

The Immediate Effect of Crude Miswak Extract against Some Bacterial isolates from Gingivitis

Received :11/11/2014

Accepted :13/1/2015

Hayder Ali Muhammid¹, Hayder Naji Ayyez², Amaal Muhammed Khudaier¹, Jassim M. Khalaf⁴

¹Department of Microbiology, College of Vet. Medicine, Kerbala University, Kerbala, Iraq.

²Department of Microbiology, College of Vet. Medicine, Al-Qadissiya University, , Al-Qadissiya, Iraq.

³Department of Vet. Surgery, College of Vet. Medicine, Kerbala University, Kerbala, Iraq.

E.mail: drhayder93@gmail.com

Abstract: This study included the isolation and identification of some bacterial species which are considered as members of gingivitis in the student of Veterinary Medicine College in the University of Kerbala, Iraq . These include the of *Streptococcus* spp. was more involved in the third stage 70% percentage and *Staphylococcus* spp. was more isolated in the second stage 40% percentage by using Blood Agar medium and Manitol Salt agar, respectively. Identification of these bacteria was performed depending on morphological and biochemical criteria, the study includes also the effect of the aqueous extract of *Salvadora persica* in the concentrations (20%, 15% and 10%) on the isolated bacteria which showed antimicrobial effects on these species under the study.

Biology Classification: QR75-99.5

Keyword: *Salvadora persica* , *Streptococcus* spp., *Staphylococcus* spp., gingivitis

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). It belongs to the Kingdom- Plantae, Division- Magnoliophyta, Class- Magnoliopsida, Order- Brassicales, Family : Salvadoraceae It is locally called as kharijal; BENG—Jhal;, 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 benzophenanthridine 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 evaluate the crude extraction of miswak as anti-bacterial activity against bacteria isolated from student gingivitis.

Selection of subject

Forty selected gingivitis samples from both gender (male and female, 20 cases for each, aged between 20-25 years) who undertaken studying in the Veterinary Medicine, Kerbala University, Iraq. The study also included ten (10) healthy who have no gingivitis. 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

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 μm in diameter (Sigma, USA), then crude extract was 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 miswak fluid around the well (6).

water tube at 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 (7), 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 (8), the swabs was rolled on the gingivitis 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 37C° for 24 to 48hrs . The biochemical

Cultural and Biochemical

Characteristic

The growing colonies on Blood agar and Maintol salt agar were examined by naked eye concerning the color , shape and size of *Streptococcus spp.* and *Staphylococcus*

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

Results and Discussion

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 (70 %) from both gender. On the other hand there are high frequency of *streptococcus* occurrence in male third stage (13.33%) followed by fourth stage (11.66%), but second stage was observes as same. Regarding they are high frequency of *Staphylococcus spp* as (40 %) in the second stage isolated from both gender followed by (30%) and (10%) in third and fourth stage, respectively. but no bacterial

characters of bacterial species was determined by using IMVEC tests (9).

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) (10).

applied, after overnight incubation at 37°C, zones of growth inhibition are observed around each well (11).

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.

The results of the conventional methods carried out on gingivitis 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 (12).The colonies of *Staphylococcus spp* on blood agar were circular , smooth , convex and pale in color, this procedure with (13).

Depending on the number of *Streptococcus spp* isolates detected by

College.(Table 1) .

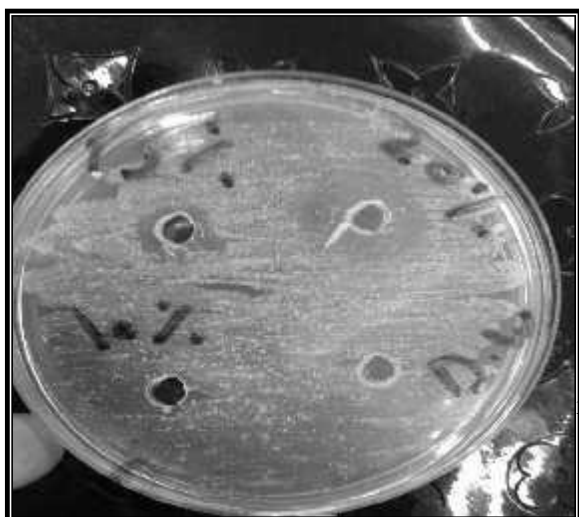
of *Staphylococcus* isolates was observed in the first stage of Veterinary Medicine

