

Antibacterial Activity of Siam Grass Extract (*Chromolaena odorata L.*) Against *Streptococcus mutans* and *Pseudomonas aeruginosa* Bacteria

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ABSTRACT

Infection is one of the main disease problems in both developed and developing countries. The prevalence of nosocomial infections reaches 15.74% and dental caries infections reach 88.8% caused by *Pseudomonas aeruginosa* and *Streptococcus mutans* bacteria. To overcome bacterial infections, people usually use antibiotics, but the use of antibiotics that are not appropriate and according to indications can cause antibiotic resistance or Antimicrobial Resistance (AMR). Therefore, this study aims to test the effectiveness of siam weed leaf extract (*Chromolaena odorata L.*) in inhibiting the growth of bacteria that cause infections. Siam weed leaf extract is made by maceration process with 96% ethanol solvent accompanied by extract quality control and testing of active compound levels. Antibacterial activity testing was carried out by disc diffusion method to see and measure the area of the inhibition zone. The results of the study found that siam weed leaf extract has good water content, total ash content, and weed acid insoluble ash content, which are below 10%, 16.6%, and 0.7%. The antibacterial activity test of Siam leaf extract obtained an inhibition zone diameter of 15.1 mm in *Streptococcus mutans* bacteria and 18.2 mm in *Pseudomonas aeruginosa*. In addition, Siam leaf extract also qualitatively contains active secondary metabolite compounds such as flavonoids, tannins, phenols, alkaloids, saponins, and steroids. This concludes that siam leaf extract has characteristics that meet the requirements, contains secondary metabolite compounds and has antibacterial activity against *Pseudomonas aeruginosa* and *Streptococcus mutans*.

Keywords: Chromolaena odorata L., Disc Diffusion, Pseudomonas aeruginosa, Streptococcus mutans, Antibacterial

Introduction

Medicinal plants are identified through human observation and are known to contain compounds that are useful for preventing or curing diseases and performing certain biological functions (Kumontoy *et al.*, 2023). One of the medicinal plant species commonly found in Indonesia is the Siam weed (*Chromolaena odorata L.*). The pharmacological activities of Siam weed include antibacterial, astringent, antispasmodic, antihypertensive, and anti-inflammatory properties (Jumain *et al.*,

2023; Lamangatjo *et al.*, 2018; Putra & Mukhlisah, 2023) . Siam weed is a medicinal plant that has the potential to act as an antibacterial which can inhibit several bacteria that cause nosocomial infections, including *Pseudomonas aeruginosa* (Ikuta *et al.*, 2022; and Hidayatullah, 2018). Bacteria that cause dental caries include *Streptococcus mutans* , *Streptococcus sobrinus* , *Streptococcus gordonii* , *Lactobacillus* and *Actinomyces* (Eva *et al.*, 2023) .

In an effort to combat various diseases caused by bacterial infections, people often use antibiotics. However, inappropriate and inappropriate use of antibiotics can lead to antibiotic resistance, or Antimicrobial Resistance (AMR) (Novard *et al.*, 2013; Jati *et al.*, 2019; and Andika *et al.*, 2020). As an alternative, the use of natural remedies can be considered as a treatment solution (Jumiarni *et al.*, 2017) . According to research (Ernawati & Jannah, 2021), The antibacterial activity of Siam weed against *Pseudomonas aeruginosa* bacteria produces an average inhibition zone at a concentration of 20-100% w/v of 7.33-15.63 mm. Meanwhile, research (Salma *et al.*, 2024) also stated that Siam weed extract has antibacterial potential against *Pseudomonas aeruginosa* bacteria , with results showing an average inhibition zone of 10-13.5 mm.

Although there have been many studies on the antibacterial activity of Siam grass extract against *Pseudomonas aeruginosa* , the results are still inconsistent. Furthermore, studies on the antibacterial activity of Siam grass extract against *Streptococcus mutans* bacteria are still rare. Therefore, the author is interested in conducting research on testing the antibacterial activity of Siam grass extract against *Streptococcus mutans* and *Pseudomonas aeruginosa* bacteria using the disc diffusion method.

