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Research Report

Antibacterial efficacy of *Salvadora persica* as a cleansing teeth towards *Streptococcus mutans* and *Lactobacilli* colonies

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ABSTRACT

Background: *Salvadora persica* is a traditional chewing stick for cleaning teeth that it is known Siwak. Several studies have demonstrated the antimicrobial effects of *Salvadora persica*. **Purpose:** This study was aimed to examine the effectiveness of *Salvadora persica* in several modified preparation against the salivary *Streptococcus mutans* and *Lactobacilli*. **Methods:** A single-blind, randomized clinical trial study with crossover design was used. The study comprised of 5 groups, per group consisted of 14 healthy dental students who had good oral hygiene. Each participant was given 5 intervention to clean their teeth using, electric toothbrush modified with siwak, electric toothbrush with siwak toothpaste (colgate kayu sugi toothpaste), electric toothbrush with general toothpaste (colgate total toothpaste), original siwak chewing stick and normal saline. The wash out periode each intervention was 3 days. Patients' saliva was used to quantify the levels of *Streptococcus mutans* and *Lactobacilli* using caries risk test (CRT) kit from Vivadent. **Results:** The results showed that there was a reduction in *Streptococcus mutans* and *Lactobacilli* risk score after cleansing different intervention except electric toothbrush modified with siwak. However, there was no significant difference for *Streptococcus mutans* ($p=0.158$) and *Lactobacilli* ($p=0.396$) risk score reduction when comparison was done between the groups. **Conclusion:** The original siwak chewing stick has antimicrobial effects similar to toothbrushing with general toothpaste and *salvadora persica* toothpaste. However, electric toothbrush modified with siwak has no effect on microbial reduction.

Key words: *Salvadora persica*, Siwak, *Streptococcus mutans*, *Lactobacilli*, antibacteri

ABSTRAK

Latar belakang: *Salvadora persica* adalah pembersih gigi tradisional yang lebih dikenal dengan sebutan Siwak. Beberapa penelitian menunjukkan bahwa *Salvadora persica* memiliki daya antibakteri. **Tujuan:** Penelitian ini bertujuan untuk mengetahui efektivitas *Salvadora persica* dalam berbagai bentuk sediaan untuk membersihkan gigi terhadap bakteri *Streptococcus mutans* dan *Lactobacilli* dalam saliva. **Metode:** Desain penelitian yang digunakan adalah single-blind, randomized clinical trial study dengan crossover. Lima kelompok perlakuan melakukan pembersihan gigi, tiap kelompok terdiri dari 14 mahasiswa kedokteran gigi, sehat dan memiliki kebersihan mulut yang baik. Tiap subyek diberi 5 macam perlakuan untuk membersihkan gigi menggunakan sikat gigi elektrik dimodifikasi dengan siwak, sikat gigi elektrik dengan pasta gigi mengandung ekstrak siwak (colgate kayu sugi), sikat gigi elektrik dengan pasta gigi umum (colgate total), siwak asli, dan salin. Saliva pasien digunakan untuk menganalisa pengurangan banyaknya bakteri *Streptococcus mutans* dan *Lactobacilli* sebelum dan sesudah perlakuan menggunakan Caries Risk Test (CRT) dari Vivadent. **Hasil:** Hasil menunjukkan adanya penurunan skor *Streptococcus mutans* dan *Lactobacilli* setelah pembersihan gigi dibandingkan sebelumnya. Sementara itu tidak ada perbedaan signifikan terhadap penurunan skor *Streptococcus mutans* ($p=0.158$) dan *Lactobacilli* ($p=0.396$) ketika dibandingkan antar kelompok. **Kesimpulan:** Mengunyah kayu siwak mempunyai efek antimikrobia yang hampir sama dengan menyikat gigi menggunakan kombinasi pasta gigi biasa dan pasta gigi yang mengandung siwak, akan tetapi menyikat gigi dengan sikat elektronik yang dimodifikasi dengan siwak dapat menurunkan jumlah bakteri.

Kata kunci: *Salvadora persica*, Siwak, *Streptococcus mutans*, *Lactobacilli*, antibacteria

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INTRODUCTION

Plaque bacterial is solely responsible for the initiation and progression of caries and periodontal diseases. The mechanical and chemical methods are available for maintenance teeth. Currently toothbrushes and dentifrices are mainly used for cleaning teeth. The traditional toothbrush or chewing stick (siwak), is used in many developing countries as the traditional means for oral hygiene.¹ It is prepared from the roots, twigs and stem of *Salvadora persica*. The stems and roots are spongy and can easily be crushed. Pieces of the root are usually scented and become soft when soaked in water. It is chewed on one end until they become frayed into a brush. The brush-end is used to clean the teeth in a manner similar to the use of a toothbrush. The promotion of good oral health by siwak is mainly attributed mechanical cleansing, but may also be due in part to built-in antiseptics.^{2,3}

