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Research Article

Formulation and Antimicrobial Evaluation of Miswak (*Salvadora persica L.*) Chewing Stick Aqueous Extract Lozenges

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ABSTRACT

The aims of this study to formulate and evaluate the antimicrobial activity of *S. persica* aqueous extract lozenges. *S. persica* aqueous extract lozenges were prepared by heating and congealing technique. Antimicrobial activities for *S. persica* aqueous extract and *S. persica* aqueous extract lozenges were determined using the well diffusion method. The formulation of *S. persica* lozenges was performed and its quality was evaluated. *S. persica* aqueous extract and *S. persica* aqueous extract lozenges were exhibited significant antibacterial and antifungal activities against clinical isolated oral pathogens. The highest antibacterial activity of; (85 mg/ml); 25 ± 0.78 mm, 30 ± 0.77 mm, 21 ± 0.83 mm and 18 ± 0.86 mm were obtained against *E. coli*, *P. aeruginosa*, S. aureus and *S. mutans*, respectively. Also, the antifungal activity of *S. persica* extract showed that the highest activity 12 ± 0.79 mm with concentration (85 mg/ml) against *C. albicans*. Moreover, *S. persica* extract lozenges were showed antimicrobial activities with the zone of inhibitions (85 mg/ml); 30 ± 0.83 mm, 20 ± 0.85 mm, 18 ± 0.78 mm and 10 ± 0.85 mm were obtained against *E. coli*, *P. aeruginosa*, S. aureus and *C. albicans* respectively. This study draws attention toward an easy and time saving formulation process of *S. persica* lozenges as suitable dosage form that may effectively control oral and throat pathogens.

Keywords: *Salvadora persica* extract; Lozenges; Antimicrobial; Oral hygiene

INTRODUCTION

Oral hygiene is the main portal for the body health most crucial component of our overall general health. It is a determinant factor for life quality as both are associated strongly [1,2]. The data mined about oral health from Global Burden of Disease Study in 2018 revealed that oral diseases are affect nearly 3.5 billion people globally [3], which can pose a serious health charge for many countries and affect individuals throughout their lifetime and influencing sleep, eating habits, and social factors [4]. Oral hygiene is diminished by many pathogenic microbes which affected the oral region by various damages that they may expand and cause serious disease [2]. Therefore, prevention and take control of oral pathogens is an important factor to maintain good oral hygiene [5]. These opportunistic pathogens are frequently isolated from the oral cavity include *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (S. aureus), *Streptococcus pneumoniae* (S. pneumoniae), *Enterobacter cloacae* (E. cloacae), *Acinetobacter baumannii* (A. baumannii), *Stenotrophomonas maltophilia* (*S. maltophilia*), and *Streptococcus agalactiae* (*S. agalactiae*) [6]. Several traditional medicinal plants have been proven for their activity and use in the prevention and treatment of different oral diseases [7]. Natural products have a unique diversity in chemical structure responsible for their pharmacological activities which in most cases are due to combined effects of the active constituents within the medicinal plant [8]. Historically, the first known natural product for oral hygiene tool was the chewing stick, the Miswak, derived mainly from Arak tree that grows in Saudi Arabia. It is obtained from (*Salvadora persica L.*) of the family Salvadoraceae, widespread in Middle Eastern, some Asian and African cultures traditionally utilize it as tooth-powder and tooth-cleaner. The World Health Organization (WHO) recommended the use of Miswak in 1986 and in 2000 in an international consensus report on oral hygiene [9,10]. *S. persica* has antibacterial activity [11]; antifungal properties [12]; antiviral activity [13]; dental plaque control [14]; antioxidant; antilipidemic and antidiabetic activity [15]. The major active constituents isolated from stem of *S. persica* for good oral hygiene are tannins, silica, alkaloids, volatile oil, sulphur monocline, organic sulphur compounds, chlorides, calcium, β-sitosterol and saponins (Table 1). Lozenges are dosage forms manufactured in a simple way, which can deliver an active ingredient as they disintegrate slowly in the oral cavity [16]. Lozenges considerable has advantages over oral sprays or gargles, which provide fast, effective and sustainable

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delivery of active ingredients representing a good vehicle for active constituents, therefore highlighting their potential benefits for mouth and throat medication with palatable mean and high patient adherence [17].

