

# The Immediate Effect of Crude Miswack Extraction against Some Bacterial isolates from Gingivitis

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## Abstract:

The study includes the isolation and identification some bacterial species which are considered as members of dental caries in the student of Veterinary Medicine College in the University of Kerbala, Iraq . These include *Streptococcus* spp and *Staphylococcus* spp. on Blood Agar medium and Manitol Salt agar , respectively. Identification of these bacteria was performed depending on morphological and biochemical crieteria, The study includes also the effect of the aqueous extract of *Salvadora persica* by the concentrations (20% and 15%) on the isolated bacteria which showed antiseptic effect on these species under the study.

## الخلاصة:

تضمنت الدراسة عزل وتشخيص بعض الجراثم المتواجدة في التهابات اللثة لدى بعض طلاب كلية الطب البيطري جامعة كربلاء، العراق والتي تشمل جراثيم *Streptococcus* spp وجرثيم *Staphylococcus* spp على وسط Blood agar ووسط Manitol Salt agar ،على التوالي. وشخصت باستخدام الصفات الشكلية والكيميائية. كما تضمنت الدراسة تحديد التأثير المثبط للمستخلص المائي للسواك بتركيز (20% و 15%) والذي اظهر فعالية تثبيطية على الانواع في قيد الدراسة.

## **Introduction**

Miswak is the most widely used chewing stick, which is prepared from the roots or twigs of *Salvadora persica*, and is used in middle-eastern and eastern African cultures(1). Various components of *Salvadora persica* have been reported to have beneficial biological properties, including significant antibacterial and antifungal activity(2,3). Primary analysis of *Salvadora persica* showed that it contains trimethyl amines, salvadorin, chloride, fluoride, silica, sulfur, mustard oil, vitamin C, resin and small amounts of tannin and saponine. These ingredients have anti-bacterial and anti-febrile properties and protect gums against irritation. Fluoride has anti-caries properties; Silica is abrasive and removes plaques and stains, and mustard oil and sulfur show anti-bacterial effects. In addition the resin creates a protective layer on the surface of the enamel and tannin has a contractive effect on the mucous membranes thus inhibits the transfer of glycosyl. Some chewing sticks contain such alkaline compounds as anthraquinones (Acacia Arabica), fagaronine and benzophenathridine with anti-bacterial properties(4,5). There were several facts viewed for Miswak when the Prophet of Allah Mohammed (Peace be upon him and his family) spoke with his cousin Imam Ali (Peace be upon him) on a Miswak (O Ali, three things better for your memory and remove the phlegm: chewing gum, cleaning the teeth with a special stick called 'miswak', and reciting the Quran, The aim of this study was to evaluation the crude extraction of miswak as anti-bacterial activity against bacteria isolated from human mouth.

## **Materials and methods**

### **Preparation of Crude Extract activity of miswak**

The usual method of crude Miswak (Saudi toothbrush) extract preparation is by sun drying the sticks for few days. The sticks are then cut into small pieces and ground to powder with a mill or a household grinding machine. The powder

is then mixed with sterile deionised water. The extract is allowed to soak for 48 hours. Then the mixture is centrifuged(Hettich –Germany) and filtered by milipore filter 0.45  $\mu\text{m}$  in diameter(Sigma, USA), then crude extract was classified and disturbed well on the inoculated plate in to three concentration 10%, 15% and 20% by distal water for each well, Although it is possible to left the media for 5-10 minute in 20°C before incubation period at 24-48 hours, this important to distribution all miswack fluid around the well (figure 1).



