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Antibacterial Effect of Siwak (*Salvadora persica*) Against *Pseudomonas aeruginosa*

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Original Research Article

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Abstract: *Pseudomonas aeruginosa* is a dangerous bacterium causing nosocomial infections and difficult to treat because of its broad resistance to antibiotics. Siwak sticks, root of Arak tree (*Salvadora persica*) has been widely used since 7.000 years ago and has many benefits including antibacterial effect. Thus, research on whether siwak (*Salvadora persica*) stick extract has antibacterial effects need to be done by measuring the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). This experimental research was intended to explain such effect. This research used the dilution test of siwak (*Salvadora persica*) ethanol extract against *Pseudomonas aeruginosa*. Growth of bacteria on eleven different concentrations of each tube (80%, 70%, 60%, 50%, 40%, 20%, 10%, 5%, 2.5%, 1:25%, 0.625%) was observed by viewing tube clarity after incubation at 36 ^{o}C for 24 hours to measure the MIC. MBC was measured by the growth of bacteria on plates as a result of the bacteria cultivation from each tube. Replication was performed four times and the results were analyzed descriptively. In the MIC test, tubes with concentration of 80% to 20% were clear and tubes of 10% to 0.625% were cloudy. In MBC test, tube with concentration of 80% to 60% were not overgrown with bacteria and tube of 50% to 0.625% were overgrown with bacteria. In conclusion, MIC of siwak (*Salvadora persica*) stick extract against *Pseudomonas aeruginosa* is 20% and the MBC value of siwak (*Salvadora persica*) extract against *Pseudomonas aeruginosa* is 60%.

Keywords: Siwak (Salvadora persica), Pseudomonas aeruginosa, anti-bacterial test, minimum inhibition concentration, minimum bactericidal concentration.

INTRODUCTION

Siwak stick (*Salvadora persica*) has been used by the Babylonians 7,000 years ago and is also used by the Greeks, the Roman Empire, and the Ancient Egypt. There are variety of siwak stick, but the most commonly used is siwak stick of Arak trees (*Salvadora persica*) which is effective to prevent the formation of dental plaque and has antibacterial effect [1, 2]. From gas chromatography-mass spectrometry (GC-MS) analysis, there are 17 essential oil components of siwak root (*Salvadora persica*) with 70% main constituent is benzyl isothiocyanate (BITC) that potently kills Gram positive bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes* [3, 4]. Siwak stick (*Salvadora persica*) is recommended in Islam to be used as a dental cleaning tool every day. As noted in the hadith "If not burden my people, indeed I would have ordered them to use siwak every time before salah." (Bukhari (2/374/887), Muslim (1/220/252) and Tirmidhi (1/18 / 22) see Shahihul jami ' Hadith 5315).

Pseudomonas aeruginosa is a Gram negative, rod-shaped bacteria, causing 10-20% of nosocomial infections, and can infect almost all parts of the body [5]. As many as 136 patients with bacteremia caused by Pseudomonas aeruginosa showed a mortality rate of 39%, while antibacterials given to 47 patients were only 27.7% effective [6].

In 2010 it was reported that more than 50% specimens of *Pseudomonas aeruginosa* were resistant to 14 kinds of antibiotics, including ampicillin, erythromycin, amoxicillin, cefuroxime, ceftriaxone and gentamicin which are the first-line antibiotics, and 19.2% were resistant to third-line antibiotic, meropenem [7].

Based on antibacterial test, siwak stick extract solution (*Salvadora persica*) can inhibit the growth of *Streptococcus mutans* and *Bacteroides melaninogenicus* [8, 9]. It has been reported also that siwak stick extract (*Salvadora persica*) inhibited the growth of 6 pathogenic oral bacterias, among others,

Staphylococcus aureus, *Streptococcus mutans*, *Streptococcus faecalis*, *Streptococcus pyogenes*, *Lactobacillus acidophilus*, and *Candida albicans* [10].

Based on these explanation, it should be investigated whether siwak stick extract (*Salvadora persica*) has antibacterial effect against *Pseudomonas aeruginosa*.

The objectives of this research are knowing the antibacterial effect of siwak stick extract (*Salvadora persica*) against *Pseudomonas aeruginosa* in vitro, measuring the minimum concentration of siwak stick extract (*Salvadora persica*) which can inhibit the growth of *Pseudomonas aeruginosa*, and measuring the minimum concentration of siwak stick extract (*Salvadora persica*) which can kill *Pseudomonas aeruginosa.*

MATERIALS AND METHODS

This is a true experimental research to test the antibacterial effect of siwak stick (*Salvadora persica*) against *Pseudomonas aeruginosa* using dilution method of antibacterial test. The materials used in this research was *Pseudomonas aeruginosa* bacteria, siwak extract, ethanol, distilled water, nutrient broth and nutrient Agar. The Pseudomonas aeruginosa bacteria was obtained from Microbiology Department of Medical Faculty on Universitas Airlangga. The origin of the siwak stick was Saudi Arabia and was obtained from a seller in Indonesia. Ethanol was used for extracting the siwak stick. Distilled water was used as siwak extract solvent in the dilution test. Nutrient broth was used as bacteria nutrient source.

