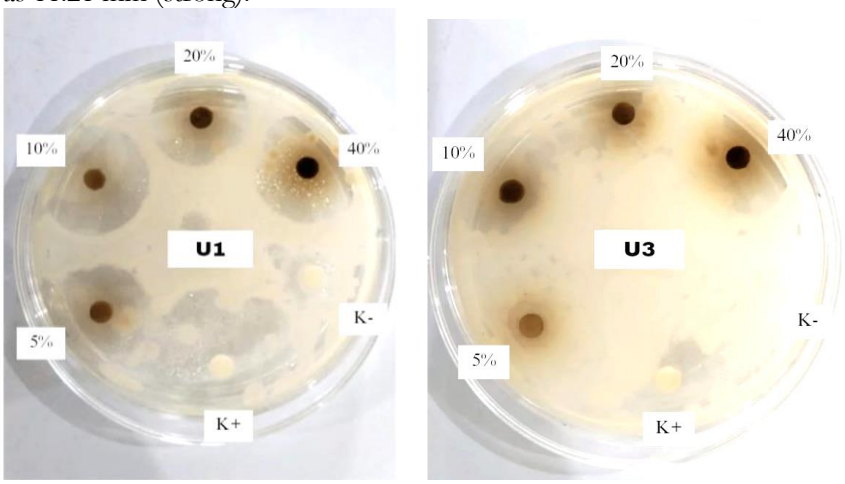
1. Result

Phytochemical screening was conducted to identify the secondary metabolites present in the ethanol extract of *Miana* leaves that have antibacterial potential. The results showed that *Miana* leaf ethanol extract contains flavonoids, tannins, and saponins (Figure 1).

These findings are supported by research conducted by Fati *et al.* (2020), which stated that *Miana* leaves contain active compounds such as saponins, flavonoids, and tannins, which play a role as antibacterial agents. However, alkaloid testing yielded negative results. Differences in phytochemical screening results may be influenced by the type of solvent, extraction method, compound concentration, plant growth location, plant age, and differences in test method sensitivity

Based on Table 2**,** *Miana* leaf ethanol extract at 5%, 10%, 20%, and 40% concentrations was able to inhibit the growth of *S.pyogenes* with varying inhibition zone diameters. The mean inhibition zone for *S. pyogenes* at 5% concentration was 9.83 mm (moderate), 10% concentration was 10.76 mm (moderate), 20%

concentration was 12.42 mm (strong), and 40% concentration was 15.66 mm (strong). Based on Table 3, *Miana* leaf ethanol extract at 5%, 10%, 20%, and 40% concentrations was also able to inhibit the growth of *S.mutans*. The mean inhibition zone for *S. mutans* at 5% concentration was 4.73 mm (weak), 10% concentration was 6.54 mm (moderate), 20% concentration was 7.66 mm (moderate), and 40% concentration was 11.21 mm (strong).



(a) (b)

Figure 1. Comparison of antibacterial inhibition zones of Miana (*Coleus scutellarioides*) leaf ethanol extract against (a) *Streptococcus pyogenes* and (b) *Streptococcus mutans*. The concentrations used: 5%, 10%, 20%, and 40%, showing a concentration-dependent inhibitory effect compared to the controls.

Table 1. Phytochemical Screening Results of Miana Leaf Ethanol Extract

Phytochemical test	Reagent	Result
Alkaloids	Mayer	(-) No precipitated formed
	Wagner	(-) No reddish brown precipitated formed
	Dragendorff	(-) No orange precipitate formed
Flavonoids	13.8	(+) Orange color formed
Tannins	18.05	(+) dark green color formed
Saponins	27.15	(+) stable foam formed

Table 2. Inhibition zone diameter (mm) of *S. pyogenes*

Treatment	P1	P2	P3	P4	Mean±SD	Inhibitory Category
K-	0	0	0	0	0	No inhibition
K1	11.1	9.8	9.45	9	9.83±0.78	Moderate
K2	12.4	10.65	9.9	10.1	10.76±0.98	Moderate
K3	13.8	12.05	12.05	11.8	12.42±0.80	Strong
K4	18.05	14.2	17.05	13.35	15.66±1.94	Strong
K+	27.15	31.15	29.65	26.3	28.56±1.93	Very Strong

Table 3. Inhibition zone diameter (mm) of *S. mutans*

Treatment	P1	P2	P3	P4	Mean±SD	Inhibitory Category
K-	0	0	0	0	0	No inhibition
K1	2.35	4.3	6.8	5.5	4.73±1.65	Weak
K2	4.7	5.1	8.25	8.1	6.54±1.64	Moderate
K3	5.85	7.1	8.9	8.8	7.66±1.27	Moderate
K4	9.8	10.6	11.8	12.65	11.21±1.09	Strong
K+	12.95	17.45	19.3	19.5	17.30±2.64	Strong

Notes : K- : Negative control (1% DMSO); K+: Positive control (Amoxicillin antibiotic); K1: 5% ethanol extract concentration; K2: 10% ethanol extract concentration; K3: 20% ethanol extract concentration; K4: 40%

ethanol extract concentration; P1: First repetition; P2: Second repetition; P3: Third repetition; P4: Fourth repetition.

