

Comparative Study of the Antibacterial Properties of *Salvadora persica* and Mouth Washes against Oral Bacteria

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Abstract

Oral health is an integral part of overall health. In many traditional cultures, plastic-bristle brushes are not used; herbal chewing sticks are used instead. Due to the continued interest in identifying efficient antiplaque agents that could be used daily without side effects, the comparative antibacterial efficacy of *Salvadora persica* and two brands of mouth washes against pathogenic bacteria including *Streptococcus* species, *Staphylococcus aureus*, *Enterococcus* species and *Escherichia coli* were studied. Dried stems of *Salvadora persica* were extracted using methanol. The antibacterial activities of methanol extract of *S. persica* and two brands of mouth washes were determined using agar well diffusion method. The minimum inhibitory concentration was determined using tube broth dilution method subcultured on a freshly prepared nutrient agar plate to determine the minimum bactericidal concentration. Methanol extract of *Salvadora persica* inhibited all the test bacteria at the highest concentration tested (400 mg/ml); with the highest zone of inhibition observed against *Staphylococcus aureus* (15.8 mm), followed by *Enterococcus* species (12.3 mm), *Streptococcus* species (10.7 mm) and *Escherichia coli* (10.4 mm). Mouth washes A and B exhibited highest antibacterial activity at the highest concentration used (400 mg/ml) against *Staphylococcus aureus* (18.6 mm, 16.2 mm) followed by *Streptococcus* species (16.3 mm, 14.6 mm), *Escherichia coli* (15.0 mm, 14.7 mm) and *Enterococcus* species (13.5mm, 14.1mm) respectively. Mouth wash A showed highest bactericidal activity compared to mouth wash B and *Salvadora persica*. The methanol extract of *Salvadora persica* had an MBC of 200 mg/ml which was similar to those of commercial mouth washes examined in this study. It has antibacterial effects and could be used as therapeutic agent and a potent oral hygiene tool. We therefore, recommend further pharmacological studies of *Salvadora persica* to discover non-synthetic tooth paste and mouth washes for maintaining good oral health.

Keywords: *Salvadora persica*, mouth washes, antibacterial activity, pharmacological, oral health.

Introduction

Good oral hygiene is the key to good oral and systemic health. Dental plaque has been proved by extensive research to be paramount factor in initiation and progression of gingival and periodontal diseases. Dental plaque formation begins with the accumulation of gram positive and

gram negative bacteria aggregation. Since bacterial plaque is the principal causative factor in gingival and periodontal diseases, the most rational method towards the prevention of periodontal diseases would be regular effective removal of plaque by personal oral hygiene protocol. Chemical agents which have antiseptic or antimicrobial action have been used, with variable success, to inhibit plaque formation and the development of gingivitis. Among these are; phenolic compounds, Bisbiguanides, pyrimidines, quaternary ammonium compounds, oxygenating agents, halogens, heavy metal salts [1]. Among these agents, chlorhexidine is the most effective antiseptic for plaque inhibition and prevention of gingivitis when used twice daily as mouth rinse [2]. But in oral use as a mouth rinse chlorhexidine has been reported to have a number of side effects. In order to overcome such side effects, the World Health Organization [3] had recommended and encouraged the possible use of natural products such as herb and plant extracts. A number of clinical studies have shown the effects of using mouth washes extracted from herbs [4, 5, 6, 7] in the prevention of dental plaque accumulation and subsequent gingival inflammation.

Salvadora persica is a medicinal plant whose roots have been used by many people in Africa, South America, Middle East and Asia as natural toothbrush or chewing stick. It is locally called *miswak* and belongs to the family Salvadoraceae. It has been demonstrated that extracts of *S. persica* improved gingival health and inhibited growth of carcinogenic bacteria [8]. Previous in vitro studies have reported the antibacterial and antifungal effects of *S. persica* on carcinogenic bacteria and periodontal pathogens including *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus faecalis*, *Streptococcus pyogenes*, *Lactobacillus acidophilus*, *Pseudomonas aeruginosa*, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Haemophilus influenzae* and *Candida albicans* [9, 10, 11, 12]. Much effort has focused on examining the inhibitory effect of *S. persica* on oral organisms but information concerning the comparative

antibacterial activity of *S. persica* and mouth washes against oral organisms is scarce. Therefore, this study aimed to compare the antibacterial properties of *S. persica* and mouth washes against oral organisms.

