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Evaluation Of Antibacterial Property Of Fresh Miswak Extract And 0.5% Sodium Fluoride Impregnated Miswak Extract Against Streptococcus Mutans And Lactobacillus Acidophilus – An Invitro Study

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Abstract: ***Streptococcus mutans and Lactobacillus acidophilus are the two oral microorganisms most commonly implicated in causation of oral infections. Above mentioned oral microorganisms have grown resistant to routinely used antimicrobials. There is a need for an antibacterial agent which is effective, safe, economical and culturally acceptable. Salvodora persica commonly known as Miswak is one such plant product which has been used from ancient time. Miswak is derived from a shrub known as Salvadora persica found mostly in Saudi Arabia and parts of the Middle East. It has shown to possess promising inhibitory effect on many of the oral microorganisms. On extensive review of dental literature, there was scarcity of studies which had tried to assess antibacterial potential of Plain and Miswak extract impregnated with 0.5% NaF against S.mutans and L. acidophilus; hence, the present study was designed.***

Keywords: Miswak, 0.5% NaF, Antimicrobial, Efficacy

I. INTRODUCTION

Dental caries continues to be a veritable scourge for mankind from time immemorial and prevention still seems to be the best panacea. It is generally accepted that oral hygiene maintenance through regular removal of dental plaque and food deposits is an essential factor in the prevention of dental caries and periodontal disease. Methods for oral hygiene maintenance vary across different countries and cultures. Despite the widespread use of toothbrushes and toothpastes, majority of the population still use indigenous oral hygiene aids like chewing sticks because of its easy availability, simplicity of usage, cultural acceptability and cost effectiveness. The Year 2000 Consensus Report on Oral Hygiene states that chewing sticks may have a role to play in the promotion of oral hygiene.

The World Health Organization has recommended and encouraged the use of sticks as an effective and alternative tool for oral hygiene[2,3]. This recommendation is also consistent with the principles of the Primary Health Care approach that focuses on prevention, community participation, and the use of appropriate technology. Thus an evaluation of antimicrobial efficacy of plain and impregnated Miswak sticks can open new vistas in prevention of oral diseases.

Miswak is an extensively used oral hygiene aid in Africa, Asia, Middle East and South American region. Miswak is

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derived from a shrub known as *Salvadora persica* found mostly in Saudi Arabia and parts of the Middle East. Miswak contains chemical compounds like Tri-methyamin, salvadrin, chloride, fluoride, silica, sulfur, mustard, Vitamin C and a small amount of saponine and tannin. Many studies have reported the antimicrobial activity of Miswak against pathogens implicated in dental caries and periodontal pathogens.

Discovery of fluoride has changed the motto of dentistry from rendering conservative treatment to a preventive approach. Fluoride has made its strong niche in the arsenals for prevention of dental caries. Many conventional oral hygiene aids like wooden toothpicks, dentifrices and dental floss have been impregnated with fluoride to prevent dental caries. Recently a study conducted by Baeshen and Birkhed et al concluded that Miswaks impregnated with 0.1% NaF or a maximum of 0.5% NaF provided optimum release of fluoride in saliva and is safe to use on daily basis. Thus Miswak could act as a safe vehicle for fluoride release on daily basis for prevention and control of dental caries.

There exists paucity in the literature about antimicrobial efficacy of fluoride impregnated Miswak extract against cariogenic pathogens. In this regard, the present invitro study aimed to assess and compare the antimicrobial efficacy of Miswak impregnated with 0.5% NaF against non-impregnated Miswak on *Streptococcus mutans* and *Lactobacillus acidophilus.*

II. MATERIALS AND METHODS

Study design: In vitro Experimental study

COLLECTION OF MISWAK STICKS

Packets of fresh Miswak sticks were purchased from a local market of Davangere city, out of which 30 sticks were randomly chosen from the packets and made sure that sticks resembled each other with respect to colour and shape. The chewing sticks were later identified by a Botanist.

PREPARATION OF MISWAK EXTRACT

Fresh Miswak sticks were cut into two equal parts. One part of the stick was used to prepare plain Miswak extract and other half of the stick was used for impregnation with 0.5% of sodium fluoride. The chewing sticks were sun dried for 2 weeks at 30^{o}C before extract preparation. The sticks were cut into small pieces and ground to a coarse powder in a ball mill and then into fine powder using an electric grinder. Miswak extract was prepared by cold maceration method using ethyl acetate, ethanol followed by distilled water as solvents in a sequential order. Order of the solvents was selected based on their polarity. The powder was kept separately in sterile, dry screw-capped bottles, which were stored in a cool place.

