



Original article

Evaluation of antifungal activity of Miswak (*Salvadora persica*) and toothpaste against oral cavity *Candida* species

Hamida S. El Magrahi^{1*}  , Abir M. Ben Ashur¹  , Shada M. Agha¹ , Shahed A. Khaleel¹ ,
Arij M. Mousa²  , Ahmed E. Atia³  , Manal O. Abuagela²  , Eman A. Abdulwahed¹  ,
Rehab S. Jerbi⁴  , Najia N. Alwaseea²  , Fawzia A. Mohamed²   and Eman A. Alaqeli⁵  

¹Department of Medical Laboratories Sciences, Faculty of Medical Technology, University of Tripoli, Tripoli,

²Department of Public Health, Faculty of Medical Technology, University of Tripoli, Tripoli,

³Department of Anesthesia and Intensive Care, Faculty of Medical Technology, University of Tripoli, Tripoli,

⁴Department of Community Medicine, Faculty of Medicine, University of Tripoli, Tripoli,

⁵Department of Health Services Administration, Faculty of Public Health, University of Benghazi, Benghazi, Libya

*Author to whom correspondence should be addressed

Received: 24-02-2023, **Revised:** 18-03-2023, **Accepted:** 23-03-2023, **Published:** 31-03-2023

Copyright © 2023 El Magrahi et al. This is an open access article distributed under the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

HOW TO CITE THIS

El Magrahi et al. (2023) Evaluation of antifungal activity of Miswak (*Salvadora persica*) and toothpaste against oral cavity *Candida* species. *Mediterr J Pharm Pharm Sci*. 3 (1): 69 - 76. <https://doi.org/10.5281/zenodo.0000000>

Keywords: Antifungal activity, *Candida* species, Miswak (*Salvadora persica*), toothpaste

Abstract: Around the world, several oral health measures have been implemented. The most popular method is to use a toothpaste. However, chewing sticks are still used in many cultures around the world in a conventional manner. Chewing sticks have a mechanical cleansing action similar to a toothbrush in addition to their antimicrobial effect. The purpose of this study is to evaluate the effect of Miswak on the growth of *Candida* species and to compare it to that of toothpaste. A cross-sectional study was carried out on 120 selected randomly volunteers from January to April, 2022. Two groups of participants were used for two weeks. The participants were separated into a group used Miswak and a group use ordinary toothpaste. Samples were collected before and after two weeks in these two groups. Samples were immediately processed for microbiological phenotypic conventional methods and in vitro susceptibility testing of the microbial isolates to antimicrobial. The findings show that a significant effect of Miswak against an oral cavity *Candida* species. Total *Candida* count was 27.5 ± 18.48 , $P = 0.001$ (Mean $\pm$ SD) from Miswak group and 247.0 ± 90.14 , $P = 0.979$ (Mean $\pm$ SD) in toothpaste group. The most frequent *Candida* isolates organisms were *C. albicans*: 22 (36.67%) in the Miswak group and 24 (40.0%) in the toothpaste group, the second isolate was *C. dubliniensis*: 04 (6.69%) in the Miswak group and 12 (20.0%) in toothpaste group followed by *C. glabrata*: 02 (3.33%) in Miswak group. In conclusion, Miswak has a considerable antifungal impact immediately and after two weeks of use.

Introduction

The oral cavity is a habitat for a large number of microorganisms' species that coexist with one to another as a normal microbiota [1]. There are more than 20 species of candida, the most common opportunistic oral fungus associated with oral candidal infection, which colonize from 20.0% to 80.0% of adults without an evidence of infection [2, 3]. *Candida albicans* are the predominant oral cavity species in medically compromised and otherwise healthy individuals [4]. Oral candidiasis is a major condition that arises due to some predisposing factors such as poor oral hygiene, immune-suppression, nutritional deficiencies, long-term of use antibiotic/radiation therapy, dental prostheses, diabetes mellitus and a high carbohydrate diet or cigarette smoking [5]. Oral health has been linked to a number of chronic and systemic disorders and good oral hygiene is vital for disease prevention [6]. Modern dental care tools are designed to provide mechanical and chemical means through regular removal of plaque and food residues from the surface and spaces among the teeth. One of the most popular ways to maintain oral hygiene is by using a toothbrush and dentifrices. Other notable methods include chewing gum, mouthwash and dental floss [7]. In many countries, chewing sticks made from the twigs, stems or roots of various plant species are chosen and prepared as natural methods of brushing teeth [8]. *Salvadora persica* is the most commonly used plant for this practice [9]. This plant is commonly known as the Miswak tree. Due to its broad association with the practice, the Arabic word Miswak, which means tooth cleaning stick, becomes a common name for *S. persica* [10]. Due to their ability to defend against some oral infections and their potential to advance oral health, chewing sticks of *S. persica* have been recommended for use in oral hygiene by the World Health Organization [11]. *S. persica* has been comprising a wide variety of organic and inorganic compounds within its extract. Among the organic compounds were glycoside, saponins, flavonoids, alkaloids, tannins, benzyl derivatives, phenol compounds and organic acids