Methodology

This research used post only control group design method that only collected the antibacterial activities by inhibition zone's data in the end of the experiment. The inhibition zone data was then categorized according to the strength of its antibacterial activity. Each sample groups treated with 4 times replication to get results data validation.

Equipment

The tools used in this study were a set of maceration tools, analytical balance (Sartoris CP224S), spatula, Erlenmeyer flask (Pyrex), maceration bottle, aluminum foil, funnel (Pyrex), evaporator flask (Pyrex), evaporator cup, watch glass, pipette, blender, and standard laboratory glassware, crucible, weighing bottle, Erlenmeyer flask (Pyrex), test tube (iwaki and Pyrex), test tube rack, Petri dish, stirring rod, measuring cup (pyrex), autoclave, inoculation needle, tweezers, micropipette, pipette tip (onemed), Bunsen burner, sterile cotton, vortex (Labnet), electric stove, refrigerator (Sanyo Medicoool), oven (BIOBASE), Muffle furnace (BIOBASE), Laminar air flow (EACI), and incubator (BIOBASE).

Material

The materials used in this study were Siamese grass granules, 96% ethanol solvent, MHA (Mueller Hilton Agar) media, chloramphenicol antibiotics, sterile distilled water, physiological NaCl, DMSO, Dragendorff reagent, Lieberman-Bouchardat reagent, NaOH, sulfuric acid, chloroform, anhydrous acetic acid, FeCl₃, 70% ethanol, spirits, *Streptococcus mutans* and *Pseudomonas aeruginosa*.

Extract Preparation

The extract was prepared using the maceration method using 96% ethanol as a solvent. A 1000 g sample was reduced in size and placed in a container, with the addition of 10 L of solvent. Soak for the first 6 hours, stirring occasionally, then let stand for 24 hours. The maceration was separated, and the resulting pulp was then re-macerated with the same repetition. The extract was obtained through an evaporation process using a rotary evaporator. Next, phytochemical screening was carried out to identify flavonoids, tannins, phenols, alkaloids, saponins, and steroids.

Phytochemical Screening

1. Flavonoid Compound Identification Test

Dissolve 1 gram of Siam weed extract in 100 mL of distilled water, then filter using filter paper. Filter 5 mL of the test solution, then add 0.05 grams of magnesium, 1 mL of concentrated HCl, and shake vigorously. A color change, whether red, yellow, or orange, indicates the presence of flavonoids in the sample (Safriana *et al.*, 2021) .

2. Tannin Compound Identification Test

Dissolve 1 gram of Siam grass extract in 100 mL of distilled water, then filter using filter paper. Filter 1 mL of the test solution, then add 3 drops of 10% FeCl₃ solution and shake vigorously. If the color changes to dark blue or blackish green, this indicates the presence of tannin compounds in the sample (Jati *et al.*, 2019) .

3. Phenol Compound Identification Test

Dissolve 1 gram of Siam weed extract in 100 mL of distilled water and then filter. Mix 1 mL of the filtrate with 2 mL of 96% ethanol. Then, add 3-4 drops of 10% FeCl₃ to the mixture. A blackish-green color change indicates the presence of phenolic compounds in the sample. (Susanti *et al.*, 2014) .

4. Alkaloid Compound Identification Test

Dissolve 1 gram of Siam grass extract in 100 mL of distilled water and then filter it using filter paper. Then, add 5 mL of ethanol to 1 mL of the sample solution and heat for 5 minutes. Add a drop of concentrated HCl and Mayer's reagent to the first test tube. Then, add a drop of Dragendorff's reagent to the second test tube. Observe the color change. If a reddish-brown precipitate forms in the Wagner test, a white precipitate in the Mayer test, and a red or orange precipitate in the Dragendorff test, this indicates the presence of alkaloid compounds in the sample (Safriana *et al.*, 2021) .