In vitro studies have indicated that *Salvadora persica* contain substances that possess plaque inhibiting and antibacterial properties against several types of cariogenic bacteria which are frequently found in the oral cavity. The growth and acid production of these bacteria is thus inhibited.⁴ Various studies shows that *Streptococcus mutans* were involved with the initiation of caries, whereas the *Lactobacillus* were associated with the progression of the lesion.^{5,6} Studies also have shown a positive correlation between the concentrations of *Streptococcus mutans* in stimulated saliva and their level in dental plaque. Salivary counts of *Streptococcus mutans* and *Lactobacilli* are also positively correlated with caries activity.⁷ Good oral hygiene habits can prevent or retard the development of dental caries.⁸

The anti-microbial and cleaning effects of siwak have been attributed to various chemicals detectable in its extracts. These effects are believed to be due to its high content of vitamin C, salvadorine, salvadourina, alkaloids, trimethylamine, cyanogenic glycosides, tannins, saponins and salts mostly as chlorides in aqueous extract.^{1,9}

The investigation has been done to assess the antimicrobial activity on salivary *Streptococcus mutans* and *Lactobacilli*.¹⁰ This study compared between cleaning teeth using siwak chewing stick and a toothbrush without toothpaste. However siwak consists of a chemical substantial that can reduce the bacteria, but it is not known the efficacy of siwak in several modified preparation for example as a toothpaste with siwak extract, customized in electric toothbrush to replace the brush. Therefore, this study, was done to examine the effectiveness of *Salvadora persica* (siwak) in several modified preparation against the salivary *Streptococcus mutans* and *Lactobacilli*.

MATERIALS AND METHODS

This was a randomized clinical trial study with cross-over design, conducted for 3 weeks at School of Dental

Sciences, University Sains Malaysia (USM) and ethical approved by Human Ethic Committee USM (USMKK/PPP/JEPeM [(206.3(4))]). This study was conducted in 5 groups, each group consist of 14 subjects. The subjects were randomly treated with 5 interventions. The interval period or wash out period between each intervention is 3 days.¹¹

All the subjects were male and female dental students who had given their consents to involve in this study. The selected subjects were medically healthy with no systemic diseases and had not used any antibiotics or antiseptic mouthwash during the last two weeks. The subjects also had good oral hygiene (DMFT less than 1) and had more than 20 natural teeth. Stimulated whole saliva flow rate for each subjects were at ± 1.6 ml/sec. Smokers, pitting (amelogenesis imperfecta), periodontitis and pregnant woman were not included in the study.

The subjects were given a briefing on how to brush and use electric toothbrush, as well as how to use siwak chewing stick and scaling was done before interventions. The 5 intervention groups are: 1) electric toothbrush modified with siwak customized (Figure 1); 2) electric toothbrush+Siwak toothpaste (Colgate kayu sugi); 3) electric toothbrush+general toothpaste (Colgate total) as a positive control; 4) normal saline (0.9% NaCl) mouthwash as a negative control; 5) original siwak chewing stick.

Saliva was collected for 5 minutes after one minutes of pre-stimulation by chewing paraffin wax. Saliva was collected at 2 minutes interval for a total period of six minutes (5 minutes saliva collection+1 min pre-stimulated). Saliva was collected before and after interventions to investigating the efficacy of intervention to againts *Streptococcus mutans* and *Lactobacilli*.

The caries risk test (CRT) Vivadent (Ivoclar, Germany) was used to quantity bacterial colony. It was sufficiently

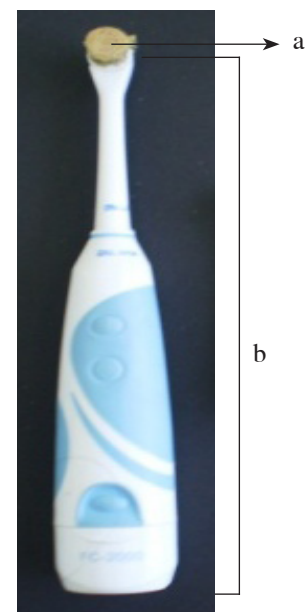


Figure 1. Electric toothbrush modified with siwak customized. a) siwak, b) electric toothbrush.

sensitive to provide a low, medium or high cariogenic bacterial challenge. The kit comes with two sided selective media sticks that assess *Streptococcus mutans* on the blue side and *Lactobacilli* on the green side. The samples were incubated at 37° C for 48 hours.

Growth density of the bacteria was evaluated under good lighting conditions by the naked eye and as per manufacturer's instructions. Bacterial growth was then scored by comparing with standards expressed in colony forming units (cfu) provided by the manufacturers as follows: *Streptococcus mutans* scoring: 0 = very low colonies are detected; 1 = low, colonies growth are $< 10^5$ cfu; 2 = medium, colonies growth are $> 10^5$ but $< 10^6$ cfu; 3 = high, colonies growth are $\geq 10^6$ cfu. *Lactobacilli* Scoring: 0= very low colonies are detected; 1= low, colonies are $\approx 10^5$ cfu; 2= medium, colonies are $\approx 10^4$ cfu; 3 = high, colonies are $\approx 10^5$ cf. The data were then be collected by single blind and SPSS version 12 for analysis.

