

## Antibacterial Activity of Ethanolic Extract Siwak Stem (*Salvadora persica*) Against *Staphylococcus aureus* ATCC 25923

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### ABSTRACT

*Staphylococcus aureus* is one of the infectious bacteria that can cause infection. These bacteria frequently infect the mouth due to the abundance of food waste. Mouth infections caused by bacteria can result in an unpleasant odor, and this infection can be treated using *Salvadora persica* stem, often known as Siwak. *Salvadora persica* contains Saponins, Alkaloids, Tanins, and Fenols. This study is an investigation of antibacterial activity of *Salvadora persica* stems against *Staphylococcus aureus* ATCC 25923 growth. Extraction method used maceration with ethanol as a solvent. The group of extract compounds was identified using various chemical reagents. *Salvadora persica* stem extract was prepared in various concentrations of 6,25%, 12,5%, 25%, and 50% using *dimethyl sulfoxide* 2% (DMSO) as thinner. *Chlorhexidine* 0,2% was a positive control, and DMSO 2% was a negative control. Antibacterial activity test was conducted by diffusion method. Analysis data used one-way ANOVA test. The results was that ethanolic extract of the siwak stem contains several compounds: saponins, alkaloids, tannins, and phenols. The result showed that *Salvadora persica* stem extracts has antibacterial activity against *Staphylococcus aureus* ATCC 25923. Antibacterial activity test against *Staphylococcus aureus* from laboratory culture showed average of largest inhibition zone diameter on 50% concentration with 20,5 mm diameter and smallest inhibition zone at 6,25% concentration with 14 mm diameter.

**Keywords:** Antibacterial, *Staphylococcus aureus*, *Salvadora persica* stem, Etanol.

## INTRODUCTION

*Staphylococcus aureus* bacteria are circular. their, diameter ranges from 0.7 to 1.2  $\mu\text{m}$ . These are facultative anaerobes without flagella, and do not produce spores. Gram staining method reveals that *Staphylococcus aureus* is gram-positive and typically has a grape-like clustered structure. *Staphylococcus aureus* generates catalase and coagulase enzymes that can ferment mannitol and thrives at 37°C and pH 7.4. *Staphylococcus aureus* germs are a typical component of human skin and mucous membranes. This bacteria is an opportunistic pathogen capable of infecting both people and animals (Pantosti, 2012).

*Streptococcus mutans*, *Streptococcus viridians*, *Staphylococcus epidermidis*, *Staphylococcus pneumonia*, and *Staphylococcus aureus* are some bacteria typically found in human oral cavity. Oral cavity infections caused by *Staphylococcus aureus* are characterized by necrosis, stomatitis, inflammation, and abscess formation. Bacterial infections of the oral cavity include dental caries, gingivitis, periodontitis, and various odontogenic infectious diseases, most notably abscesses (Robertson & Smith, 2009). *Staphylococcus aureus* isolation rates vary depending on the group studied, with reported carriage rates ranging from 24% to 84% in healthy adult dentate oral cavities and an incidence of 48% in the denture-wearing population. In addition, the bacteria cause several unique oral illnesses (e.g., angular cheilitis, parotitis, staphylococcal mucositis). Recent evidence also suggests that *S. aureus* may play a role in dental implant failure (McCormack *et al.*, 2015).