**Figure 1: Procedure of miswack extraction beginning from grind as small pieces till the end of diluents preparation.**

### **Selection of subject**

Forty (40) selected dental caries from both gender(male and female, 20 cases for each, aged between 20-25 years) who undertaken studying at the Kerbala students in Veterinary Medicine, Kerbala University, Iraq. The study also included ten (10) healthy who have no dental caries. medical history information were taken from the students before starting the isolation of bacterial caries which determined by dentist. All samples were placed in sterile peptone water tubeat plastic bags, It was prepared by dissolving 5 gm peptone and 5 gm sodium chloride in 1000 ml of

D.W. after that the pH was adjusted to 7.5 (6), labeled and transported to the laboratory in portable coolers at 4 °C, to be processed within 3 – 4 hours of collection. Regarding the procedure used modifying according to method (7), the swabs were rolled on the dental caries mixing with saliva, then soaked in pepton water broth at 37±2 °C for 24 hours overnight. Subsequently, a loopful of broth was streaked on surface of (Blood agar plates with 15% NaCl, Manitol Salt agar) then incubated at 37°C for 24 to 48hrs. The biochemical characters of bacterial species was determined by using IMVEC tests (8).

### **Cultural and Biochemical Characteristic**

The growing colonies on Blood agar and manitol salt agar were examined by naked eye concerning the color, shape and size of *Streptococcus spp* and *Staphylococcus spp*, respectively. Also they are several biochemical processing were depended on the carbohydrate fermentation for *Streptococcus spp* as (Sucrose, Lactose and Raffinose) and enzyme production as (Catalase and Oxidase)(9).

### **Well -Diffusion test**

The well diffusion test is the most commonly used method of testing the sensitivity of a microorganism to an antimicrobial agent, the bacterial isolate to be tested is seeded over the entire surface of an Muller Hinton agar plate with Standard Macferland's Solution and Miswack-impregnated full-well are applied, after overnight incubation at 37°C, zones of growth inhibition are observed around each well(10)

### **Statistical analysis**

In order to determine the statistical significance among different variables, SPSS program (Statistical Program for Social Sciences) version 11 was used. Chi – square was applied to test the obtained results.

## Results and Discussion

The results of the conventional methods carried out on dental caries isolation showed that all tests were able to detect *Streptococcus spp* and *Staphylococcus spp*, after 24-48 hrs of incubation period. The colonies of *streptococcus spp* on blood agar were small , circular , pink color, partial hemolysis on blood agar with 15% NaCl also fermentation of some carbohydrate this procedures agree with (11).The colonies of *Staphylococcus spp* on blood agar were circular , smooth , convex and pale in color, this procedure with (12).

Depending on the number of *Streptococcus spp* isolates detected by culture methods, the differences were found in the frequency of *Streptococcus spp* prevalence in students both gender at Veterinary Medicine College. *Streptococcus* prevalence was more frequently observed in third stage (5 %) from both gender. On the other hand there are high frequency of *streptococcus* occurrence in male third stage (13.33%) followed by second stage (11.66%), but first and fourth stage was observes as same. Regarding they are high frequency of *Staphylococcus spp* as (10 %) in the second stage isolated from female followed by (6.66%) and (3.33%) in third and fourth stage, respectively. but no bacterial of *Staphylococcus* isolates was observed in the first stage of Veterinary Medicine College.(Table 1) .