RESULTS

The reduction of *Streptococcus mutans* was presented in Table 1 that showed in all group except modified siwak. The comparisons of the *Streptococcus mutant* risks score within five groups using repeated measures ANOVA revealed no significant effects. The values of comparisons were $W=0.545$, $F(4, 10)=2.085$, $p=0.158$, partial eta square=0.455. The descriptive statistics of mean and standard deviation for the *Lactobacillus* risk score was presented in table 2. However the *Lactobacilli* risk score after using different agents for cleansing teeth i.e siwak, colgate total, colgate kayu and saline sugi had reduction except for modified siwak as well. The comparison within five groups

Table 1. Descriptive statistics for *Streptococcus mutans* risk score

	n	Mean	SD
Modified miswak	14	0.1	0.53
Colgate kayu sugi	14	-0.1	0.66
Colgate total	14	-0.2	0.70
Saline	14	-0.3	0.73
miswak chewing stick	14	-0.4	0.93

Table 2. Descriptive statistics of *Lactobacillus* risk score

	n	Mean	SD
Modified miswak	14	0.1	0.73
Colgate kayu sugi	14	-0.3	0.61
Colgate total	14	-0.4	0.63
Saline	14	-0.3	0.73
Siwak chewing stick	14	-0.4	0.76

using repeated measures ANOVA revealed no significant effects. The values of comparisons were $W=0.689$, $F(4, 10)=1.130$, $p=0.396$, partial eta square=0.311. It means that the modified siwak is not effective to reduce *Streptococcus mutans* and *Lactobacilli*.

DISCUSSION

Modified siwak was prepared by cutting the end of siwak chewing stick for about 1 cm and we stick it to the electric toothbrush. Study by Hairudin shows that different length and size of siwak exert different effects,¹² so the modified siwak released less chemical substance when compared to original siwak chewing stick. Almas¹⁰ in his study reported that siwak had reduction *Streptococcus mutans* better than toothbrushing without toothpaste. Siwak clean the teeth by releasing chemical substance and mechanical cleansing action meanwhile toothbrushing without toothpaste has only mechanical cleansing action.

The number of bacteria after cleansing teeth using original Siwak, electric toothbrush with Colgate Total and Colgate Kayu Sugi toothpaste had no significance difference in reduction of the *Streptococcus mutans* and *Lactobacilli* risk score. It mean that the siwak had same effect as tooth brushing with toothpaste in reducing the numbers of bacteria. Many studies reported that toothpastes have antimicrobial activity both in vitro and in vivo.¹³ However *Salvadora persica* contains antimicrobial anionic components such as sulphate (SO₄²⁻), chloride (Cl⁻) and thiocyanate (SCN⁻). SCN⁻ acts as a substrate for lactoperoxidase to generate hypothiocyanite (OSCN⁻) in the presence of hydrogen peroxide. OSCN⁻ has been demonstrated to react with sulfhydryl groups in bacterial enzymes which in turn lead to bacterial death. Acid production in human dental plaque in vitro has been reported to be inversely proportional to the concentration of OSCN⁻ in the test system, while supplementing saliva with hydrogen peroxide and SCN⁻ inhibited acid production.⁹

However, the use of miswak has also been reported to inhibit the formation of dental plaque chemically, and exert antimicrobial effect against many oral bacteria. In vitro studies have demonstrated that aqueous extracts of miswak have growth-inhibitory effects on several micro-organisms.¹⁴ Using the checkerboard DNA-DNA hybridization (CKB) method, miswak may have a selective inhibitory effect on the levels of certain bacteria in saliva, particularly several oral *Streptococci* species.¹⁵

The subjects that involved in this study had good oral hygiene (DMFT 0) and this can influence to the result. Previous studies have shown that subjects with low caries tended to have higher mean flow rates of unstimulated parotid saliva compared to those in the higher caries group.¹⁶ Flow rates of saliva can influence the numbers of microorganism in the oral cavity. Natural flow of saliva will detach microorganism not firmly attached to an oral surface. Salivary components such as mucins can aggregate

certain bacteria which facilitates their removal from the mouth. Mucins are high molecular weight glycoprotein containing more than 40% of carbohydrate. Other salivary components that act as antimicrobial are Lysozyme, Lactoferrin, Salivary peroxidase enzyme and Histidine-rich polypeptide.¹⁷ The other hand the continuous supply substance of *Salvadora persica* can increase the salivary flow rate from the stem.¹⁴

The result proved that the cleansing teeth using original siwak chewing stick have similarity effectiveness with siwak toothpaste, general toothpaste to reducing *Streptococcus mutans* and *Lactobacilli*. Suggestion for further research should be carried out in larger sample size with the high risk karies condition. Controlled clinical trials are needed to find out the effect of *Salvadora persica* on cariogenic microorganisms for a prolonged period of time to assess the substantively of the tested material.

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