[12]. For inorganic compounds, i.e. anionic compounds such as fluoride, chloride, sulphate, thiocyanate and nitrate have been identified [13]. Long fibers and the specific complexity of phytochemicals and minerals offer it an advantage as a tool for oral and dental health care by supplying all of the necessary means of mechanical and chemical cleaning [14]. Miswak at the time of usage stimulates saliva production and buffer sits pH [15]. These ingredients have the ability to remineralize dental hard tissue, stimulate the gingiva and treat inflammatory gums. Chewing sticks also contain volatile oils, tannic acid, sulphur and sterols which contribute to anti-septic, astringent and bactericidal properties. It reduces plaque formation, has anti-cariious properties, eliminates bad odors and improves taste sensation [16]. It contains a strong antimicrobial activity [17] as well as protecting from pathogens that enter the body through the mouth [18]. Thus. The present study aimed to assess the effect of Miswak on the growth of *Candida* species and to compare with toothpaste use.

Materials and methods

Study design: This study was conducted at University of Tripoli, Faculty of Medical Technology, Department of Medical Laboratory Sciences, from January to April, 2022, and was approved by the Research Ethics Committee of the University (2021). It was performed on 120 volunteers who divided into two main groups. Group Miswak uses, whereas, the other group uses toothpaste for two weeks, three times daily.

Study population: 120 subjects aged 15 - 65 years (78 females and 42 males) consented to participate and were enrolled in the study. During the two weeks, neither antibiotic nor antiseptic mouthwash had been utilized by the chosen participants. The study excluded smokers and none of the individuals had ever utilized Miswak. There were 60 volunteers split between the two groups. Data were collected by questionnaire including age, sex and oral condition health.

Sample collection culture and identifications:
Sampling was carried out two times:

Miswak group: The first sample was before using Miswak and after utilizing Miswak for two weeks, the second sample was taken. Volunteers were instructed about the Miswak use, advised to use it three times daily and shown how to keep it fresh by cutting off the edge of the Miswak every day and storing it in the refrigerator at night.

Toothpaste group: The first sample was taken before using toothpaste and the second sample was taken after using toothpaste for two weeks as directed. By using an oral concentrated rinse, samples were acquired by washing the mouth for 30 seconds with 10 ml of sterile water. The mouthwash liquid is then placed in conical tubes and placed in an insulated container until it is microbiologically processed. The sample suspension was washed three times in sterilized 0.10 M phosphate-buffered saline pH 7.4 (PBS) by centrifugation at 3 500 rpm. The pellet was then resuspended in 0.1 mL of PBS and 100 μ l aliquots were inoculated by spiral plating system onto the surface of Sabouraud's dextrose agar with an antibiotic. The growth of the colonies on the plates was counted and expressed as the number of Candida colony forming units per milliliter (CFU per ml) of rinse after 24 - 72 hours of incubation at 30 °C [19]. Yeasts species identified by germ-tube production, micromorphology and chlamydospores production on corn meal agar plus 0.1% Tween 80. On corn meal agar, all isolates tested positive for germ tube test and chlamydospores and Tween 80 was identified as *C. albicans* or *C. dubliniensis*, Sunflower seed agar. Candida species were identified by chromogenic media [20].

Statistical analysis: The raw data were entered into spreadsheets in Excel and then imported into SPSS software version 26 (IBM Corp, Armonk, N.Y, USA). All data were expressed as Mean and standard

deviation (Mean $\pm$ SD). Descriptive statistics were used to calculate the frequency distribution, mean, standard deviation and median. For the total number of microorganisms in the two groups, *t*-test was used. The level of significant difference was set at **p* < 0.05, ***p* < 0.01 as highly significant and ****p* < 0.001 as very high significant.