Table 1: The active constituents from stem of *S. persica* and their pharmacological benefits for oral hygiene.

Active constituents	Pharmacological activity
Tannins (tannic acid)	Reduces plaque and gingivitis.
Silica	An abrasive material to remove stains on teeth.
Alkaloids (salvadorine)	Bactericidal effect and stimulate the gingiva.
Essential oils	Antimicrobial activity and stimulates the flow of saliva, which acts as a buffering agent.
Benzylisothiocyanate	Chemo-preventive, antiviral, antibacterial and anti-fungal agents.
Vitamin C	It helps in tissue healing and repair.
Fluoride	Anti-decay effects.
Chloride	Inhibits calculus formation and help in removing stains from the teeth.
Calcium	Its saturation of saliva inhibits demineralization and induces the re-mineralization of tooth enamel.
Sulfur	Bactericidal effect.

Therefore, this study aimed to formulate *S. persica* aqueous extract in form of lozenges and evaluate their formulation properties as well as antimicrobial activity against different bacterial species and fungi, specifically *Candida albicans*. As we are targeting oral hygiene the choice of lozenges will be more reasonable compared to the other solid dosage forms [18-28].

MATERIALS AND METHODS

Plant materials

S. persica sticks were collected from Al Azazi area, Gezira state, Sudan. The studied plant part was identified and authenticated by Medicinal and Aromatic Plants Research Centre, Faculty of Pharmacy, University of Gezira and Agricultural Research Corporation (ARC), Wad Medani, Sudan.

Preparation of plant extract

The collected sticks were cut into small pieces (0.5 cm-1.0 cm) and dried in an air-open shade, then milled into coarse powder using electrical blender (Moulinex Blender the genuine 400 W, France). The powdered plant material (286.4 g) was macerated in 1.30 liter of cold distilled water at refrigerator for 72 hours with occasional shaking; then filtered using Whatman filter paper (Whatman® glass microfiber filters, 90 mm Grade GF/B; Aldrich, Germany) twice. Filtrate was evaporated using rotary evaporator at 60°C (Heidolph, Germany) until dryness to yield a green mass, followed by freeze-drying (LYO GT2, SRK-Systemtechnik GmbH, Germany) and was stored until use.

Qualitative phytochemical screening

S. persica extract was subjected to phytochemical screening for their different constituents such as; carbohydrates and/or glycosides, alkaloids and/or nitrogenous compounds, flavonoids, tannins and/or phenolic compounds, sterols and/or triterpenes, saponins and sulfur compounds [29].

Formulation of *S. persica* hard candy lozenges

Two standard model of hard candy lozenges formula based on different excipients were prepared by heating and congealing technique [30]. The compositions of model formulations are given in Table 2. For formula I, the candy base was prepared by dissolvingof fine powder sugar in one-third amount of distilled water in candy base cooker. This had been continued till the temperature reached 200°C. Liquid glucose was then poured slowly and temperature lowered to 150°C in order to add the methyl cellulose till the mixture became candy mass. Sodium citrate powder and amaranth red (coloring agent) were added to the candy mass with continuous stirring. Then the mass was removed from the cooker and then menthol powder flavor and *S. persica* extract were added and mixed thoroughly. Finally, the mass was poured in pre-calibrated mold and left for 15 minutes to congeal. The same procedure was repeated for formula II with sodium carboxymethyl cellulose (NaCMC) instead of methyl cellulose (MC) (Table 2).

Table 2: The compositions of *S. persica* extract hard candy lozenges standard formulation.

Ingredients (Single lozenge)	Formula I	Formula II
Sugar	67.5 g	67.5 g
Liquid glucose	28.1 g	28.1 g
Methylcellulose 1% (MC)	2 g	0
Sodium carboxymethylcellulose 1% (CMC)	0	2 g
Acidulent (Sodium Citrate)	1 g	1 g
Flavor (Menthol)	0.67 g	0.67 g
Color (Amaranth)	0.03 g	0.03 g
S. persica extract	75 mg	75 mg

Quality control analysis of *S. persica* extracts lozenges

Moisture content analysis: Gravimetric method for moisture analysis was followed, in which sample (1 g) will be weighed and placed in vacuum oven at 60°C-70°C for 12-16 hours. Final weight will be subtracted from initial and differences in moisture content were calculated [31].