5. Saponin Compound Identification Test

1 gram of Siam weed extract was dissolved in 100 mL of distilled water, then filtered using filter paper. Mix 0.5 mL of the solution with 5 mL of hot water in a test tube and shake the tube for 10 seconds until foam forms on the sample. Add 1 drop of

2N HCl and shake again vertically for 10 seconds. If foam forms measuring 1-10 cm and lasts for 5 minutes, this indicates the presence of saponin compounds in the sample (Jati *et al.*, 2019) .

6. Identification Test for Steroid and Triterpenoid Compounds

A total of 0.1 grams of Siam weed extract in 10 mL of chloroform was filtered using filter paper. 2 mL of the filtrate was mixed with 0.5 mL of anhydrous CH₂COOH and 2 mL of concentrated H₂SO₄ (Liebermann-Burchard method), which were added through the wall of the test tube. If a brown or purple ring forms at the solution boundary, the sample is positive for containing steroids. Conversely, if a greenish-blue ring forms, the sample contains triterpenoid compounds (Rahmasiahi *et al.*, 2023).

Preparation for Antibacterial Activity Testing

Preparation of Mueller–Hinton Agar Medium

To prepare 320 mL of Mueller–Hinton Agar (MHA), 12.16 g of Mueller–Hinton Agar powder was weighed and transferred into an Erlenmeyer flask. The powder was dissolved in 320 mL of distilled water to obtain sufficient medium for 16 Petri dishes, each containing 20 mL of agar, filled to the disposable plate limit. The solution was heated until complete dissolution was achieved, then sterilized using an autoclave at 121 °C for 15 minutes for disinfection purposes (Abadia, 2025).

Preparation of Test Bacterial Suspensions

Cultures of *Pseudomonas aeruginosa* and *Streptococcus mutans* were collected using a sterile inoculating loop (one loopful). The bacterial cells were then suspended in a test tube containing 10 mL of 0.9% sodium chloride (NaCl) solution until the turbidity matched the McFarland standard corresponding to 1.5×10^8 CFU/mL (0.5 McFarland standard) (Abadia, 2025).

Antibacterial Activity Assay of Siam weed extract

Three treatment groups were used in the antibacterial activity assay were a 40% (w/v) Siam weed extract as the test treatment, distilled water as the negative control, and chloramphenicol (10 mg) as the positive control. Mueller–Hinton Agar medium was prepared in 16 Petri dishes, allowing four replications for each bacterial strain. Each disposable Petri dish received the assigned treatment according to its respective group.

Bacterial suspensions were inoculated onto the Mueller–Hinton Agar plates using the streak plate method. Using sterile forceps, a 6 mm diameter paper disc was immersed in Siam weed extract and then placed with gentle pressure onto the surface of the agar medium previously inoculated with *Pseudomonas aeruginosa* and *Streptococcus mutans*. The plates were incubated at 37 °C for 24 hours until inhibition zones formed. The diameters of the inhibition zones were measured using a vernier caliper, and the inhibitory activity was subsequently analyzed (Abadia, 2025).

Results and Discussion

Siam Grass Extract (*Chromolaena odorata* L.).

Extraction of 96% ethanol extract was carried out using maceration and two remaceration processes. The resulting filtrate was concentrated using a rotary

evaporator at 50°C and 60 rpm. The extraction yield of the simplex was 292.5 g. Maceration was chosen as the extraction method to avoid damage to secondary metabolite compounds that can be denatured at high temperatures (Wendersteyt *et al.*, 2021). The use of 96% ethanol solvent was chosen because it is more selective, non-toxic, and has good absorption capacity (Mujipradhana *et al.*, 2021). This temperature was chosen because under vacuum conditions, ethanol can evaporate more easily and quickly (Muiz *et al.*, 2021).