Results

In this study, of the 120 adult participants, 78 were females (65.0%) and 42 were males (35.0%). The mean age of the participants was 34.2 ± 17.42 years old (range: 15 - 65 years). In the two groups, 60 participants were Miswak users and 60 participants were toothpaste users. The present findings of the biochemical tests used in this study to identify isolated strains were given in **Table 1**. The results show that there is statistically significant difference between the collected samples after two weeks of using Miswak compare to toothpaste according to the microbial growth on Sabouraud's dextrose agar. Where that Miswak is more effective than toothpaste, showed a significant decrease in the total number of colonies for each sample with a correlation coefficient. This shows that there is a positive correlation between the Miswak and the overall number of candida species with *p* < 0.001. **Tables 2** and **3** show the effect of Miswak on the candida. Thus, the total candida count was 27.50 ± 18.48 , IQR = 30, *p* = 0.001 from Miswak group and 247.0 ± 90.14 , IQR = 110, *p* = 0.979 in toothpaste group. **Table 4** depicts the distribution of all the organisms from the Miswak group and toothpaste group that has been isolated. The most frequent Candida isolated organisms were *C. albicans* 22 (36.67%) in the Miswak group and 24 (40.00%) in the toothpaste group, the second isolate was *C. dubliniensis* 04 (6.687%) Miswak group and 12 (20.0%) in toothpaste group followed by *C. glabrata* 02 (03.33%) in Miswak group.

Table 1: Phenotypic and microscopic characteristics of isolated candida species

Types	Microscopic characteristics	Chlamydospores	Colonies on chromogenic media
<i>C. albicans</i>	G +ve, spherical or semi-spherical and germ tube in human serum	+	Light green colonies
<i>C. dubliniensis</i>	G+ve, spherical or semi-spherical and germ tube in human serum	+	Bluish green colonies
<i>C. glabrata</i>	G +ve, oval.	-	Smooth creamy

Table 2: Candida count in the different brushing groups

Group, each n = 60	Time	Mean ± SD	Median	IQR	P value
Miswak group	Before	253 ± 97.12	249.5	100	0.001
	After	27.5 ± 18.48**	30	30	
Toothpaste group	Before	247.78 ± 89.89	240.5	111	0.979
	After	247.0 ± 90.14	238	110	

IQR = Interquartile Range

Table 3: Comparison between Miswak and toothpaste through cultural growth before and after use

Demographic Data	Candida colonization (CFU per ml)			
	Count	No	10 - 90 CFU/ml	> 105 CFU/ml
<i>Miswak group, n = 60</i>				
Before using Miswak	< 200	08	08	00
	200 - 500	18	10	08
	> 500	02	00	02
Total number		28	18	10
After 2 weeks of using Miswak	< 200	04	04	00
	200 - 500	00	00	00
	> 500	00	00	00
Total number		04	04	00
<i>Toothpaste group, n = 60</i>				
Before using toothpaste	< 200	12	08	04
	200 - 500	22	14	04
	> 500	02	04	02
Total number		36	26	10
After 2 weeks of using toothpaste	< 200	12	07	04
	200 - 500	21	14	04
	> 500	02	04	02
Total number		35	25	10

Table 4: Microorganisms isolated from the mouth of the test group

Type of microorganisms	Isolated species	Miswak group no = 60	Toothpaste group no = 60
Candida	<i>C. albicans</i>	22 (36.67%)	24 (40.0%)
	<i>C. dubliniensis</i>	04 (06.67%)	12 (20.0%)
	<i>C. glabrata</i>	02 (03.33%)	00
Total		28 (46.67%)	36 (60.0%)

Discussion

Good oral hygiene maintenance is a key to prevent oral diseases [21]. Miswak is used in the Arab world and represented by flexible and strong sticks [8, 9]. This study conducted for the first time in Libya to investigate the anti-candida activities. The findings showed that Miswak displays certain antifungal activity compared to toothpaste particularly against *C. albicans*, *C. dubliniensis* and *C. glabrata* strains. The highest candida count in colony forming units was recorded in toothpaste group indicating the least antifungal activity while the least candida count was recorded in Miswak indicating the highest antifungal activity. A significant difference between Miswak before and after using while there no significant difference between the toothpaste group before and after using. Several studies assessed Miswak and its effect on oral health. Miswak was containing several medicinal properties and scientifically was proved to be helpful even when used alone and without other methods of cleaning teeth [22 - 27]. It has also been indicated that Miswak released a variety of beneficial chemicals such as fluoride, saponins and sterol which possess antimicrobial activity. Chewing minimizes Miswak contamination [28, 29]. Miswak Chewing stick stimulates salivary flow. Saliva plays a vital role in maintaining homeostasis by balancing the pH in the oral cavity [30, 31]. As a result, using Miswak can reduce the risk of oral microbial contamination and translocation [32, 33]. The antimicrobial activity of released phytochemicals reduces the number of candida [27, 34]. Studies have indicated that Miswak possesses antifungal properties compared to ordinary

