ISSN - 2250-1991 | IF : 5.761 | IC Value : 79.96

Original Research Paper Surgery

Anti-bacterial Activity of Salvadora Persica (chewing stick) On Streptococcus Mutans Isolate From Patients Attending University Of Maiduguri Teaching Hospital Dental unit.

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ABSTRACT

Background: Plants have been used as mouth cleaning sticks for centuries in many countries across many countries to maintain oral hygiene. Use of chewing sticks is known to use mechanical activity in cleaning different part of mouth and removing stubborn microbes and food leftover.

Aim: The aim of the study is to investigate the immediate effect of Miswak extract on Streptococcus Mutans and other cariogenic Bacteria.

Method: The Salvadora Persica stick were cut in to pieces using sharp knife and later grounded to powder. Samples were collected from tooth swab of six patients diagnosed with Dental Caries attending Dental unit of university of Maiduguri Teaching Hospital.

Results: The result obtained from the study showed that the water extract of the *S. persica* showed clear zone of inhibition on chocolate agar plate but ethanol extract showed no zone of inhibition on the tested organism on chocolate agar plate.

KEYWORDS Miswak, water extract, Streptococcus mutans, Chocolate agar,

INTRODUCTION

From prehistoric times, medicinal plants have been identified and used by mankind for its therapeutic values.[1] Nature has been a source of medicinal agents forthousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine. The plant, based on traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Owolabi *et al*., 2007). According to the World Health Organization (WHO, 1987) "a medicinal plant" is any plant, which in one or more of its organ contains substances that can be used for the therapeutic purposes or which, are precursors for the synthesis of useful drugs.[2] This definition distinguishes those plants whose therapeutic properties and constituents have been established scientifically and plants that are regarded as medicinal but which have not yet been subjected to thorough investigation.

The genus *Salvadora* belongs to the family Salvadoraceae, comprising of three genera (i.e. Azima, Dobera & Salvadora) and 10 species distributed mainly in the tropical and subtropical region of Africa and Asia.[3] In Pakistan, this family is represented by a single genus *Salvadora*, with two species i.e., *S. persica L*., and S. oleoides Decne [4, 5]

Surprisingly, despite the widespread use of miswak since ancient times, relatively little scientific attention has been paid to its oral health beneficial effects. In (1987, WHO) encouraged the developing nations to use miswak for oral hygiene because of tradition, availability and low cost.[6] Recently, it was concluded that chewing sticks may have a role to play in the promotion of oral hygiene and that evaluation of their effectiveness warrants further research.

MATERIALS AND METHODS

Salvadora persica, which is a plant used as chewing stick (miswak) was used on oral pathogens collected from patient attending University of Maiduguri Teaching hospital, Dental unit. The samples collected were tooth swab of six patients diagnosed with Dental caries. All practical was done in the Laboratory of the Department of Microbiology, University of Maiduguri in January 2016. All samples were collected with the use of sterile swab sticks from the carious lesion of the teeth. The swab was then returned to its case, labeled and taken to the laboratory.

S. persica chewing sticks (miswak) were bought from a local market in Maiduguri, Borno state, Nigeria. Aqueous extraction was performed following the method of (Al-Mas 2001) and (Prachant et al 2007), where the sticks were cut using a sharp knife and grounded to powder using food blender. 200g of the powder was mixed with 400ml of sterile deionized water and allowed to soak for 24hrs at 60oC on a shaking water bath. The mixture was then centrifuged at 10000 rpm for 3 min and the supernatant collected was filtered using cotton wool and evaporate using rotary evaporator for extraction. Finally, the extract was dried on tray for complete dryness overnight and stored at -20°c until further use.

The ethanol extraction, 300g of the powder was mixed with 1000ml of the ethanol solvent and allowed to soak for 24hrs at 50°C on a shaking water bath. Following that, the mixture was centrifuged at 10000 rpm for 3 min and the supernatant collected was filtered using cotton wool and evaporate using rotary evaporator for extraction. Finally, the extract was dried on tray for complete dryness overnight and stored at -20°C until further use.

CULTURE TECHNIQUE

Streak plate method for isolating pure culture of single specie was used. The swab was smeared on a small section at the edge of agar plate and was then discarded. A sterile loop was used to drag through the swab-smeared area and streaking the area not inoculated for discrete colonies. The petri dishes were incubated in an averted position to avoid condensed inside the plate cover from falling onto the surface of the medium and causing bacterial colonies to mix. MacConkey Agar, Nutrient Agar (Titan Biotech Limited, India), Blood Agar and Chocolate Agar were used

Observations were made by checking each plate for growth of bacterial colonies after 24hrs of incubation period.

Gram staining was done by making a thin smear of each sample on

a clean grease-free glass slide, air drying and heat fixing by passing briefly over flame three times. Each smear was covered with Crystal Violet for a minute, and then Lugols Iodine for a minute too then washed. The dyed smeared was then decolorized with acetone until the color ceased to flow out. Finally, the smear was covered with neutral red for 1 minute, washed and air dried. Each slide was then viewed under microscope using oil immersion at x 100.