Determination of weight variations and diameter: The average weight of the lozenges was determined by individually weighing of 20 lozenges of each formulation using an analytical balance (W3100A-210, Accuris™, USA), individual weight was compared to the average weight and subsequently the mean weights and the respective standard errors were calculated. Also, the diameter was measured for 20 lozenges [32].

***In vitro* dissolution experiment:** *In vitro* dissolution test was conducted based on United State Pharmacopeia method II [33] with little modifications using USP apparatus II (RC-6 Dissolution tester, Guoming® , China). Artificial saliva pH 6.8 at 37 ± 0.5°C was taken as dissolution media. Each lozenge was immersed in 1000 mL vessel and the rotation speed was set to 100 rpm. At different time intervals (5, 10, 15 and 20 minutes) a 5 mL sample was withdrawn and replaced with 5 ml fresh artificial Saliva as shows in Table 3. UV/Visible spectroscopy was used to determine the absorbance at wavelength 275 nm (7205 UV/Visible spectrophotometer, Jenway, UK). The samples were then determined spectrophotometrically based on the λ_{max} [34].

Table 3: The composition of artificial saliva.

Ingredients	Quantity
Sodium chloride	8.000 g
Potassium dihydrogen phosphate	0.190 g
Sodium hydrogen phosphate dihydrate	2.984 g
Phosphoric acid	ad pH 6.80
Demineralized water	ad 1 L

Antimicrobial activity

Tested microorganisms: Tested microorganisms were clinically isolated microorganisms including four bacterial strains; *E. coli* (ML-UG/B130024), *P. aeruginosa* (ML- UG/B130025), *S. aureus*, streptococcus mutans (ML-UG/B130028) and one fungal strain C. *albican* (ML- UG/F130001); and identified by the Microbiology Department Laboratory at Faculty of Pharmacy, University of Gezira, Wad Medani, Sudan.

Microorganisms growth condition and antimicrobial assay: The antibacterial and antifungal activities of *S. persica* aqueous extract and *S. persica* aqueous extract lozenges were determined using the well diffusion method. Petri plates containing 20 ml of, nutrient agar for bacteria (Darwin Biological, UK) or malt extract for fungus (Himedia, India), agar medium was seeded with 1-3 days culturesof microbial inoculums. Wells (6 mm in diameter) were cut off from agar and 50 µl of the samples in different concentrations and standard antibiotics were tested and then incubated at 37°C for 24-48 hours (bacterial strains) and for 3-5 days (fungal strain).The antibacterial and antifungal activities were determined by measurement of the diameter of the inhibition zone surrounding the well [35].

Data analysis

All the obtained data were expressed as means ± Standard Error of Means (SE) and analyzed using analysis of variance (ANOVA). Comparisons with the control groups were made using One-way ANOVA. The level of statistical significance was set at $p<0.05$

RESULTS AND DISCUSSION

Extraction and phytochemical screening

S. persica stick aqueous extraction process was yielded 34.8 g of dry green extract (12.15% w/w). The qualitative preliminary phytochemical screening indicated that *S. persica* extract contain tannins and/or phenolic compounds, sulfur compounds, carbohydrates and/or glycoside and Saponins (Table 4). On the other hand, alkaloids and/or nitrogenous compounds, flavonoids, and sterols and/or triterpenes were absent. These phytochemical results can support possible presence of *S. persica* extract biological activities [11,18,28].

Table 4: The phytochemical screening results of *S. persica* extract.

Test	Result
Sulfur compounds	+
Tannins	+
Glycoside	+/-
Alkaloids	-
Flavonoids	-
Flavones	-
Saponins	-

***Key:** (+) =Present; (-) =Absent.

Formulation of *S. persica* lozenges

All the formulated lozenges were showed good physical appearance (Figure 1), thereby attractive and unpleasant taste masking were found in *S. persica* lozenges. Based on the general physical texture, lozenges contain MC were more solid compared to that one containing NaCMC which lead to more chewable lozenge as the salt act as hygroscopic agent resulting in increased moisture content. The addition of hydrocolloid; NaCMC and MC; directly affected the formulated lozenges in term of solid nature, chewing capability and extended the release of extract (Figure 1) [36].

