

Effectiveness of siwak salvadora persica extract to aggregatibacter actinomycetemcomitans as one of pathogenic bacteria causing periodontal disease



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Abstract

Objective: Periodontal disease is one of oral and dental diseases which most commonly found in humans caused by several factors, one of them due to the accumulation of bacterial plaque. Aggregatibacter actinomycetemcomitans is a bacterial pathogen that frequently causes periodontal disease.

Material and Methods: Sample of *A. actinomycetemcomitans* colonies in MHA medium. Twenty four samples were divided into six treatment groups, they were 5 groups given the extract siwak with concentration of 3.125%, 6.25%, 12.5%, 25%, 50% and 1 group treated with control DMSO 5%. Inhibition zone

was measured after 48 hours incubation at 37°C and measured using caliper. To inhibit the growth of these bacteria can be done by using natural ingredients that contain anti-bacterial agent.

Results: Data analysis was performed using Kruskal Wallis test. The mean diameter of inhibition zone at concentrations of 3.125%, 6.25%, 12.5%, 25% and 50% were 6.4 mm, 7.0 mm, 7.2 mm, 7.9 mm and 8.6 mm.

Conclusion: Siwak extract can inhibit the growth of *A. actinomycetemcomitans* which is one of pathogenic bacteria causing periodontal disease.

Keywords: Siwak extract, Aggregatibacter actinomycetemcomitans, Periodontal disease

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Introduction

Periodonsium is tooth-supportive tissue which consist of gingiva, periodontal ligament, cementum and alveolar bone. It is divided into two parts i.e. gingiva that has main function in protecting the underlying tissue and connective part which consist of periodontal ligament, cementum and alveolar bone.¹ According to Basic National Health Survey in 2013, national prevalence of dental and oral problems was 25.6%. Sixteen provinces had dental and oral problems above the national value, they are Aceh 30.5%, DKI Jakarta 29.1%, Jawa Barat 28.0%, DI Yogyakarta 32.1%, Jawa Timur 28.6%, Nusa Tenggara Barat 26.9%, Nusa Tenggara Timur 27.2%, Kalimantan Selatan 36.1%, Sulawesi Utara 31.6%, Sulawesi Tengah 35.6%, Sulawesi Selatan 36.2%, Southeast Sulawesi 28.6%, Gorontalo 30.1%, West Sulawesi 32.2%, Maluku 27.2% and Maluku Utara 26.9%.²

Damage to the periodontal tissues is usually due to the accumulation of bacterial plaque on the tooth crown which then extends into the gingival sulcus and impair the adjacent gingiva.³ Most common bacteria which found in people suffering periodontal disease are porphyromonas gingivalis, treponema denticola, tannerella forsythia and *A. actinomycetemcomitans*.¹

A. actinomycetemcomitans is one of the bacteria found in periodontal disease, including a

group of gram-negative bacteria, facultative anaerobes, coccobacillus, non-motile, colorless and capnophilic.^{4,5} To inhibit the growth of these bacteria can be done in various ways, one of them is using natural ingredients from plants that contain antibacterial agents such as siwak.³

Siwak or *salvadora persica* is a medical plant whose roots have been widely used by many people in Africa, South America, Middle East and Asia. Siwak is a plant that is very popular in the Middle East. The World Health Organization (WHO) recommends and advocate siwak as an effective tool for oral hygiene, with softwood fiber mechanical action and its chemical therapeutic action.⁶ Siwak contains essential oils and a variety of other chemical compounds, i.e. Anorganic compound, such triethylamine, alkaloids, flavonoids, anthraquinone, tannins, saponins, sterols, vitamin C and inorganic compounds, such chloride, calcium, a large amount of fluoride, silica and sulfur, the chemical components of essential oils contained in miswak leaves such benzyl nitrile, eugenol, thymol, isothymol, eucalyptol, isoterpinolen and beta-caryophyllen.⁷

The research results of inhibition test of siwak on porphyromonas gingivalis showed that siwak wood have inhibitory activity against porphyromonas

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gingivalis. Therefore, the authors are interested in investigating the effectiveness of siwak against *A. actinomycetemcomitans* which is one of the pathogenic bacteria causing periodontal disease.

Material and Methods

This was laboratory experiments research conducted at the Phytochemistry Laboratory Faculty of Pharmacy, Hasanuddin University and Microbiology Laboratory Faculty of Medicine, Hasanuddin University. Siwak wood mashed then diluted with 96% ethanol for five days. Then filtered using filter paper and the filtrate was evaporated using a rotary evaporator in order to obtain a thick extract.

Siwak extract was made into five different concentration which were 3.125%, 6.25%, 12.5%, 25% and 50% with DMSO 5% as negative control.