BIOCHEMICAL TESTS

Catalase test

A loopful of a bacterial culture was smeared onto a drop of hydrogen peroxide on a clean, grease-free slide. No gas bubble from the culture indicates a negative reaction.

Oxidase Test

A filter paper soaked with 1% Kovacs oxidase reagent and a loopful of bacterial culture was then smeared on the treated filter paper and observed for color change. No color change within 10 to 30 seconds indicates negative reaction

Antibiotic Resistance Profile Determination

This procedure was done using a puncture. Four plates containing chocolate agar were streaked and punctured. Two plates were picked at random, ethanol solvent of Salvadora persica extract was added to one of the plate that was picked. To the other plate, powdered form of the ethanol extract of the Salvadora persica was added to the plate. Both plates were incubated at 37oc for 24hrs.

The remaining two plates were used for aqueous and powdered form of the Salvadora persica extract. One of the plates was added with aqueous solution of the extract and the other plate was added with powdered form of the extract. Both plates were incubated at 37°C for 24hrs.

RESULTS

Figure 1: A photograph showing the growth of oral pathogen on chocolate agar

TABLE 1: PERCEPTION YIELD OF THE ETHANOL AND AQUEOUS EXTRACT OF DRIED STEM OF SALVADORA PERSICA

Weight of Powdered Stem for extraction (g)	800
Weight of Ethanol (g)	200
Weight of Distilled water (g)	300

The table shows and identifies the isolate from a carious tooth of a patient attending Dental clinic in UMTH. Six samples were collected and three had good growth while three had no growth on blood agar. Further screening involves gram stain showed the three sample where gram positive Cocci in chain.

TABLE 2: GRAM STAIN

S/N	SAMPLE NO.	GP	GN
•	S1	+	
•	S2	+	
•	S3	+	

KEYS

S2 = Sample 1, S3 = Sample 2, S5 = Sample 3, GP = Gram Positive, GN = Gram Negative, + = Present

The table below showed and confirmed the samples tested using biochemical test to be Gram Positive Streptococci.

TABLE 3: BIOCHEMICAL TEST

S/N	SAMPLE NO.	GLUC	LAC	SUC	MAN	SORB	CAT	MOT
•	S1	+	+	+	-	+	-	-
•	S2	+	+	+	-	+	-	-
•	S3	+	+	+	-	+	-	-

KEYS

S2 = Sample 1, S3 = Sample 2, S5 = Sample 3, GLUC = Glucose, LAC = Lactose, SUC = Sucrose. MAN = Manitol, SORB = Sorbitol, CAT = Catalase, MOT = Motility.

The table below shows the sensitivity of the isolate on the extract of S. Persica. The two extracts were Ethanol and Distilled water.

TABLE 3:

EXTRACT	ZONE OF INHIBITION
ETHANOL	0
s DISTILLED WATER	3.2mm

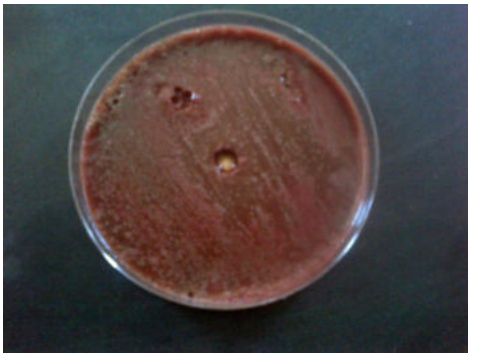

Figure 2: A photograph of Ethanol extract on tested organism showing no zone of inhibition of the inoculated S. mutans on chocolate agar plate

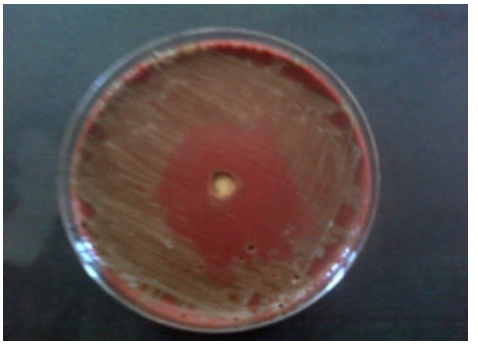

Figure 3: A photograph of aqueous extract showing zone of inhibition of the inoculated S. mutans on chocolate agar plate.

PHYTOCHEMICALS ANALYSIS RESULT

TABLE 4: Result of phytochemical screening of stem, leaves and roots of *S. persica*.