Siwak stick (*Salvadora persica*) was cleaned, then cut into small pieces, 1-2 mm thick. Then siwak sawdust (*Salvadora persica*) was inserted in the extractor shaker, ethanol 96% was added to siwak (*Salvadora persica*) until it was submerged then shake for 2 x 24 hours. After that, the material was filtered so that the first clear yellowish filtrate was obtained. The siwak dregs (*Salvadora persica*) was returned to the extractor, then 96% ethanol was added again. Furthermore, shake back for 2 hours, then filter. The second filtrate was obtained and was added into the first filtrate. The filtrate then put into vacuum evaporator at a temperature of 50-60 ° C to separate the ethanol solvent by evaporation and obtained brownish yellow siwak stick extract (*Salvadora persica*).

It was taken 0.1 mL of 0.5 Mc Farland (1.5 x 10^8 CFU mL^{-1}) from the cultures of *Pseudomonas aeruginosa* at 24 hours old, then it was inserted into a test tube containing nutrient broth and incubated at 37 ° C for 24 hours. The study was begun by preparing 13 pieces of tubes labeled Control Tube 1 (CT1), CT2, Test Tube 1 (TT1) to TT11. The first stage was adding the CT1, TT2 until TT8 0.5 mL of nutrient broth with bacteria.

The second stage was adding 0.4 mL of siwak stick extract (*Salvadora persica*) 100% and 0.1 mL of distilled water to CT2, TT1 and TT2 thus it was obtained siwak stick extract (*Salvadora persica*) 80% in 0.5 mL of extract solution in CT2 and TT1 and siwak stick extract (*Salvadora persica*) 40% in 1 mL of extract solution in TT2. As much as 0.5 mL of extract solution in TT2 was taken and was added to TT3. As much as 0.5 mL of solution in TT3 was taken and added to TT4 afterwards. Similarly done taking 0.5 mL of solution from the previous tube and added to the next tubes until TT8. Finally, as much as 0.5 mL of extract solution in TT11 was taken out and was discarded. So that each tube contained a number of 0.5 mL of extract solution with different concentrations.

TT9 (70%) was filled with 0.35 mL of siwak stick extract (*Salvadora persica*) plus 0.15 mL of distilled water and bacteria. Tube TT10 (60%) was filled with 0.3 mL of siwak stick extract (*Salvadora persica*) plus 0.2 mL of distilled water and bacteria. And TT11 (50%) was filled with 0.25 mL of siwak stick extract (*Salvadora persica*) plus 0.25 mL of distilled water and bacteria. In the end, all thirteen reaction tubes were incubated for 24 hours in a temperature of 37^o C.

Measurement of minimum inhibitory concentration (MIC) was done by looking at the smallest siwak stick extract (*Salvadora persica*) concentration in the tube which was able to inhibit the growth of bacteria. The observations were done visually. After that, all tubes were cultured on Agar plate in order to measure the minimum bactericidal concentration (MBC). Minimum bactericidal concentration (MBC) was measured by looking which Agar plate were overgrown with *Pseudomonas aeruginosa*. The smallest concentration which was not overgrown with bacteria was the MBC value. Replication of this test was done four times.

RESULTS AND DISCUSSION

From MIC test, the results obtained from tubes with concentration of 80%, 70%, 60%, 50%, 40% and 20% were clear, meaning the bacteria did not grow. The tubes with concentration of 10%, 5%, 2.5%, 1.25% and 0.625% looks cloudy, meaning the bacteria could grow. It can be concluded that the minimum inhibitory concentration (MIC) value of siwak stick extract (*Salvadora persica*) against *Pseudomonas aeruginosa* was 20%. At a concentration of at least 20%, siwak stick extract (*Salvadora persica*) can inhibit the growth of *Pseudomonas aeruginosa*.