*Staphylococcus aureus* causes oral illnesses, typically treated with antibiotics and painkillers. Antibiotics can be used to treat oral cavity disorders, but the inappropriate use of antibiotics will lead to resistance (Mohammed, 2012). *Salvadora perisca* or known as the toothbrush tree has been widely used to clean the mouth of Asian, African countries, and Middle Eastern countries. The parts of the plants used include roots, stems, and twigs. Besides, the stems are also used as toothpick materials to maintain oral hygiene (Al-Ayed *et al.*, 2016). Miswak sticks can be modified into chewing sticks and utilized as teeth and mouth cleansers. Chewing sticks have been widely used for over 7000 years due to their simplicity, availability, and religious significance. The Prophet Muhammad and His followers used chewing sticks to clean their teeth and mouth (Abhary & Al-Hazmi, 2016). Terpenoids, trimethylamine, alkaloids including salvadorin, chloride, lots of fluoride and silica, sulfur, vitamin C, tannins, saponins, flavonoids, and steroids are only some of the chemicals found in siwak stem extract. The ethanolic extract from Siwak stems contains active components like saponins, alkaloids, tannins, and phenols. Miswak's salvadorin compounds, a type of alkaloid component found in dried root bark, can inhibit *Staphylococcus aureus* bacteria due to the compound's alkaloid nature (Amal, 2018). The ethanolic extract of miswak stems (*Salvadora persica*) was shown to inhibit the development of *Porphyromonas gingivalis* with a MIC of 2.254% (v/v), per the findings of a study by (Kamil *et al.*, 2013). This study aimed to identify the chemical group and antibacterial activity of the ethanolic extract of miswak stems (*Salvadora persica*) against *Staphylococcus aureus* ATCC 25923.

## RESEARCH METHOD

From February through June 2019, the research was done in the Laboratory of Phytochemistry and Microbiology at Setia Budi University in Surakarta. This study is an in vitro analytical research laboratory experiment.

### Materials

The siwak plant's stems were acquired from a siwak wood producer in Surakarta, Central Java, Indonesia. *Staphylococcus aureus* ATCC 25923's bacterial culture was acquired from Setia Budi University's Microbiology Laboratory in Surakarta. Brain Heart Infusion (BHI) and Mueller Hinton Agar (MHA), two bacterial growth and antibacterial test mediums, were bought from Merck in Darmstadt, Germany. Chemical reagents of several kinds, including 96% ethanol, chloroform, aqua dest, 2% DMSO, 10% ammonia solution, concentrated HCl, acetic anhydride, 1% FeCl, hydrogen peroxide solution, physiological NaCl, BaCl<sub>2</sub>, and 10% H<sub>2</sub>SO<sub>4</sub>, were bought from Sigma Aldrich in Singapore.

### Extract of Siwak Plant Stem

Siwak stem powder was weighed at 300 grams and then placed in a maceration bottle with 3 litres of 96% ethanol solvent, resulting in a 1:10 ratio (300 grams of powder plus 3 litres of ethanol solvent). After two days, the maceration products were filtered using filter paper and occasionally shaken, to obtain the filtrate. In order to get a proper concentration of Siwak stem extract, the filtering results were processed using a rotary evaporator at a temperature of 40°C until the ethanol solvent was used up. The extract was placed in a dark bottle and covered with aluminium foil. Siwak stem extract was prepared serially at doses of 50%, 25%, 12.5%, and 6.25% using 2% DMSO (dimethyl sulfoxide) (Agustin *et al.*, 2018; Djamal *et al.*, 2020; Rukmana, 2015; Seran *et al.*, 2020).

### Siwak Stem Extract Qualitative Compound Identification

The goal of the identification of chemical content is to confirm that the chemical composition of the siwak stem extract is accurate. By examining the presence of saponins, alkaloids, tannins, and phenolics, the class of compounds in the Siwak stem extract was identified qualitatively. The chemical compounds are identified using a wide range of chemical reagents.

The saponin group was detected by preparing 2 ml of the extract mixed with 10 ml of aquadest, it was shaken and added by 2N HCl. The group of alkaloid chemicals can be identified using 2 ml of extract, 2 ml of hot HCl, and 3 drops of Dragendorff's reagent. The group of tannin compounds was identified by dissolving 2 ml of the extract in 10 ml of aquadest and adding 3 drops of FeCl<sub>3</sub> 1%. Phenolic detection was conducted by dissolving 2 ml of the extract in 10 ml of distillate water, and adding five drops of 5% FeCl<sub>3</sub> (Rukmana *et al.*, 2020) (Rukmana *et al.*, 2019).