2. Discussion

The results indicate that increasing the concentration of *Miana* leaf ethanol extract enhances the inhibition zone diameter. This is due to the higher concentration of active antibacterial compounds at higher extract concentrations. Additionally, increased concentration enhances the penetration of active compounds into bacterial cells, which disrupts cellular metabolism and leads to bacterial cell death (Lingga et al., 2015). Differences in inhibition zones between *S.pyogenes* and *S.mutans* may be due to bacterial type, as each bacterium exhibits different sensitivities to the extract concentrations.

Factors influencing the antibacterial activity of a substance include bacterial density, disc placement time, incubation temperature, incubation duration, medium thickness, bacterial suspension turbidity, and media composition (Hastuty *et al.*, 2018). The positive control (amoxicillin) produced a larger inhibition zone compared to the *Miana* leaf ethanol extract for both *S.pyogenes* and *S.mutans*. The average inhibition zone for amoxicillin was 28.56 mm for *S. pyogenes* (very strong inhibition) and 17.30 mm for *S. mutans* (strong inhibition). Amoxicillin is a broad-spectrum antibiotic with high oral bioavailability. The β-lactam ring in amoxicillin exerts antibacterial activity by targeting susceptible Gram-positive and Gram-negative bacteria (Sofyani *et al.*, 2018). The mechanism of action of amoxicillin involves inhibiting the final stage of bacterial cell wall synthesis, causing cell rupture and preventing bacterial cell wall formation by binding to one or more penicillin-binding proteins (Pratiwi, 2019).

The antibacterial activity of *Miana* leaf ethanol extract against *S.pyogenes* and *S.mutans* is attributed to its secondary metabolite content, including flavonoids, tannins, and saponins. These bioactive compounds exhibit antibacterial properties. Flavonoids inhibit DNA replication and bacterial membrane function, leading to bacterial cell damage and death.

The mechanism of flavonoids enhances cytoplasmic membrane permeability in *Streptococcus pyogenes* and *Streptococcus mutans*, thereby exerting an inhibitory effect on Gram-positive bacteria (Charmelya, 2023). Tannins act as antibacterial agents by forming stable complexes with proteins, resulting in bacterial protoplasm coagulation (Rijayanti, 2014). They target polypeptides in the bacterial cell wall, leading to cell lysis due to osmotic or physical pressure, ultimately causing bacterial death (Setiawan, 2017). Saponins reduce water surface tension, leading to foam formation. They act by increasing cell membrane permeability, resulting in hemolysis and bacterial cell leakage (Putri *et al.*, 2023).

The antibacterial activity of Miana leaves observed in this study is consistent with previous research, although inhibition zone diameters vary depending on the extract concentration, solvent used, and target bacterial strains. Effectiveness against *S.mutans* a study focusing on oral pathogenic bacteria found that Miana leaf ethanol extract inhibited *S. mutans* with an average zone of 9.80 mm at a 25% concentration (Putri *et al.*, 2023). Comparison with other Gram-Positive Bacteria Miana extract has shown even stronger activity against other Gram-positive bacteria like *Staphylococcus aureus*. Research using a 5% concentration of Miana extract in a solid soap formulation produced an inhibition zone of 18.4 mm (Asjur *et al.*, 2024).

The Shapiro-Wilk normality test showed a p-value > 0.05**,** indicating that the data were normally distributed. The homogeneity test also yielded a p-value > 0.05, confirming data homogeneity. The One-Way ANOVA test produced **a** p-value (sig) = 0.000 < 0.05**,** indicating significant antibacterial activity and differences in inhibition zone diameters at 5%, 10%, 20%, and 40% ethanol extract concentrations against *S.pyogenes* and *S. mutans*.

Conclusion

There is antibacterial activity from the ethanol extract of Miana leaves at concentrations of 5%, 10%, 20%, and 40% in inhibiting the growth of *S.pyogenes* and *S.mutans* using the disk diffusion method. Miana leaf ethanol extract exhibits significant antibacterial activity against *S. pyogenes* and *S. mutans* in a dose-dependent manner. *S. pyogenes* showed higher sensitivity with inhibition zones ranging from 9.83 to 15.66 mm (moderate to strong), while *S. mutans* displayed inhibition zones between 4.73 and 11.21 mm (weak to strong) across the 5% to 40% concentration range.

Author Statements

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Author's contributions: Klara Aprillia contributed to the conceptualization, data collection, laboratory experimentation, and original draft preparation. Sister Sianturi contributed to the methodology, formal analysis, supervision, and final review and editing of the manuscript. All authors have read and approved the final version of the manuscript

Generative AI: Not applicable.

Data availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request

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