toothpaste, thus, preventing oral candidiasis [35, 36]. Where the Naeini et al. [37] reported from an *in vitro* study that alcoholic extract of Miswak have strong to moderate activity, on oral Candida species, including *C. albicans*, *C. dubliniensis* and *C. glabrata*. More, hexane components in roots of Miswak were found robust against *C. albicans* [37]. In current study, an assay after two weeks was conducted to illustrate the difference between Miswak use and toothbrushes with toothpaste. Where the results showed that Miswak affected candida in all the investigated strains. Miswak was much more effective than toothpaste in eliminating candida immediately and after two weeks of use. These results indicate that the extracts of Miswak contain compounds with a therapeutic potential against different Candida strains, and hence, they can potentially be utilized as therapeutic agents. The significance of this study came from an *in vivo* assessment of Miswak on oral candida that was followed up on for two weeks. In case of both treatments are combined or used, Miswak has antibacterial and antifungal activities as well as preventing plaque. Toothpaste contains fluoride which prevents tooth decay and thus, the use of both for one month may reach an ideal level of oral hygiene and dental health. On the other hand, more studies are needed to verify the use of Miswak as an effective mean to improve oral hygiene tools and fungicidal therapeutically agent for *C. albicans*.

Conclusion: Miswak reduces substantially the total oral fungus more than toothpaste, thus, Miswak use can limit the risk of oral candida species contamination and translocation.

Acknowledgments: The authors would like to thank all the involved participants for their support to carry out the study.

Data availability statement: The raw data that support the findings of this article are available from the corresponding author upon reasonable request.

Author contributions: HSE conceived, designed, collected and performed the analysis of data. SMA & SAK contributed in data collection and analysis. All authors drafted, revised the final version of the manuscript and have approved the final version of the manuscript and agreed to be accountable for its contents.

Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical issues: Including plagiarism, informed consent, data fabrication or falsification and double publication or submission have completely been observed by authors.

Author declarations: The authors confirm that all relevant ethical guidelines have been followed and any necessary IRB and/or ethics committee approvals have been obtained.