Methodology

Collection of samples

Dried stems of *Salvadora persica* and two brands of mouth washes were purchased from a local market in Owerri, Imo State. The samples were immediately transported to the laboratory for microbiological analysis.

Preparation of extract

The extract of *S. persica* was prepared using the method described by [13, 14]. Exactly 80g of grinded stems of *S. persica* were soaked in 200 ml methanol in a sterile well capped flask for 72 hours at room temperature and then filtered using Whatman filter paper. The filtrate was evaporated to dryness in a water bath at 40°C and the extract was stored in a sterile container until when needed.

Test organisms

The test bacteria: *Streptococcus* species, *Staphylococcus aureus*, *Enterococcus* species and *Escherichia coli* were collected from the Federal Medical Centre, Owerri, Imo state and preserved in agar slants. The test organisms were subcultured on sterile media and re-identified using standard microbiological and biochemical tests.

Determination of antibacterial activity

The antibacterial activities of methanol extract of *S. persica* and two brands of mouth washes were determined using agar well diffusion method described by [15]. Muller Hilton agar was prepared and poured into sterile Petri dishes and allowed to solidify. The plates were inoculated with the test bacteria and a 6 mm diameter cork borer was used to make three wells into each Petri dish and 0.1 ml methanol extract of *S. persica* and the two mouth washes were introduced. The plates are incubated at 37°C for 24 hours. Clear zones around the bored holes are indicative of the inhibition of the bacteria by the extract of *S. persica* and the mouth washes.

Determination of minimum inhibitory concentration (MIC)

The MICs of methanol extract of *S. persica* and two brands of mouth washes were determined using tube broth dilution method described by [16]. Different concentrations; 400, 200, 100, 50 and 25 of methanol extract of *S. persica* and the mouth washes were constituted using twofold serial dilution. The test tubes were inoculated with the test bacteria and incubated at 37°C for 24 hours. Tubes were analyzed visually. The lowest

concentration at which no turbidity occurred was recorded as the MIC value.

Determination of the minimum bactericidal concentration (MBC)

The MBCs of methanol extract of *S. persica* and two brands of mouth washes were determined using the method described by [16]. A loopful of inoculum was taken from each MIC tube and subcultured on a freshly prepared nutrient agar plate and incubated at 37°C for 24 hours. The MBC was recorded as the concentration of the extract of *S. persica* and the two brands of mouth washes that did not show any growth on nutrient agar plates.

Results

The result of the antibacterial activity of methanol extract of *Salvadora persica* and two brands of mouth washes against the test bacteria is shown in Table 1. Methanol extract of *Salvadora persica* inhibited all the test bacteria at the highest concentration used (400 mg/ml); with the highest zone of inhibition observed against *Staphylococcus aureus* (15.8 mm), followed by *Enterococcus* species (12.3 mm), *Streptococcus* species (10.7 mm) and *Escherichia coli* (10.4 mm). The results showed that the methanol extract of *S. persica* exhibited a stronger antibacterial activity against Gram-positive than Gram-negative bacteria. Mouth washes A and B exhibited highest antibacterial activity at the highest concentration used (400 mg/ml) against *Staphylococcus aureus* (18.6 mm, 16.2 mm) followed by *Streptococcus* species (16.3 mm, 14.6 mm), *Escherichia coli* (15.0 mm, 14.7 mm) and *Enterococcus* species (13.5mm, 14.1mm) respectively.

Table 1: Antibacterial activity of methanol extract of *Salvadora persica* and two brands of mouth washes

Test bacteria	Inhibition Zone Diameter (mm) at 400 mg/ml		
	<i>Salvadora persica</i>	Solution A	Solution B
<i>Staphylococcus aureus</i>	15.8	18.6	16.2
<i>Streptococcus</i> species	10.7	16.3	14.6
<i>Escherichia coli</i>	10.4	15.0	14.7
<i>Enterococcus</i> species	12.3	13.5	14.1

The MIC and MBC values of methanol extract of *S. persica* and two brands of mouth washes against the test bacteria are presented in Table 2. Methanol extract of *S. persica* had lowest MIC (200 mg/ml) against *S. aureus* and *Streptococcus* species. Mouth wash A had lowest MIC (200 mg/ml) against all test bacteria except *Enterococcus* species (400 mg/ml) whereas mouth wash B had lowest MIC (200 mg/ml) against all test bacteria except *S. aureus* (400 mg/ml). The lowest MBC

value was seen for *Streptococcus* spp. and *E. coli* (100 mg/ml) for mouth wash A. Methanol extract of *S. persica* and mouth wash B had MBC value of 200 mg/ml.

Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of methanol extract of *S. persica* and two brands of mouth washes

Sample	Test Bacteria	Concentration	Growth on plates	MIC	MBC
Salvadora persica	<i>Staphylococcus aureus</i>	400	-	200	200
		200	-		
		100	+		
		50	+		
		25	+		
	<i>Streptococcus</i> species	400	-	200	200
		200	-		
		100	+		
		50	+		
		25	+		
	<i>Escherichia coli</i>	400	-	400	400
		200	+		
		100	+		
		50	+		
		25	+		
<i>Enterococcus</i> species	400	-	400	200	
	200	-			
	100	+			
	50	+			
	25	+			
Solution A	<i>Staphylococcus aureus</i>	400	-	200	200
		200	-		
		100	+		
		50	+		
		25	+		
	<i>Streptococcus</i> species	400	-	200	100
		200	-		
		100	-		
		50	+		
		25	+		
	<i>Escherichia coli</i>	400	-	200	100
		200	-		
		100	-		
		50	+		
		25	+		
<i>Enterococcus</i> species	400	-	400	200	
	200	-			
	100	+			
	50	+			
	25	+			
Solution B	<i>Staphylococcus aureus</i>	400	-	400	400
		200	+		
		100	+		
		50	+		
		25	+		
	<i>Streptococcus</i> species	400	-	200	200
		200	-		
		100	+		
		50	+		
		25	+		
	<i>Escherichia coli</i>	400	-	200	200
		200	-		
		100	+		
		50	+		
		25	+		
<i>Enterococcus</i> species	400	-	200	200	
	200	-			
	100	+			
	50	+			
	25	+			

Antimicrobial resistance has always been a global health concern challenging treatment of oral infections caused by bacterial pathogens. This problem has opened a wide range of research studies investigating the possible use of natural plant products in the treatment of oral infections. In the present study, the two brands of mouth washes were selected based on their medicinal uses in the treatment of oral diseases. The methanol extract of *S. persica* exhibited a stronger antibacterial activity against Gram-positive than Gram-negative bacteria. These results are in variance with previous findings from other studies [9, 11]. In their study, the extract of *S. persica* exhibited rapid and strong bactericidal effect against Gram-negative bacteria but low effect on Gram-positive bacteria. The authors speculated that extract of *S. persica* might penetrate through the outer bacterial membrane and possibly interfere with the bacterial redox systems and thus hamper the ability of the bacterium to maintain its membrane potential [17]. The methanol extract of *S. persica* had promising MIC values against test bacteria. Previous studies have reported that *S. persica* extracts were effective against *S. aureus*, *Streptococcus mutans*, *Streptococcus pyogenes*, *E. faecalis*, and *P. aeruginosa* [12, 18]. However, mouth wash A showed highest bactericidal activity compared to mouth wash B and *S. persica*. The methanol extract of *S. persica* showed the ability to inhibit the test bacteria compared to those of commercial mouth washes examined in this study suggesting that both the methanol extract of *S. persica* and the mouth washes are equally effective. Similar results were also obtained by other researcher, although, the dilution of the ethanol extract from miswak wood had different concentrations the result was similar inhibition [19]. Overall, mouth wash A was the most effective on the test bacteria. However, the use of commercial mouthwash containing alcohol should be avoided because a high concentration of alcohol has been proven to be able to cause lesions hyperkeratotic, both in humans and in laboratory animals [20]. Chlorhexidine is a popular ingredient of commercial mouthwash because they prevent dental plaque caused by bacteria. But, root extract of *S. persica* could inhibit bacteria growth with no side effects. Miswak wood does not cause tooth discoloration, bad taste and burning in the oral cavity compared with chlorhexidine [21]. It is cheap, readily available in urban and rural areas of the countries. The results of the study demonstrated that methanol extract of *S. persica* as well as the two brands of mouth washes inhibited the growth of several carcinogenic and pathogenic bacteria.

Conclusion

The research result concluded that methanol extract of *S. persica* as well as the mouth washes have inhibitory effects on the test bacteria. However,

Discussion

mouth washes were more effective in inhibiting the test bacteria compared to methanol extract of *S. persica*. Among the mouth washes evaluated, mouth wash A was found to be more effective than mouth wash B against the test bacteria. *S. persica* extract can be a good alternative to the mouth washes since it is inexpensive and readily available.

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