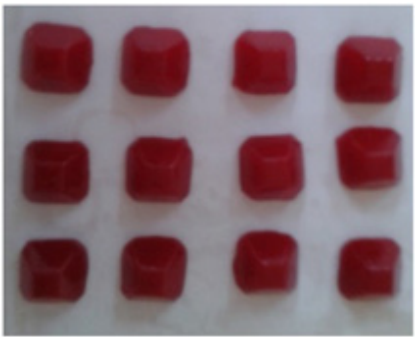

Figure 1: *S. persica* extract lozenges physical appearance.

Quality control analysis of *S. persica* extract lozenges

The average weight of *S. persica* lozenges was found (3.228 g ± 0.206 g), while the mean of lozenges diameter was found (16 mm ± 0.75) as shown in Table 5, which found to be within the standard limits.

Table 5: Weight and diameters of *S. persica* lozenges.

S. NO.	Weight of lozenges in grams ± SE	Diameter in cm ± SE
1	3.2100 ± 0.0008	1.6 ± 0.28
2	3.1539 ± 0.059	1.5 ± 0.35
3	3.3539 ± 0.07	1.6 ± 0.34
4	3.5746 ± 0.010	1.7 ± 0.28
5	3.0143 ± 0.014	1.5 ± 0.35
6	3.0646 ± 0.017	1.6 ± 0.34
Average	3.2285	1.6
SD	0.2069	0.075

***Abbreviation:** SE: Standard Error; SD: Standard Deviation

The moisture content of all the formulations found to be below 2% ± 0.3 (Table 6). This is due to suitable amounts of water soluble polymer and less water uptake. For *in vitro* drug release, randomly selected six lozenges were tested. The percentage release of *S. persica* extract from lozenges was presented in Table 7 and Figure 2. The results obtained were showed extended release of the *S. persica* extract over a period of 20 minutes. All the six lozenges at the first 5 minutes were released about 11%-14% ± 0.16%-0.6% of the total content. The cumulative amount was released increased gradually up to 100% after 20 minutes. The extend release behavior mostly is due to the presence of the hydrocolloid polymer in the formula. The results were obtained by Abdoun and Alenizi, 2019 when formulated a clotrimazole in polymer showed that the drug is released in 30 minutes was 97.45% ± 0.7% and 91.76% ± 0.83% from guar gum methyl cellulose lozenges respectively, while 97.31% drug release in 7 minutes from lozenges containing no polymer [37].

Antimicrobial assay

The antibacterial and antifungal activities of *S. persica* extract and *S. persica* extract lozenges (Formula II) were identified using well-diffusion method. The antibacterial activity of *S. persica* extract showed that the highest zone of inhibitions (85 mg/ml); 25 ± 0.78 mm, 30 ± 0.77 mm, 21 ± 0.83 mm and 18 ± 0.86 mm were obtained against *E. coli*, *P. aeruginosa*, S. aureus and *S. mutans*, respectively (Table 8 and Figure 3), which significantly higher than zone of inhibition of antimicrobial standards (cefuroxime and chlorhexidine) against bacterial strains. Also, the antifungal activity of *S. persica* extract showed that the highest activity 12 ± 0.79 mm with concentration (85 mg/ml) against *C. albicans*. The antimicrobial activity of *S. persica* which from this study was higher than that study investigated the effect aqueous extracts of *S. persica* against oral pathogens; with zone of inhibitions (200 mg/ml); 10.8 mm, 18.4 mm, 19.3 mm and 12.4 mm were obtained against *P. aeruginosa*, *S. aureus*, *S. mutans*, and *C. albicans* respectively [38]. On the other hand, *S. persica* extract lozenges showed that the highest zone of inhibitions (75 mg/ml); 30 ± 0.83 mm, 20 ± 0.85 mm and 18 ± 0.78 were obtained against *E. coli*, *P. aeruginosa* and S. aureus respectively (Table 8 and Figure 3), which higher than zone of inhibition of antimicrobial standards (cefuroxime and chlorhexidine) against the same bacterial strains.