References

1. Muzyka BC, Epifanio RN (2013) Update on oral fungal infections. Dental Clinics of North America. 57 (4): 561-81. doi: 10.1016/j.cden.2013.07.002.
2. Hebbar PB, Pai A, Sujatha D (2013) Mycological and histological associations of Candida in oral mucosal lesions. Journal of Oral Science. 55 (2): 157-160. doi: 10.2334/josnurd.55.157.
3. Sharma U, Patel K, Shah V, Sinha S, Rathore VPS (2017) Isolation and speciation of candida in type II diabetic patients using chrom agar: A microbial study. Journal of Clinical and Diagnostic Research. 11 (8): DC09-DC011. doi: 10.7860/JCDR/2017/24864.10394.
4. Sardi JCO, Gullo FP, Pitangui NS, Fusco-Almeida AM, Mendes-Giannini MJS (2013) In vitro antifungal susceptibility of *Candida albicans* isolates from patients with chronic periodontitis and diabetes. Clinical Microbiology. 2: 1. 1000103. doi: 10.4172/2327-5073.1000103.
5. Sharma A (2019) Oral candidiasis: An opportunistic infection: A review. International Journal of Applied Dental Sciences. 5 (1): 23-27.
6. Kassebaum NJ, Smith AGC, Bernabé E, Fleming TD, Reynolds AE, Vos T, Murray CJL, Marcenes W, Abyu GY, Alsharif U, Asayesh H, Benzian H, Dandona L, Dandona R, Kasaeian A, Khader YS, Khang YH, Kokubo Y, Kotsakis GA, Lalloo R, Misganaw A, Montero P, Nourzadeh M, Pinho C, Qorbani M, Rios Blancas MJ, Sawhney M, Steiner C, Traebert J, Tyrovolas S, Ukwaja KN, Vollset SE, Yonemoto N (2017) Global, regional, and national prevalence, incidence, and disability-adjusted life years for oral conditions for 195 countries, 1990-2015: a systematic analysis for the global burden of diseases, injuries, and risk factors. Journal of Dental Research. 96 (4): 380-387. doi: 10.1177/0022034517693566.
7. Van Leeuwen MP, Rosema NA, Versteeg PA, Slot DE, Hennequin-Hoenderdos NL, Van der Weijden GA (2017) Effectiveness of various interventions on maintenance of gingival health during 1 year—a randomized clinical trial. International Journal Dental Hygiene. 15 (4): e16-e27. doi: 10.1111/idh.12213.
8. Niazi FM, Naseem ZK, Zafar MS, Almas K (2016) Role of *Salvadora persica* chewing stick (Miswak): A natural toothbrush for holistic oral health. European Journal of Dentistry. 10 (2): 301-308. doi: 10.4103/1305-7456.178297.
9. Qureshi AA, Qureshi AA, Dohipoide A, Jamadar NN (2016) Effects of Miswak-*Salvadora persica* on oral health. Al Ameen Journal of Medical Sciences. 9 (4): 215-218.
10. Haque MM, Alsareii SA (2015) A review of the therapeutic effects of using Miswak (*Salvadora Persica*) on oral health. Saudi Medical Journal. 36 (5): 530-543. doi: 10.15537/smj.2015.5.10785.
11. Halawany HS (2012) A review on Miswak (*Salvadora persica*) and its effect on various Aspects of oral health. The Saudi Dental Journal. 24 (2): 63-69. doi: 10.1016/j.sdentj.2011.12.004.
12. Aumeeruddy MZ, Zengin G, Mahomoodally MF (2018) A review of the traditional and modern uses of *Salvadora persica* L. (Miswak): Toothbrush tree of Prophet Muhammad Journal of Ethnopharmacology. 213: 409-444. doi: 10.1016/j.jep.2017.11.030.
13. Rizvi A, Zafar MS, Farid WM, Gazal G (2014) Assessment of antimicrobial efficacy of MTAD, sodium hypochlorite, EDTA and chlorhexidine for endodontic applications: An in vitro study. Middle East Journal of Scientific Research. 21 (2): 353-357. doi: 10.5829/idosi.mejsr.2014.21.02.524.
14. Kumar SV (2019) Indigenous oral hygiene aids. Scholars Journal of Applied Medical Sciences. 7 (3): 1267-1269. doi: 10.36347/sjams.2019.v07i03.081.
15. Pachava S, Chandu VC, Yaddanapalli SC, Dasari AB, Assaf HM (2019) Comparing caries experience between Azadirachta indica chewing stick users and toothbrush users among 35-44-year-old rural population of Southern India. Journal of International Society of Preventive and Community Dentistry. 9 (4): 417-422. doi: 1004103/jispcd. JISPCD_428_18.
16. Malik AS, Shaikat MS, Qureshi AA, Abdur R (2014) Comparative effectiveness of chewing stick and toothbrush: A randomized clinical trial. North American Journal of Medical Sciences. 6 (7): 333-337. doi: 10.4103/1947-2714.136916.