Table 1. Results of Extract Evaluation Test

NO	Test	Results	
		Mean ± SD	
		Form : Thick Extract	
		Color : Green	
1	Organoleptic Test	Aroma : Siam weed leaves have a distinctive aromatic aroma.	
		Taste : Bitter	
2	Water Content Test	7.78% ± 0.28	
3	Total Ash Content Test	0.095% ± 0.01	
4	Acid Insoluble Ash Test	0.09% ± 0.01	
5	Percentage Result Test	29.25%	

Table 2. Phytochemical Screening Results

Merge	Reagent	Discoloration		Results
		Before	After	
Flavonoid	Mg + concentrated HCl	Yellowish green	Yellow	+
Saponin	Aquadest + HCl 2N	Yellowish green	Foam is stable for 5 minutes	+
Tannin	FeCl ₃ 10 %	Yellowish green	Dark green	+
Steroid	Acetic acid anhydrous	Yellowish green	A brownish or purple ring forms.	+
Triterpenoid	concentrated H ₂ SO ₄	Yellowish green	No color change	-
Alkaloid	Dragendorph	Yellowish green	A red or orange precipitate forms	+
	Mayer	Yellowish green	White precipitate forms	+
Phenol	Ethanol + FeCl ₃ 10 %	Yellowish green	Dark green	+

Description: (+) Contains phytochemical compounds
(-) Does not contain compounds

Organoleptic testing is carried out to ensure the quality and safety of the extract or preparation produced (Octavia *et al.*, 2023). The water content test results were obtained from the extract thickening process. The purpose of the water content test is to remove residual water. The water content test for the 96% ethanol extract of golden pothos leaves reached 7.78%, which meets the extract water content requirement of a maximum of 10%. (Ministry of Health of the Republic of Indonesia,

2022). The total ash content test results are obtained from calculations based on the weight of the test sample, expressed in %w/w. The total ash content test is carried out to provide a general overview of the mineral content that does not evaporate during the extract combustion process (Agustien *et al.*, 2024). The lower the total ash content, the higher the purity level of an extract. The required total ash content is less than 16.6% (Ministry of Health of the Republic of Indonesia, 2008). In the total ash content test of 96% ethanol extract of golden pothos leaves, an average of 0.095% was obtained, so the purity of the extract meets the requirements because the average percentage is still below 16.6%. The acid-insoluble ash content test in golden pothos extract aims to determine the content of acid-insoluble impurities and can describe the level of purity and cleanliness in extract processing. The acid-insoluble ash test functions to indicate the presence of mineral or metal contamination that is not acid-soluble in an extract. (Agustien *et al.*, 2024). In the acid insoluble ash content test of 96% ethanol extract of golden pothos leaves, an average of 0.09% was obtained, so the purity of the extract met the requirements of below 0.7%. (Ministry of Health of the Republic of Indonesia, 2008).

The yield test was conducted with a value of 29.25% from the maceration process. This high yield is due to the use of a temperature of 50°C during the extract concentration process (Waluyo *et al.*, 2022). A smaller temperature increase will reduce the possibility of solvent evaporation. Higher extract yields indicate that the content of soluble secondary metabolite constituents in the raw material also increases (Senduk *et al.*, 2020). This results also related to the metabolite compounds contained in extract. Phytochemical screening functions to identify the secondary metabolite content contained in the extract, so that it can be identified which secondary metabolites have the potential to be antibacterial (Andika *et al.*, 2010; Senduk *et al.*, 2020; and Putra & Mukhlisah, 2023). In this study, based on **Table 2**, phytochemical screening of 96% ethanol extract of golden pothos leaves showed positive results for flavonoids, tannins, phenols, alkaloids, saponins, and steroids.