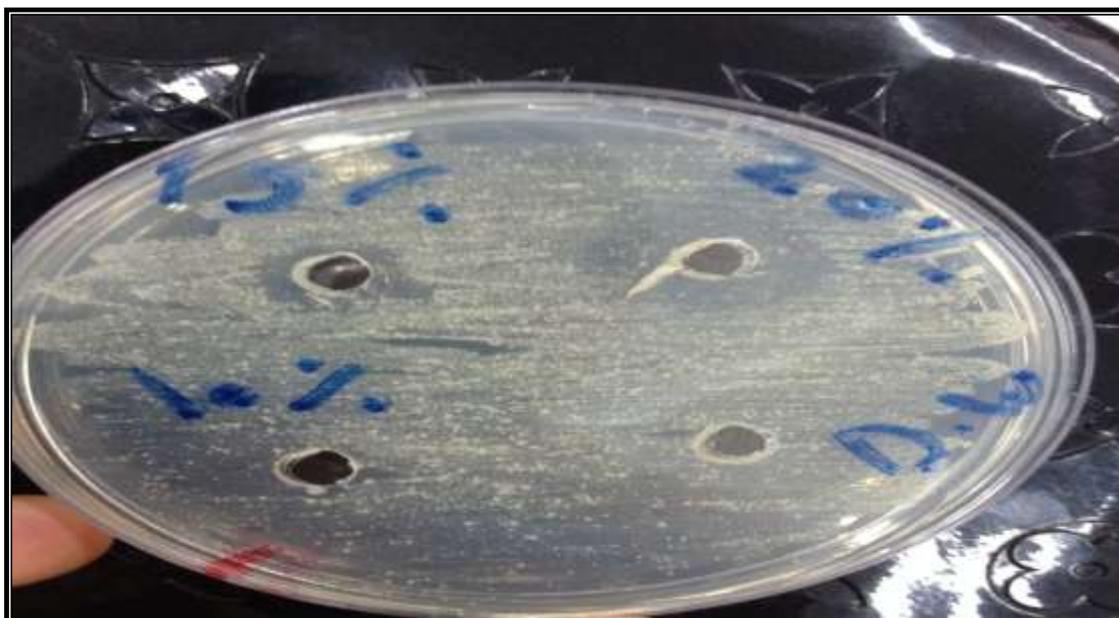
**Table (1): Percentage of different positive test of some bacterial profile in the veterinary students stages:**

Dental Caries (40)								
Type of bacterial isolate	First stage		Second stage		Third stage		Fourth stage	
	Male	female	Male	female	Male	female	Male	female
	5	5	5	5	5	5	5	5
<i>Streptococcus spp</i>	1	2	2	2	4	3	1	0

<i>Staphylococcus spp</i>	0	0	1	3	1	2	0	1
X <sup>2</sup> (calculated) = 12.798, Degrees of freedom= 12, Yates' chi-square= 5.291								
Non significant								

The bacterial isolates also accomplished in vitro susceptibility toward water miswack extract by using disc diffusion method, four and three isolates from male and female ,respectively. All tested isolates from dental caries showed high susceptibility (100%) toward Miswack (20%) Concentration as (20 mm) in diameter followed by (14mm) and (10mm) toward Miswack as (15 %) and (10%) Concentration, respectively. On the other hand , these isolates revealed varying percentage of susceptibility (Fig 2).

In the present study, also the most frequently isolated are *Staphylococcus spp*, the result resemble to (12 and13)was obtained similar results when they analyzed the frequency with which bacteria occurred in dental caries by using water miswack extraction.



**Figure (2) Miswack susceptibility for bacterial isolates causing dental caries**

In vitro studies testing this crude Miswak extract conclude Miswak has considerable antibacterial effect, which increases with extract concentrations (11

and 13). Water Miswak extract has an inhibition zone of (20mm) on *Streptococcus mutans* and (24mm) on *Staphylococcus aureus* (14).

In other studies with water extract, inhibition zones on *Streptococcus faecalis* of 3mm (5% Miswak extract) 4 mm (10% Miswak extract), and 7 mm (50% Miswak extract) are reported: the (50%) extract had (3 mm) inhibition zone on *S. mutans*(15and16). Aqueous Miswak extracts of( 15%) and (20%) concentrations have a fungistatic effect on *Candida albicans*for up to 48 hours (17). Different alcohol extracts of *S. persica*have potent antifungal activity on different Candidal species.

In a study on different extraction solutions, ethanol extract of *Salvadora persica* root is the most potent and *S. mutans*is the most susceptible strain (18). However, the results from these studies cannot be compared as the Miswak sources and the concentrations predations are different.

There is a direct relationship between dental caries and the intake of carbohydrates, the most cariogenic sugar is sucrose, and the evidence for its central role in the initiation of dental caries includes increases in the caries prevalence of isolated populations with the introduction of sucrose-rich diets. Whenever possible, *Salvadora persica* should be used to reduce the incidence of possible side-effects of some drugs for dental caries, emergence of resistant bacteria and drug costs(19)

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