Table (1): Percentage of different positive test of some bacterial profile in the veterinary student's stages:

No. of Students	<i>Streptococcus spp</i> 10 samples		Total	Percentage (%)	<i>Staphylococcus spp</i> 10 samples		Total	Percentage (%)
	Male	Female			Male	Female		
	5	5			5	5		
First stage	1	2	3	30%	0	0	0	0
Second stage	2	2	4	40%	1	3	4	40%
Third stage	4	3	7	70%	1	2	3	30%
Fourth stage	1	0	1	10%	0	1	1	10%
Statistical analysis	X ² (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 gingivitis 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 1). In the present study, also the most frequently isolated are *Staphylococcus spp*, the result resemble to (13 and14) was obtained similar results when they analyzed the frequency with which bacteria occurred in gingivitis by using water miswack extraction



isolates

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* (15).

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* (16 and 17). Aqueous Miswak extracts of (15%) and (20%) concentrations have a fungistatic effect on *Candida albicans* for up to 48 hours (18). Different alcohol extracts of *S. persica* have potent antifungal activity on different *Candidal* species (19).

In a study on different extraction solutions, ethanol extract of *Salvadora persica* root is the most potent and *S. mutans* the most susceptible strain (20). 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 gingivitis and the intake of carbohydrates, the most cariogenic sugar is sucrose, and the evidence for its central role in the initiation of gingivitis 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 gingivitis, emergence of resistant bacteria and drug costs (21).

References

1-Elvin-Lewis, M. **1980**. Plants used for teeth cleaning throughout the world. *J. Prev. Dent.*, 6:61–70.

2-Al-Bagieh, N. H.; Idowu, A. and Salako, N.O. **1994**. Effect of aqueous extract of miswak on the in vitro growth of *Candida albicans*. *J. Microbiol.* 80:107–13.

3-Almas, K.; Al-Bagieh, N.H. and Akpata, E.S. **1997**. In vitro antibacterial effect of freshly cut and 1-month-old Miswak extracts. *Biomed Lett* 56:145–9.

4-Amin, T.T. and Al-Abad, B.M. **2008**. Oral hygiene practices, dental knowledge, dietary habits and their relation to caries among male primary school children in Al Hassa, Saudi Arabia. *Int. J. Dent. Hyg.* 6:361-370.

5-Al-Otaibi, M.; Al-Harthy, M.; Söder B.; Gustafsson, A. and Angmar-Månsson, B. **2003**. Comparative effect of chewing sticks and toothbrushing on plaque removal and gingival health. *Oral Health & Preventive Dentistry*, 1:301-307.

6- Hassan, S. H. **2012**. A review on miswak (*Salvadora persica*) and its effect

on various aspects of oral health, Saudi Dent J. 24(2): 63–69.

7-Colle, J.G.; Miles, R.S.; and Wan, B. **1996**. Tests for the identification of bacteria. In: Mackie and McCartney Practical Medical Microbiology. Eds. Collee J.G.; Fraser A.G.; Marmion B.P. and Simmons A. 14th ed. Edinburg: Churchill Livingstone. pp: 131-50.

8-Bhatt, P.A.; Pratap, A. and Jha, P.K. **2012**. Study of size-dependent glass transition and Kauzmann temperatures of tin dioxide nanoparticles. Journal of Thermal Analysis and Calorimetry, 110 (2) 535-538.

9- Brooks, G.F.; Butel, J.S. and Mores, S.A. **2004**. Jawetz, Melnick, and Adelbergs Medical Microbiology. 23rd ed., Printed in Singapore.

10- Koneman, E.W.; Allen, S.D.; Janda, W.M.; Schrecken, Berger, P.C. and Jr, W.C.W. **1997**. Color Atlas and Text Book of Diagnostic Microbiology. 3rd Ed. J.B Lippincott Comp., Philadelphia USA.