Antibacterial Activity Test Results

Siam weed extract was observed against *Streptococcus mutans* and *Pseudomonas aeruginosa* bacteria using disc diffusion method to evaluate the antibacterial effect of the extract on bacterial growth by measuring the diameter of the inhibition zone. The results of the formation of the inhibition zone diameter can be seen in **Figure 1**. Based on **Table 3**, the diameter of the positive control inhibition zone for *Streptococcus mutans* and *Pseudomonas aeruginosa* bacteria was 35.9 ± 1.7 mm and 38.9 ± 5.4 mm respectively, which were categorized as very strong. Based on **Table 3**, it can be seen that the diameter of the inhibition zone formed by the Siam grass extract against *Streptococcus mutans* at a concentration of 40% was 15.1 ± 4.4 mm, and against *Pseudomonas aeruginosa* bacteria was 18.2 ± 1.2 mm (strong category). These results are positively correlated with the concentration of the material used. The sensitivity of the test bacteria, the diffusion rate, and the concentration of the antibacterial compound are factors that determine the level of inhibition (Djumidar *et al.*, 2022). This results in better effectiveness in inhibiting bacterial growth, resulting in a wider inhibition zone diameter (Safiti *et al.*, 2023).

This is also related to the increase in antibacterial compounds such as compounds resulting from secondary metabolism. Compounds resulting from secondary metabolism (e.g., polyphenols, flavonoids, saponins, tannins, steroids, terpenoids, and alkaloids) can disrupt bacterial cell metabolism and cause death, resulting in decreased bacterial growth as the concentration increases (Alouw *et al.*, 2022).

Table 3. Results of Antibacterial Activity Test of Extracts

Tasting	Bacteria <i>Streptococcus mutans</i>		Bacteria <i>Pseudomonas aeruginosa bacteria</i>	
	inhibition zone	Category	inhibition zone	Category
Control (+) R1	34.5 mm	Very strong	46.5 mm	Very strong
Control (+) R2	36.2 mm	Very strong	34.5 mm	Very strong
Control (+) R3	34.6 mm	Very strong	38.8 mm	Very strong
Control (+) R4	38.1 mm	Very strong	35.4 mm	Very strong
Control (+) average	35.9 ± 1.7 mm	Very strong	38.9 ± 5.4 mm	Very strong
Control (-) R1	0 mm	There is none.	0 mm	There is none.
Control (-) R2	0 mm	There is none.	0 mm	There is none.
Control (-) R3	0 mm	There is none.	0 mm	There is none.
Control (-) R4	0 mm	There is none.	0 mm	There is none.
Control (-) average	0 mm	There is none.	0 mm	There is none.
Concentration 40% R1	14.9 mm	Strong	16.4 mm	Strong
Concentration 40% R2	9.5 mm	Strong	18.1 mm	Strong
Concentration 40% R3	15.7 mm	Strong	18.8 mm	Strong
Concentration 40% R4	20.2 mm	Strong	19.3 mm	Strong
Average concentration 40%	15.1 ± 4.4 mm	Strong	18.2 ± 1.2 mm	Strong

Description : (R1) Replication number 1, (R2) Replication number 1,
(R3) Replication number 3, (R4) Replication number 4,

Based on **Table 3**, the diameter of the positive control inhibition zone for *Streptococcus mutans* and *Pseudomonas aeruginosa bacteria* was 35.9 ± 1.7 mm and 38.9 ± 5.4 mm respectively, which were categorized as very strong (over 20 mm) (Salma *et al.*, 2024). Based on **Table 3.**, it can be seen that the diameter of the inhibition zone formed by the Siam grass extract against *Streptococcus mutans* at a concentration of 40% was 15.1 ± 4.4 mm, and against *Pseudomonas aeruginosa bacteria* was 18.2 ± 1.2 mm (strong category). The extract test with a concentration of 40% was used because this concentration was the lowest test concentration that could produce a strong category inhibition zone diameter (10-20 mm) in *Pseudomonas aeruginosa bacteria* (Ernawati & Jannah, 2021; Salma *et al.*, 2024).

These results are positively correlated with the concentration of the material used. The sensitivity of the test bacteria, the diffusion rate, and the concentration of the antibacterial compound are factors that determine the level of inhibition (Djumidar *et al.*, 2022). This results in better effectiveness in inhibiting bacterial growth, resulting in a wider inhibition zone diameter (Safiti *et al.*, 2023). This is also related to the increase in active antibacterial compounds such as compounds resulting from secondary metabolism. Compounds resulting from secondary metabolism (e.g., polyphenols, flavonoids, saponins, tannins, steroids, terpenoids, and alkaloids) can disrupt bacterial cell metabolism and cause death, resulting in decreased bacterial growth as the concentration increases (Alouw *et al.*, 2022).