About 200 g of the fine powder was accurately weighed in an electric balance and soaked in 100 ml of ethyl acetate solvent in a screw capped sterile bottle. The extraction procedure was done manually for a period of 24 hours. The powder was allowed to soak in each solvent for 48 hours at 4^{o}C before the mixture were centrifuged at 2,000 rpm for 10 minutes. The extract was prepared at 5%, 10%, 25%, 30%, 35%, 40%, 45% and 50% concentration respectively. The supernatant was passed through Whitman's filter paper (0.45 µm pore size). Similarly, the extract was prepared using ethanol followed by distilled water using the same procedure. The extract was further dried in a vacuum desiccator over anhydrous copper sulfate to give a dry solid of the extract for bioassay. The extract was stored at room temperature in a tightly closed container to preserve it from any contamination and decomposition. After which the antibacterial activity of Miswak extract was evaluated.

III. PREPARATION OF SODIUM FLUORIDE IMPREGNATED MISWAK EXTRACT

IMPREGNATION OF MISWAK STICKS

0.5% of sodium fluoride was used to impregnate Miswak sticks. Miswaks were immersed in a sterile bottle containing 500ml of sodium fluoride solution for a day. After impregnation, the Miswaks were removed from the bottle using forceps and placed on a filter paper for drying at room temperature overnight[1]. Same methodology discussed earlier for Miswak extract preparation was carried out to prepare 0.5% sodium fluoride impregnated Miswak.

STOCK SOLUTION PREPARATION

The solution of Miswak extract was prepared by dissolving 1gm of extract weighed using four decimal point weighing digital scale in 5ml Ringer solution. In order to prepare 50% of Miswak concentration, 2.5ml of stock solution was added to 2.5ml ringer's solution.

CULTIVATION OF MICROORGANISMS

Two types of microorganisms were considered, *Streptococcus mutans* and *Lactobacillus acidophilus.* Each was grown on 85mm blood agar plates. A suspension was prepared by transferring the colonies from blood agar plates using a sterile platinum loop to which 10ml of brain heart infusion was added. Later it was inoculated for one day at 37^{0}C. Culture tubes were shaken thoroughly to produce an even microbial suspension. Several dilutions were prepared for each microorganism to obtain a standardized number of cells per one micro liter of the culture medium using the Serial dilution technique.

IV. ASSESSMENT OF ANTIBACTERIAL ACTIVITY

The antibacterial activity of extracts was evaluated by using the Disc diffusion test and Minimum inhibitory concentration (MIC) test.

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DISC DIFFUSION TEST (DISC DIFFUSION ASSAY)

A modified agar diffusion method was used to determine antimicrobial activity. Nutrient agar was inoculated with a microbial cell suspension (200 µl in 20 ml of medium) and poured into sterile petri dishes. Sterile filter paper discs 6 mm in diameter were impregnated with 20 µl of each extract concentration. Concentrations were prepared using the ame solvents employed to dissolve the Miswak sticks (Ethanol, Ethyl acetate and Distilled water). Later sterilized via pasteurization and membrane filtration (regarding the aqueous extract) and placed on the inoculated agar surface. After pre-incubation for 2hrs in a refrigerator the plates were incubated overnight at 37 °C for 18-24 hrs. At the end of the incubation period antimicrobial activity was evaluated by measuring the zones of inhibition in terms of millimeters.

MINIMUM INHIBITORY CONCENTRATION TEST (MICRO DILUTION ASSAY)

The Minimum inhibitory concentration (MIC) values were determined in terms of microlitre (µl) with the agar dilution method. The MIC of the Miswak extracts were evaluated based on a microdilution method in 96 multi-well micro titer plates, as previously described. The dissolved extracts were first diluted to the highest concentration to be tested (12.5 mg/ml), 50µl of nutrient broth was distributed from the 2nd to the 12th well, a volume of 100 µl from each of the ethanol, ethyl acetate and aqueous extracts were initially prepared and pipetted into the 1st test wells of each micro titer line, and then 50 µl of dilution was transferred from the 2nd to the 12th well. The final concentration of the extracts adopted to evaluate antibacterial activity was included from 50 mg/ml to 0.12 mg/ml(i.e from 1st dilution to 10th dilution). Plates were wrapped loosely with cling film to ensure that bacteria did not become dehydrated and then they were placed in an incubator at 37 °C for 18-24hours. Color change was then assessed visually. Any color change from purple to pink or colorless was recorded as positive. The lowest concentration at which color change occurred was taken as the MIC value.

V. RESULTS

Type of solvent		Microorganism	Dilutions of extract 75µl	50µl	25 µl	10 µl	5 µl
Ethyl acetate extract	PLAIN MISWAK	Streptococcus mutans	R	R	R	R	R
Ethanol extract			R	R	R	R	R
Aqueous extract			26mm	25mm	16mm	R	R
Ethyl acetate extract	FLUORIDATED MISWAK		R	R	R	R	R
Ethanol extract			35mm	R	R	R	R
Aqueous extract			26mm	25mm	16mm	15mm	8mm

R= Resistant
µl= Micro liter
mm= Millimeter

Table 1: Antibacterial efficacy of plain miswak extract and 0.5% NaF impregnated miswak extract against S. mutans using disc diffusion test measured as zone of inhibition in terms of millimeters