17. Alamri HM, Sarah A, Hashish N (2018) Antimicrobial effect of Almsiwak (*Salvadora Persica*) extracts, against selected pathogenic microbes. European Journal of Pharmaceutical and Medical Research. 5 (11): 287-293.
18. Al-Sohaibani S, Murugan K (2012) Anti-biofilm activity of *Salvadora persica* on cariogenic isolates of *Streptococcus mutans*: in vitro and molecular docking studies. Biofouling. 28 (1): 29-38. doi: 10.1080/08927014.2011.647308.
19. Tooyama H, Matsumoto T, Hayashi K, Kurashina K, Kurita H, Uchida M, Kasuga E, Honda H (2015) Candida concentrations determined following concentrated oral rinse culture reflect clinical oral signs. BMC Oral Health. 15: 150. doi: 10.1186/s12903-015-0138-z.
20. Jan A, Bashir G, Fomda BA, Khangsar DA, Manzoor M, Kholi A, Shuail M, Choudhary S, Fatima A (2018) Hypertonic xylose agar medium: A novel medium for differentiation of *Candida dubliniensis* from *Candida albicans*. Indian Journal of Medical Microbiology. 35 (4): 518-521. doi: 10.4103/ijmm.IJMM_17_216.
21. Sadeghi NB, Moghimipour E, Yusef NS, Nezarat S (2018) Antifungal and antibacterial activities of polyherbal toothpaste against oral pathogens, in vitro. Current Medical Mycology. 4 (2): 21-26. doi: 10.18502/cmm.4.2.65.
22. El-Desoukey R (2015) Comparative microbiological study between Miswak and toothpaste. International Journal of Microbiological Research. 6 (1): 47-53. doi: 10.5829/idosi.ijmr.2015.6.1.9331.
23. Alili N, Türp JC, Kulik EM, Waltimo T (2014) Volatile compounds of *Salvadora persica* inhibit the growth of oral *Candida* species. Archives of Oral Biology. 59 (5): 441-447. doi: 10.1016/j.archoralbio.2014.02.001.
24. Khounghanian R, Alwakeel A, Albadah A, Almaflehi N (2018) Evaluation of the amount and type of microorganisms in tooth brushes and Miswak after immediate brushing. ARC Journal of Dental Science. 3 (1): 15-21. doi: doi.org/10.20431/2456-0030.0301005.
25. Saha S, Mohammad S, Samadi, F (2012) Efficiency of traditional chewing stick (Miswak) as an oral hygiene aid among Muslim school children in Lucknow: A crosssectional study. Journal of Oral Biology and Craniofacial Research. 2 (3): 176-180. doi: 10.1016/j.jobcr.2012.10.009.
26. Moeintaghavi A, Arab H, Khajekaramodini M, Hosseini R, Danesteh H, Niknami H (2012) In vitro antimicrobial comparison of chlorhexidine, *Salvadora persica* mouthwash and Miswak extract. Journal of Contemporary Dental Practice. 13 (2): 147-152. doi: 10.5005/jp-journals-10024-1111.
27. Alireza RGA, Afsaneh R, Seied Hosein MS, Siamak Y, Afshin K, Zeinab K, Mahvash MJ, Amir RR (2014) Inhibitory activity of *Salvadora persica* extracts against oral bacterial strains associated with periodontitis: an in-vitro study. Journal of Oral Biology and Craniofacial Research. 4 (1): 19-23. doi: 10.1016/j.jobcr.2014.01.001.
28. Fayez Niazi, Mustafa Naseem, Zohaib Khurshid, Muhammad S. Zafar, Khalid Almas. (2016) Role of *Salvadora persica* chewing stick (Miswak): A natural toothbrush for holistic oral health. European Journal of Dentistry. 10 (2): 301-308. doi: 10.4103/1305-7456.178297.
29. Tartaglia GM, Kumar S, Fornari CD, Corti E, Connelly ST (2017) Mouthwashes in the 21st century: a narrative review about active molecules and effectiveness on the periodontal outcomes. Expert Opinion in Drug Delivery. 14 (8): 973-982. doi: 10.1080/17425247.2017.1260118.
30. Abhary M, Al-Hazmi AA (2016) Antibacterial activity of Miswak (*Salvadora persica* L) extracts on oral hygiene. Journal of Taibah University for Science. 10: 513-520.
31. Albabtain R, Ibrahim L, Bhangra S, Rosengren A, Gustafsson A (2018) Chemical effects of chewing sticks made of *Salvadora persica*. International Journal of Dental Hygiene. 16 (4): 535-540. doi: 10.1111/idh.12359.
32. Ismail AD (2015) Review on chemical and biologically active components of the toothbrush tree (*Salvadora persica*). European Journal of Pharmaceutical and Medical Research. 2 (6): 12-17.
33. Ismail AD, Husham EH (2016) Survival rate of oral bacteria on toothbrush and Miswak stick. American Journal of Health Research. 4 (5): 134-137. doi: 10.11648/j.ajhr.20160405.14.
34. Naseem S, Hashmi K, Fasih F, Sharafat S, Khanani R (2014) In vitro evaluation of antimicrobial effect of Miswak against common oral pathogens. Pakistan Journal of Medical Sciences. 30: 398-403. doi: 10.12669/pjms.302.4284.
35. Vamsi S, Latha P (2014) Urolithiasis: an updated review over genetics, pathophysiology and its clinical management. International Journal of Pharmacy and Pharmaceutical Sciences. 6 (11): 23-31.

36. Baeshen H, Salahuddin S, Dam R, Zawawi KH, Birkhed D (2017) Comparison of fluoridated Miswak and toothbrushing with fluoridated toothpaste on plaque removal and fluoride release. *Journal of Contemporary Dental Practice*. 18 (4): 300-306. doi: 10.5005/jp-journals-10024-2035.
37. Naeini A, Naderi NJ, Shokri H (2014) Analysis and in vitro anti-Candida antifungal activity of Cuminum cyminum and Salvadora persica herbs extracts against pathogenic Candida strains. *Journal of Mycologie Medicale*. 24 (1): 13-8. doi: 10.1016/j.mycmed.2013.09.006.