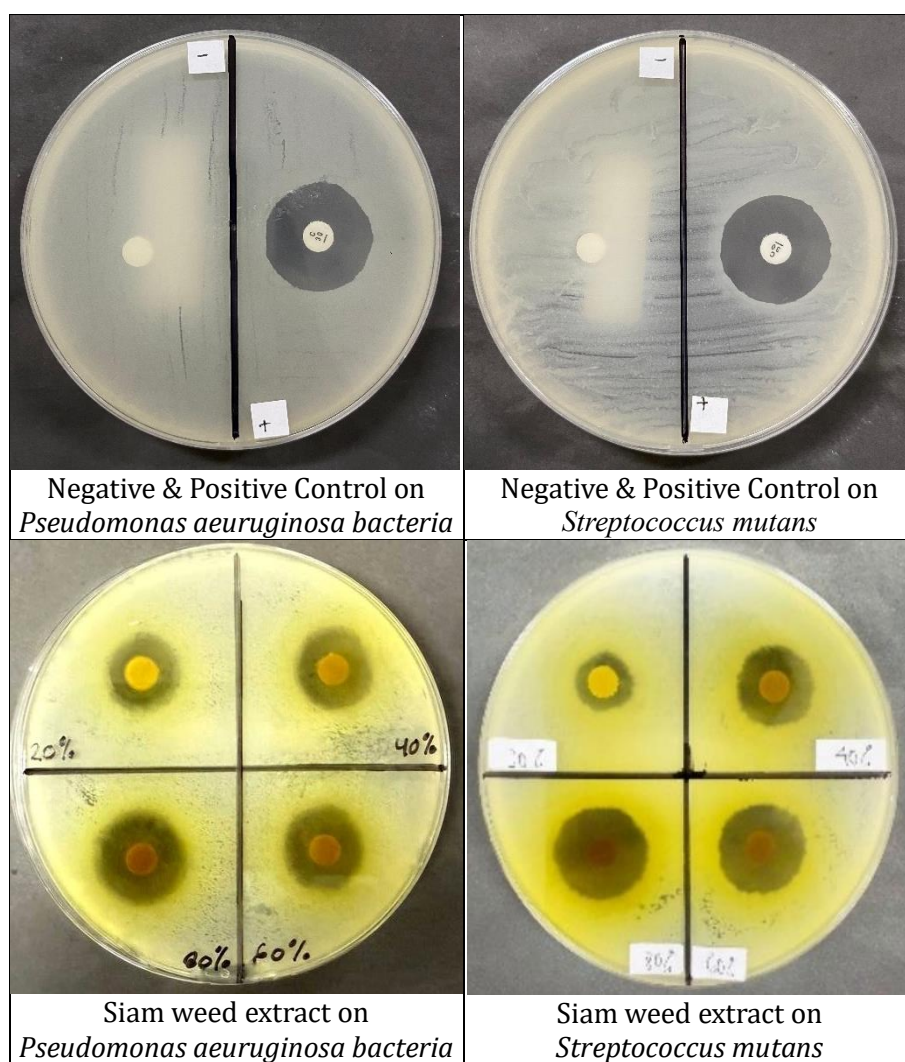


Figure 1. Results of the inhibition zone of the antibacterial activity test

Conclusion

Extract ethanol 96% of Siam weed (*Chromolaena odorata* L.) leaves has characteristics that meet the requirements, contains secondary metabolite compounds, and has antibacterial activity against the growth of *Pseudomonas*

aeruginosa and *Streptococcus mutans* bacteria. Antibacterial activity ranges from moderate to strong inhibition zone diameter.

Declaration of Competing Interest

The authors declare that they have no competing interests

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