Then, it was tested on *A. actinomycetemcomitans*, replication was performed four times according federer sample size formula. Incubation during 1x48 hour in temperature of 37°C. After that, measure the clear zone using caliper.

Results

It has been performed research about the effectiveness of siwak extracts against *A. actinomycetemcomitans*. This laboratory experiment was conducted at the phytochemistry Laboratory, Faculty of Pharmacy and Microbiology Laboratory, Faculty of Medicine, Hasanuddin University. This posttest was only with control group design conducted in November to December 2016. This research sample is *A. actinomycetemcomitans* preparations that had been cultured in the laboratory.

This study used five siwak extract concentration, the concentration of 3.125%, 6.25%, 12.5%, 25% and 50%, using 5% DMSO as a negative control. Based on the sample size formula Federer, the number of samples in each group concentration and control, respectively four samples for each group, so that the overall amount of the sample of 24 bacteria preparation. Inhibition was measured after treatment given figure 1. The size of the clear zone that occurred was a measure of inhibition power of treatment and measured using a caliper and expressed in millimeters (mm). After that, data was collected, processed and analyzed using SPSS version 22.0. Results of the study appear in the table of distribution as follows:

Table 1 shows that the differences in vast of inhibition zone in each treatment and control groups, where there are four samples of bacterial preparations for each group. Based on these data, the lowest mean of board inhibition zones was at 3.125% concentrations and the highest mean of inhibition zone was at 50% concentration. In this study, using Kruskal Wallis test it showed that p-value: 0.005 ($p < 0.05$), this means that there were significant differences in vast of inhibition zones between siwak with 3.124%, 6:25%, 12.5%, 25%, 50% concentration and control DMSO 5% in inhibiting the growth of bacteria *A. actinomycetemcomitans*. Due to the results obtained meaning-ful, then the test continues to see the differences of the various concentrations.

In table 2 comparison of two groups of concentration that has p-value < 0.05 means that there were significant differences between the two groups of those concentrations, whereas the comparison of DMSO 5% with concentration of 3.125% ($p: 0.508$), DMSO 5% with 6.25% ($p: 0.219$), the concentration of 3.125% with 6.25% ($p: 0.386$), the concentration of 3.125%

Table 1 Differences of wide of inhibition zone (mm) using siwak extract with 3.125%, 6.25%, 12.5%, 25%, 50% concentration and DMSO 5% as control

Intervention	n	Wide of inhibition zone	
		Mean \pm SD	p-value
DMSO 5%	4	6.2 \pm 0.000	0.005
3.125%	4	6.4 \pm 0.440	
6.25%	4	7.0 \pm 0.910	
12.5%	4	7.2 \pm 0.590	
25%	4	7.9 \pm 0.518	
50%	4	8.6 \pm 0.434	
Total	24	7.2 \pm 0.990	

*Kruskal Wallis Test: $p < 0.05$; significant

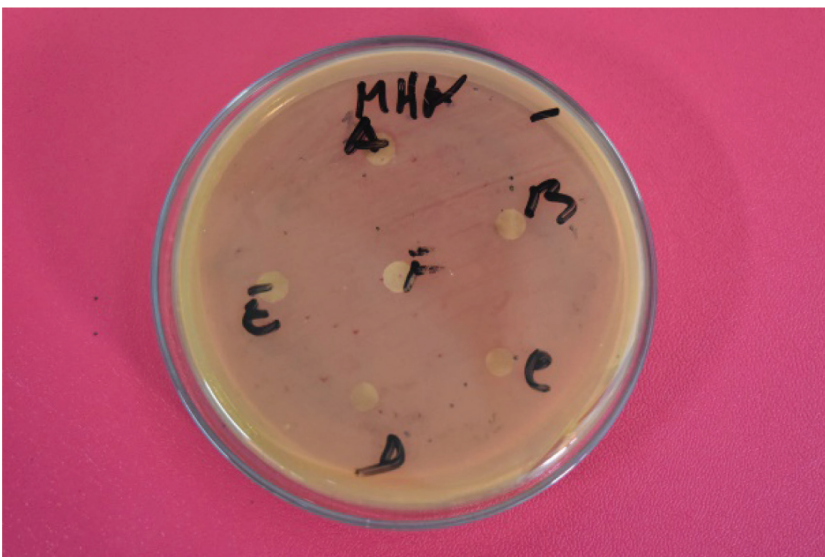


Figure 1 Inhibition zone of siwak extract on *A. actinomycetemcomitans*

Table 2 Differences between intervention groups

Groups		Mean ± SD	p – value
DMSO 5%	3.125%	6.4 ± 0.440	0.508
	6.25%	7.0 ± 0.910	0.219
	12.5%	7.2 ± 0.590	0.013
	25%	7.9 ± 0.518	0.013
	50%	8.6 ± 0.434	0.013
3.125%	6.25%	7.0 ± 0.910	0.386
	12.5%	7.2 ± 0.590	0.081
	25%	7.9 ± 0.518	0.020
	50%	8.6 ± 0.434	0.020
6.25%	12.5%	7.2 ± 0.590	0.772
	25%	7.9 ± 0.518	0.245
	50%	8.6 ± 0.434	0.020
12.5%	25%	7.9 ± 0.518	0.080
	50%	8.6 ± 0.434	0.019
25%	50%	8.6 ± 0.434	0.080