11- Vandepitte, J.; Engback, K.; Piot, P. and Heuch, C.C. **1991**. Basics Laboratory procedures in Clinical Bacteriology. World Health Organization, Geneva.

12-Shaikh, N.; Leonard E.; Martin J.M., **2010**. Prevalence of streptococcal pharyngitis and streptococcal carriage in children: a meta-analysis. Pediatrics 126 (3): e557–64.

13-Baker, J.S. **1984**. Comparison of various methods for differentiation of staphylococci and micrococci. J Clin Microbiol 19:875-9.

14- Almas, K.; Al-Bagieh, N. and Akpata, E. **1997**. In vitro antimicrobial effects of freshly cut and 1-month old miswak (chewing stick) Biomed. Lett. 56:145–149.

15-Al-lafi, T. and Ababneh, H. **1995**. The effect of the extract of the Miswak (chewing sticks) used in Jordan and the Middle East on oral bacteria. Int. Dent. J. 45:218-222.

16-Almas, K. and Al-Bagieh, N.H. **1999**. The antimicrobial effects of bark and pulp extracts of miswak, *Salvadora persica*. Biomedical Letter 60:71-75.

17-Almas, K. **2001**. The antimicrobial effects of seven different types of Asian chewing sticks. Odonto-Stomatologietropicale -No 96, pp 17-20.

18-Al-Bagieh N.; Idowu A. and Salako N.O. **1994**. Effect of aqueous extract of miswak on the in vitro growth of *Candida albicans*. Microbios. 80(323):107–113.

19- Noumi, E.; Snoussi, M.; Hajlaoui, H.; Valentin, E. and Bakhrouf, A. **2010**. Antifungal properties of *Salvadora persica* and *Juglans regia* L. extracts against oral *Candida* strains. Eur. J. Clin Microbiol. Infect. Dis.; 29(1):81-88.

20-Abd EL-Rahman H. F.;Skaug N. and George W. F. **2002**. In vitro antimicrobial effects of crude miswak extracts on oral pathogens, Saudi Dental Journal, 14:26-32.

21- Almas K, Al-Bahair K, Al-Ragabah A. **2000**. Comparative pilot study of oral health status among toothbrush and miswak users. Pakistan Oral & Dental Journal; 20 (1):35-45

تأثير المباشر لمستخلص السواك الخام ضد بعض عزلات البكتيريا من التهابات اللثة

تاريخ القبول 2015/1/13

تاريخ الاستلام 2014/11/11

حيدر علي محمد¹ حيدر ناجي عايز² امال محمد خضير¹ جاسم محمد خلف³

فرع الاحياء المجهرية, كلية الطب البيطري , جامعة كربلاء, كربلاء, العراق 1

فرع الاحياء المجهرية, كلية الطب البيطري , جامعة القادسية, القادسية, العراق 2

فرع الجراحة والتوليد, كلية الطب البيطري , جامعة كربلاء, كربلاء, العراق 3

الخلاصة:

تضمنت الدراسة عزل وتشخيص بعض الجراثيم المتواجدة في التهابات اللثة لدى بعض طلاب كلية الطب البيطري جامعة والتي كانت اكثر حدوثا في طلاب المرحلة الثالثة بنسبة 70% *Streptococcus* spp. والعراق والتي تشمل جراثيم/كربلاء Blood agar والتي كانت اكثر نسبة عزل 40% في طلاب المرحلة الثانية على وسط *Staphylococcus* spp. وجراثيم ،على التوالي. وشخصت باستخدام الصفات المظهرية والكيميوكيوية كما تضمنت الدراسة تحديد Manitol Salt agar ووسط التأثير المثبط للمستخلص المائي للسواك بتركيز (20% و 15% و 10%) والذي اظهر فعالية تثبيطية على الانواع في قيد الدراسة.

.....
الكلمات المفتاحية: السواك, المكورات المسببة, المكورات العنقودية, التهاب اللثة.