### Preparation of *Staphylococcus aureus* ATCC 25923 Test Suspension

*Staphylococcus aureus* ATCC 25923 test bacteria were sub cultured on Brain Heart Infusion (BHI) media, then it was incubated for 24 hours at 37°C. The Mc Farland standard of 1.5x10<sup>8</sup> cfu/mL was then used to standardize bacteria grown on BHI medium (Prastiyanto *et al.*, 2020) (Noor *et al.*, 2020).

### Antibacterial Diffusion Method Test

Mueller Hinton Agar (MHA), the antibacterial test medium, was sterilized and placed in a petri dish. In order to evenly spread the germs throughout the MHA medium, a sterile cotton swab was dipped into a bacterial solution with standardized turbidity and the Mc Farland standard 1.5x10<sup>8</sup> cfu/mL. A boorproof tool was used to create wells in MHA media, which was one for a positive control, and another one for a negative control. Meanwhile, the remaining wells were for extracts that were tested at concentrations of 50%, 25%, 12.5%, and 6.25%, with each hole filled 50µL. The treatment was repeated three times and then incubated at 37°C for 24 hours. Chlorhexidine 0.2% was used as a positive control, and DMSO 2% (Dimethyl sulfoxide) as a negative control (Hidanah *et al.*, 2017) (Turahman, 2019).

### Data Analysis

The acquired information was shown as the size of the inhibitory zone for each concentration of Siwak stem extract (*Salvadora persica*) against *Staphylococcus aureus* ATCC 25923. The data were examined statistically with a one-way analysis of variance (ANOVA).

## RESULT AND DISCUSSION

In this study, the water content of siwak stem powder was 9.49%. This water content is relatively low and it can inhibit the growth of microorganisms and fungi. In order to get the ethanol content out of the siwak stems, a maceration process was used. The siwak stem extraction findings are shown in table 1.

Table 1. Numbers for the ethanolic extract of Siwak stems

Siwak stem powder (gram)	Bottle Mass (grams)	Bottle mass + Ekstrakt (gram)	Mass of ekstrakt (gram)	Rendemen (%)
300	240,162	259,089	18,927	6,30%

The concentration of Siwak stem ethanolic extract was 6.30 percent. Large amounts of the extract were harvested successfully in this study. The concentration that can be expected when extracting natural elements from plants varies depends on the nature of the resource being mined. Leaves, stems, fruits, seeds, and roots are all parts of plants that may have medical applications. The more solvent and chemicals are extracted, the larger the concentration of the extract (Prastiyanto *et al.*, 2020).

Different chemical reagents were used to conduct qualitative analyses of the ethanolic extract of Siwak stems. Table 2 displays the results of a study that sought to classify the compounds present in an ethanolic extract of miswak stems.

Table 2. Results of qualitative identification of compound groups in the ethanolic extract of Siwak stems

Group of compounds	Results
Saponin	+
Alkaloid	+
Tanin	+
Fenol	+

Ethanolic extract of Siwak stems was found to have saponins, tannins, phenols, and alkaloids. Salvidorine is an example of the alkaloid class found in siwak stem extract. Salvidorine, an alkaloid compound found in dried root bark, is one of the ingredients in Siwak stem. Other ingredients include chloride, fluoride to prevent caries (a substance often added to commercial toothpaste), silica to whiten teeth, sulfur to remove dental plaque, vitamin C to treat canker sores, and resin to protect tooth enamel (Suryani & Astuti, 2016). Saponins, alkaloids, cardiac glycosides, terpenoids, and flavonoids have all been found in the ethanolic extract of siwak stems, according to earlier research.

The antibacterial properties of siwak stem extract have been well-documented. *Salmonella enterica* ATCC 5174, *Proteus vulgaris* ATCC 49132, *Klebsiella pneumonia* ATCC 27736, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 are only some of the gram-negative pathogenic bacteria that siwak stem extract can inhibit (Abdallah & Al-Harbi, 2015).