*Mann Whitney test: p<0.05; significant

with 12.5% (p:0.081), the concentration of 6.25% with 12.5% (p:0.772), the concentration of 6.25% with 25% (p:0.245), the concentration of 12.5% with 25% (p:0.080), concentration of 25% with 50% (p:0.080) has a value of p>0.05 so there were no significant difference between the groups.

Discussion

Periodontal disease is a dental and oral diseases which most commonly found in humans, caused by several factors, one of them due to the accumulation of bacterial plaque. *A. actinomycetemcomitans* is pathogenic bacteria that frequently causes periodontal disease. To inhibit the growth of these bacteria can be done by using natural ingredients that contain anti-bacterial agent. One of the natural ingredients that contain antibacterial substances is siwak.

In the study conducted by Ashshobirin et al.³ showed that siwak wood extract can inhibit the growth of bacteria *porphyromonas gingivalis*, this is due to the chemical compounds contained in siwak wood containing antibacterial activity such as saponins, flavonoids, tannins, alkaloids and terpenoid.³

Research by Santoso⁸ that the effect of the siwak extract solution can inhibit the growth of *S. mutans* in vitro and in vivo.⁸ Al-Bayaty also expressed alcohol extract of siwak produce good and safe antibacterial activity Besides of antibacterial, Kumar et al.⁹ stated that siwak also contains antiulcer, antifungal, antiparasitic and antiviral.⁹

Based on the researches above, this study was conducted in order to investigate whether siwak extract can inhibit the growth of bacteria *A. actinomycetemcomitans*, which is one of the pathogenic bacteria causing periodontal diseases.

Siwak mashed and dried and then mixed with 96% ethanol and allowed to stand for five days. Then, the results of immersion filtered using filter paper and evaporated using rotary evaporator in order to obtain thick siwak extract. Siwak extract later made into five different concentration, i.e. 3.125%, 6.25%, 12.5%, 25% and 50% by using 5% DMSO as negative control, and then dropped into the paper disc and put it on preparation of *A. actinomycetemcomitans*. Then incubated for 48 hours and measuring inhibition zone using caliper. Large zone of inhibition continues to increase with increasing concentration of siwak extract. The highest inhibition zone presented in 50% concentrations and the lowest inhibition zone was at 3.125% concentration. The higher siwak extract concentration, the higher its antibacterial activity.

The data was tested using Kruskal Wallis to determine significance of inhibition zone on different concentration of siwak extract, test to determine the differences between the six treatment groups. The results obtained have significant difference at each concentration, except DMSO 5% with siwak extract at 3.125% concentration, DMSO 5% with siwak extract at 6.25% concentration, siwak extract at 3.125% concentration with 6.25%, siwak extract at 3.125% compared with 12.5%, concentration of 6.25% with 12.5%, siwak extract at 6.25% with 25%, siwak extract at 12.5% with 25%, siwak extract at 25% with 50%.

The results showed that the siwak extract can inhibit *A. actinomycetemcomitans*, with highest concentration of 50% and the lowest concentration of 3.125%. The antibacterial activity because siwak has compounds that can inhibit the growth of bacteria, such as tannins, saponins, flavonoids, alkaloids and terpenoids with a different mechanism. Tannins and flavonoids can interfere cell's permeability by wrinkled the cell walls. Disruption permeability, the cell cannot do its activities so that growth is impaired. Saponins have OH groups that destroy bacteria by destroying the cell wall and penetrate into the cell by dissolving the lipid layer. Alkaloids cause cell wall layers are not fully formed and cause cell death by disrupting components of the peptidoglycan in the bacteria cell.³

Conclusion

Siwak extract can inhibit the growth of *A. actinomycetemcomitans* which is one of

pathogenic bacteria causing periodontal disease. The higher concentration of siwak extract, the greater its inhibitory power. The highest concentration which can inhibit the growth of *A. actinomycetemcomitans* is 50% concentration and the lowest concentration is 3.125%.

Conflict of Interest

The authors report no conflict of interest.

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