Phytochemicals	Stem	Roots	Leaves
Alkaloids	-	+	-
Flavonoids	+	-	+
Salvadourea	-	+	+
Thiocynate	+	+	+

Glycoside	-	-	-
Nitrate	+	+	-
Chloride	+	+	-

DISCUSSION

In this study, the antibacterial effect of *S. persica* on Streptococcus *mutans* was investigated. This agrees with the findings of (Almas *et., al.* 1999)[7] that *S. persica* has antibacterial activity on different types of bacteria including *mutans streptococci*. However information on the biological active compounds contributing to the reported antibacterial effects of *S. persica* are relatively limited (Wu *et., al*. 2001)[8], the production of acid by *streptococcus mutans* are inhibited by many compound such as Xylitol. This corresponds with the findings of (Kakuta *et al*, 2003)[9], that the growth and acid production of *mutans streptococci* are inhibited by different substance such as Xylitol, and is possible that Miswak may contain substances that inhibit the growth and acid production of *mutans streptococci* (Al-Otabi, *et al*, 2003).[10]

The result obtained from this study showed that the water extract of the *S. persica* showed clear zone of inhibition on chocolate agar plate but ethanol extract showed no zone of inhibition on the tested organism on chocolate agar plate. This revealed that the water extract has *in vitro* antibacterial activity against the clinical isolates tested (*S. mutans*), than the ethanol extract, this is because the ethanol solvent reduce the concentration of the active substance in S. persica such as Xylitol. The study also described that the chewing stick made from the root or small branches have been in use for over thousands of years, and currently the use of *S. persica* is more popular among Islamic communities of the world. These findings are well supported by earlier researchers (Azaizeh *et al*., 2003),[11] who reported toothbrushes prepared from the roots and small branches of *S. persica*, to be highly useful as maintainer of teeth.

The resources for oral hygiene in many developing countries, including Nigeria is limited. Therefore, use of inexpensive traditional preventive tools is well recognised and supported by W.H.O. This is also in line with a recent consensus (Consensus Statement on Oral Hygiene. Int Dent J 2000)[12], stating that "chewing sticks may have a role to play in the promotion of oral hygiene" and that "evaluation of their effectiveness warrants further research". The present study also discovered that Miswak has high amount of chloride and substantial amount silica, which agrees with the earlier research that Miswak extracts contained high amounts of chloride and substantial amounts of silica (Darout IA and *et al,* 2000).[13]

Study results in agreement with (Almas *et al*, 2000)[14]; (Darmani *et al*., 2006)[15] who examined the effects of miswak extracts on the growth of the various cariogenic microorganisms including *Streptococcus mutans*. The result showed inhibition in growth of *Streptococcus mutans*. The study result also agrees with (Al-Bayati and Sulaiman 2008)[16] who found that aqueous extract of *Salvadora persica* was more effective than ethanolic extract in inhibiting tested bacteria. But in contrast to (Moustafa *et al*., 1987)[17]; Al-Bagieh and Almas, 1997)[18] who found that ethanol extract of miswak has been shown to have a stronger microbial inhibitory effect on different microorganisms than the aqueous extract. The phytochemical screening showed the presence of Alkaloids, Flavonoids, Salvadourea, Thiocynate, Glycoside, Tannins, and Saponins in the stem *S. persica* (Table 4).this agrees with the phytochemical finding of (Darout I A, *et al* 2000)[19] who investigated the aqueous extract of stem and root of S. persica L. for some antimicrobial anionic components by using capillary electrophoresis techniques. It was reported that the root and stem extracts contain sulfate, chloride, tannins, and Saponins. The presence of these phytochemicals constituents may be responsible for some of the observed antibacterial activity of the stem extract *S. persica*

CONCLUSION

The findings suggest that miswak may also have a selective inhibitory effect on the level of certain bacteria in saliva, particularly several oral *Streptococcus* species. From this study, it can be concluded that Miswak extract has high antibacterial effect on oral pathogen, particularly *S. mutans* and also Miswak water extract showed higher efficacy of 50% on oral bacteria than the ethanol extract. In this study, it was shown that S. persica aqueous extract has significant effect in reducing the bacterial population in biofilm developed in a dynamic environment when used before and after meals.From this study, it could be concluded that water extraction of Salvadora persica is an effective antimicrobial agent when utilized clinically as a root canal irrigant in the treatment of teeth with necrotic pulps during root canal treatment.

RECOMMENDATION

1. The present study recommended that *S. persica* stem is more useful in the treatment of oral pathogen.s
2. This study also recommended that pharmaceutical industries should explored the further studies of *S. persica* own as (Asuwaki or chewing stick) in other to be able to produce and prepare an optimized preparation of the herbal extracts into modern drugs, for curing many disease including dental caries in Nigeria.
3. It also recommended that further research be conducted so as to develop suitable way of using it.
4. Further research is recommended in order to isolate the specific bioactive components in the stem which is responsible for the antibacterial activity.
5. Further research is recommended to explore the possible effect of miswak on oral tissues.
6. Public enlightment and awareness about the efficacy of use of chewing stick in our community.
7. Further research to compare the efficacy of Miswak extract and other root canal irrigant in root canal treatment should be carried out.

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