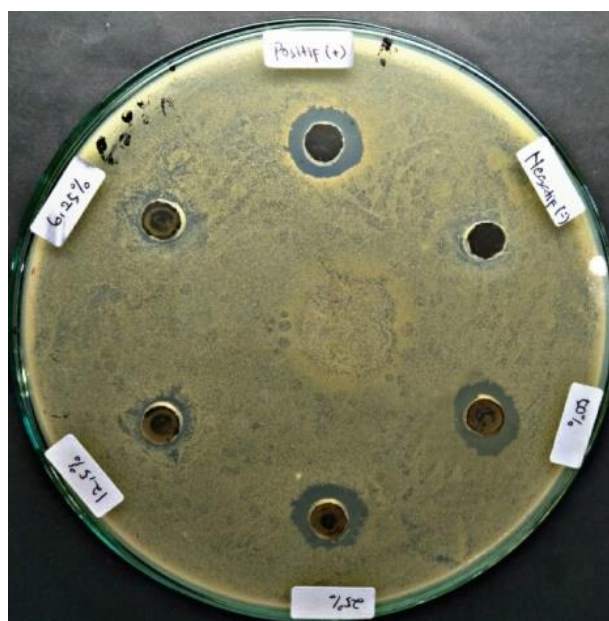


Figure 1. Results of the antibacterial activity test of the ethanolic extract of miswak stems against *Staphylococcus aureus* ATCC 25923.

The inhibitory zone against *Staphylococcus aureus* ATCC 25923 increases with increasing concentrations of Siwak stem extract, as shown in Figure 1. Growth inhibition of *Staphylococcus aureus* ATCC 25923 was measured by measuring the diameter of the inhibition zone as a function of Siwak stem ethanolic extract concentration (Table 3). The results indicates that the ethanolic extract of siwak stems has a greater ability to kill bacteria due to a higher concentration of active chemicals. Salvidorin, a member of the alkaloid family, can be detected in the ethanolic extract of siwak stems. The alkaloid family can function as an antibiotic by disrupting the formation of the bacterial cell wall's peptidoglycan component, leading to the eventual death of the cell (Shilpa *et al.*, 2018). Antibacterial active saponins are present in the ethanolic extract of siwak stems. The cell membrane is a barrier that controls what goes in and out of the cell, thus it's vitally important. When the cell membrane's ability to function is compromised, the cell dies (Rukmana *et al.*, 2019).

Table 3. The results of the calculation of the inhibition zone of the ethanolic extract of miswak stems against *Staphylococcus aureus* ATCC 25923

No	Extract Concentration	Replications (mm)			Average inhibition zone diameter (mm)
		One	Two	Three	
1.	6,25%	12,5	16,5	13	14 <sup>b</sup>
2.	12,5%	15	19,5	16,5	17 <sup>c</sup>
3.	25%	18,5	20,5	19	19,3 <sup>d</sup>
4.	50%	19	22	20,5	20,5 <sup>e</sup>
5.	Negative control	0	0	0	0 <sup>a</sup>
6.	Positive control (+)	20	23	22	21,6 <sup>e</sup>

Note: The <sup>a-e</sup> symbols indicate significant differences between treatments ( $p < 0.05$ ).

The ethanolic extract of miswak stems also contains chemicals belonging to the tannin groups. Tannins can be antibacterial because they prevent bacterial cell formation by blocking the enzymes reverse transcriptase and DNA topoisomerase. Polyphenol acts as a toxin in bacterial cells, destroying the cell wall and protein precipitation. Polyphenols have been shown to disrupt bacterial cell membranes, denature proteins, inhibit enzymes, and even cause cells to leak (Hussen *et al.*, 2019).

## CONCLUSION

The ethanolic extract of Siwak stems has been found to have alkaloid chemicals, saponins, tannins, and phenols. Ethanolic extract from siwak stems has antibacterial activity against *Staphylococcus aureus* ATCC 25923. The optimal extract concentration for inhibiting *Staphylococcus aureus* ATCC 25923 was 50 percent, with an average inhibition zone of 20.5 millimeters.

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