Table-1: Results of minimum inhibitory concentration (MIC) test

		Bacteria growth										
		80%	70%	60%	50%	40%	20%	10%	5%	2,5%	1,25%	0,625%
Replication	I	-	-	-	-	-	-	+	+	+	+	+
	II	-	-	-	-	-	-	+	+	+	+	+
	III	-	-	-	-	-	-	+	+	+	+	+
	IV	-	-	-	-	-	-	+	+	+	+	+
+ = cloudy tube (bacteria grew) - = clear tube (bacteria didn't grow)												

From MBC test, the results obtained from Agar plates with concentration of 80%, 70% and 60% were clear, meaning the bacteria was dead. The Agar plate with concentration of 50%, 40%, 20%, 10%, 5%, 2.5%, 1.25% and 0.625% were overgrown with bacteria, meaning the bacteria was not dead. It can be concluded that the minimum bactericidal concentration (MBC) value of siwak stick extract (*Salvadora persica*) against *Pseudomonas aeruginosa* was 60%. At a concentration of at least 60%, siwak stick extract (*Salvadora persica*) can kill bacteria *Pseudomonas aeruginosa*.

Table-2: Results of minimum bactericidal concentration (MBC) test

		Bacteria growth in Agar plate										
		80%	70%	60%	50%	40%	20%	10%	5%	2,5%	1,25%	0,625%
Replication	I	-	-	-	+	+	+	+	+	+	+	+
	II	-	-	-	+	+	+	+	+	+	+	+
	III	-	-	-	+	+	+	+	+	+	+	+
	IV	-	-	-	+	+	+	+	+	+	+	+
+ = Agar plate was overgrown with bacteria (bacteria was not dead) - = Agar plate was not overgrown with bacteria (bacteria was dead)												

In the extraction process of siwak stick (*Salvadora persica*), ethanol was used as a solvent, because it was widely used for extracting active ingredients from cells that are antibacterial. Thus, the active ingredient which wanted to be used in this study could be extracted well [11].

Based on the results and analysis, the MIC value of siwak stick extract (*Salvadora persica*) was 20%. Whereas the MBC value of siwak stick extract (*Salvadora persica*) was 60%. Al- Sieni [12] obtained similar result i.e., a siwak stick with methanol extract could inhibit the growth of five pathogenic bacteria (*Fusobacterium nucleatum*, *Lactobacillus casei*, *Staphylococcus epidermidis*, *Streptococcus mutans* and *Streptococcus salivarius*) at concentrations in the range of at least 50% -100%.

Siwak stick (*Salvadora persica*) has antibacterial activity because it contains a variety of active antibacterial ingredients including, alkaloids, flavonoids, benzyl isothiocyanate (BITC), benzyl nitrate, trimethylamine, butanediamide, sulfur and saponin [13, 14].

Alkaloids which are contained in many plants and have many effects on the human body such as analgesics, central nervous stimulant, antihypotension, antihypertension and antipyretic depending on their type. In addition, alkaloids also have a potent antibacterial effect. Alkaloids are direct antibacterial agents by inhibiting the synthesis of nucleic acids, inhibiting dihydrofolate reductase enzyme, inhibiting cell division, damaging the integrity of the outer cell membrane and cytoplasmic membrane. Alkaloids in low levels also enhance the activity of antibiotics by altering the permeability of cells or by blocking pumps out (efflux pump) so that the levels of antibiotics that can enter the cell are increasing. Alkaloids can also reduce the pathogenicity of bacteria by interfering the regulation of virulence genes, inhibits the sortase enzyme, damaging fimbiria and another adhesin, inhibiting biofilm formation and inhibiting the toxic effects of the bacteria [15].

Flavonoids are antibacterial agents obtained in siwak stick extract (*Salvadora persica*). It was said that the B ring of flavonoids play a role in hydrogen intercalation or bonding with bases in nucleic acids and this explains the inhibition action of DNA and RNA formation. Flavonoids also reduce the stability of bacterial cell membrane, damage cell membranes and disrupt energy metabolism processes such as antibiotics which inhibiting respiration processes to reduce the availability of energy resulting in bacterial cell death [16].

Benzylisothiocyanate (BITC) is an active ingredient which has antibacterial activity against Gram positive and negative bacteria, antifungal and anticancer [17]. Its mechanism as antibacterial remains unclear, however it was reported that BITC kills bacteria by intracellular protein aggregation, which resulted in a

major disruption of metabolic processes, and ultimately toward cell death [18].

CONCLUSION

Based on this research, it could be concluded that the siwak stick extract (*Salvadora persica*) had antibacterial activity against *Pseudomonas aeruginosa*. Minimum concentration of siwak stick extract (*Salvadora persica*) which could inhibit the growth of bacteria *Pseudomonas aeruginosa* was 20%. And minimum concentration of siwak stick extract (*Salvadora persica*), which could kill the bacteria *Pseudomonas aeruginosa* was 80%.

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