Type of solvent		Microorganism	Dilutions of extract 75µl	50µl	25 µl	10 µl	5 µl
Ethyl acetate extract	PLAIN MISWAK	Lactobacillus acidophilus	R	R	R	R	R
Ethanol extract			30mm	26mm	20mm	12mm	R
Aqueous extract			16mm	13mm	R	R	R
Ethyl acetate extract	0.5% FLUORIDATED MISWAK	Lactobacillus acidophilus	R	R	R	R	R
Ethanol extract			20mm	14mm	R	R	R
Aqueous extract			16mm	13mm	R	R	R

R= Resistant
µl= Micro liter
mm= Millimeter

Table 2: Antibacterial efficacy of plain miswak extract and 0.5% NaF impregnated miswak extract against L. acidophilus using disc diffusion test measured as zone of inhibition in terms of millimeters

VI. RESULTS

Aqueous extract of Miswak showed antibacterial activity against *Streptococcus mutans* and *Lactobacillus acidophilus* (table no.1 and 2). Aqueous extract of fluoridated Miswak showed high antibacterial activity against *streptococcus mutans* compared to plain Miswak extract (table no.1). Aqueous extracts of fluoridated Miswak showed antibacterial activity against *streptococcus mutans* at all dilutions (table no.1) and antibacterial activity was comparatively less against *Lactobacillus acidophilus* (table no.2).

Fluoridated miswak extracts were more sensitive for aqueous and ethanol extracts than plain miswak extracts and they were more effective in inhibiting the *S mutans*. All the three different solvents (Distilled water, Ethanol and Ethyl acetate) extracts were sensitive against tested microorganisms but it was found to be high with respect to aqueous extract followed by ethanol and ethyl acetate.

Aqueous extracts of 0.5% of fluoridated Miswak was highly sensitive even after eighth dilution, whereas ethyl acetate extract of fluoridated Miswak was found to be sensitive only at first dilution against *Streptococcus mutans*. On the other hand ehtanolic extracts of 0.5% fluoridated Miswak was resistant through out all dilutions (1st to 10th) against *Streptococcus mutans.*

VII. DISCUSSION

In the present study aqueous extract of Miswak showed antibacterial activity against *Streptococcus mutans* and

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Lactobacillus acidophilus (Table no.1 and 2). Aqueous extract of fluoridated Miswak showed high antibacterial activity against *streptococcus mutans* compared to plain Miswak extract (table no.1). Aqueous extracts of fluoridated Miswak showed antibacterial activity against *streptococcus mutans* at all dilutions (Table no.1) and antibacterial activity was comparatively less against *Lactobacillus acidophilus* (Table no.2). To our best knowledge present study is first of its kind hence valid comparison could not be done.

Resources for oral health care are limited in many developing countries and the need to explore and test easily available and inexpensive traditional preventive tools are recognized and supported by WHO. Miswak is a common name for S. persica, which is commonly used in Saudi Arabia and the whole of Arab world. Miswak sticks clean between the teeth and do not break, regardless of the amount of pressure applied, as they are flexible and strong. The small wicks bend to the appropriate shape to clear plaque and left over food in between teeth and do not damage the gums.

The selection of chewing sticks in the present study was based on a number of factors. The use of chewing sticks is most common in Asian countries especially in the Indian subcontinent and the Middle East region. Furthermore chewing sticks are cheap, readily available in urban and rural areas of the countries. Their taste is agreeable and pleasant and reported to have antiplaque and many other pharmacological properties. A recent survey in Pakistan showed that more than half of the rural population used chewing sticks as on oral hygiene aid.

Fresh Miswak sticks and 0.5% sodium fluoride concentration were selected in the present study. According to Baeshen et al and Birkhed et al, 2010 fresh Miswak sticks have the ability to release fluoride quickly when compared to old Miswaks. Sodium fluoride impregnated Miswak sticks were found to release more amount of fluoride when compared to non impregnated Miswaks (Baeshen et al, 2008). It is explained by the fact that the porous wood in salvodara persica can absorb and retain the sodium fluoride solution both on the surface and in porosities of the inner spongy part. The retention and release of fluoride depends on several factors, such as the degree of porosity and the nature of a Miswak. Baeshen et al found that this type of chewing stick is an interesting fluoride vehicle in countries where they are commonly used. If they will be used in the future on a broad scale, it is recommended to use fresh Miswaks impregnated in 0.5% sodium fluoride for daily use.

With better understanding of the properties and action of fluoridated Miswaks against tested microorganisms chewing sticks may well represent an equivalent or alternative tool to the toothbrush for prevention and control of dental diseases in developing countries. The use of Miswak has evolved in various cultures independent of each other. Further in vitro experimental studies have to be conducted on biofilm models. Further research and controlled clinical trials are warranted to better evaluate the effectiveness of chewing sticks and their oral health benefits.

VIII. CONCLUSIONS

- ✓ 0.5% of sodium fluoride impregnated Miswak extract showed antibacterial activity against *S.mutans* and *L. acidophilus*.
- ✓ Aqueous extract of 0.5% of sodium fluoride impregnated Miswak extract showed higher antibacterial efficacy against *S.mutans* compared to plain Miswak extract.

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