Table 6: *S. persica* lozenges moisture content (Formula II).

S. No.	Weight before (g) ± SE	Weight after (g) ± SE	% of weight loss
1	3.2100 ± 0.02	3.1600 ± 0.01	1.56%
2	3.1539 ± 0.03	3.1355 ± 0.01	0.58%
3	3.3539 ± 0.11	3.3345 ± 0.11	0.58%
4	3.5746 ± 0.10	3.5542 ± 0.10	0.57%
5	3.0143 ± 0.03	2.9950 ± 0.02	0.64%
6	3.0646 ± 0.02	3.0410 ± 0.02	0.77%

*Abbreviation: SE: Standard Error.

Table 7: The release percentage of *S. persica* extract from lozenges (Formula II).

Time (Min)	% Release ± SE					
	Lozenge 1	Lozenge 2	Lozenge 3	Lozenge 4	Lozenge 5	Lozenge 6
5	11.58 ± 0.2	11.18 ± 0.2	13 ± 0.16	13.32 ± 0.6	14.53 ± 0.6	12.74 ± 0.16
10	38.71 ± 0.13	32.35 ± 1.68	36.94 ± 1.98	38.46 ± 0.12	40.91 ± 1.90	35.71 ± 1.68
15	67.74 ± 0.35	64.71 ± 0.98	67.03 ± 0.36	73.08 ± 1.31	70.45 ± 1.32	66.67 ± 0.98
20	100	100	100	100	100	100

*Abbreviation: SE: Standard Error

Table 8: Antimicrobial activity zone inhibitions of *S. persica* extract and *S. persica* extract lozenges.

Sample	Concentration	*E. coli*	*P. aeruginosa*	S. aurues	*S. mutans*	*C. albicans*
		Zone of inhibition (mm) ± SE				
S. persica extract	12.5 mg/ml	13 ± 0.19	18 ± 0.63	11 ± 0.35	10 ± 0.75	-ve
	25 mg/ml	15 ± 0.18	20 ± 0.88	20 ± 0.66	13 ± 0.79	-ve
	50 mg/ml	23 ± 0.85	25 ± 0.81	21 ± 0.73	13 ± 0.81	10 ± 0.45
	75 mg/ml	25 ± 0.88	26 ± 0.75	20 ± 0.89	15 ± 0.65	12 ± 0.60
	85 mg/ml	25 ± 0.78	30 ± 0.77	21 ± 0.83	18 ± 0.86	12 ± 0.79
S. persica lozenges	75 mg/ml	30 ± 0.83	20 ± 0.85	18 ± 0.78	15 ± 0.88	10 ± 0.85
Cefuroxime (S)	75 mg/ml	15 ± 0.55	19 ± 0.80	20 ± 0.86	16 ± 0.86	-
Nystatin (S)	1003 IU/ml	-	-	-	-	25 ± 0.73
Chlorohexidine (S)	1%	16 ± 0.82	20 ± 0.87	26 ± 0.59	17 ± 0.43	10 ± 0.82

*Abbreviation: SE: Standard Error; -ve: Negative

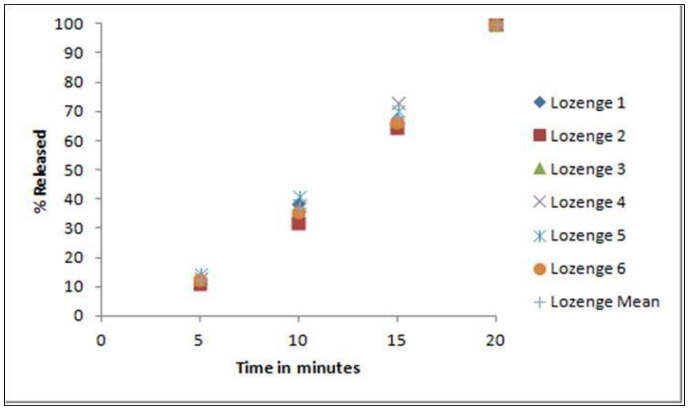

Figure 2: The release percentage of *S. persica* extract from lozenges.

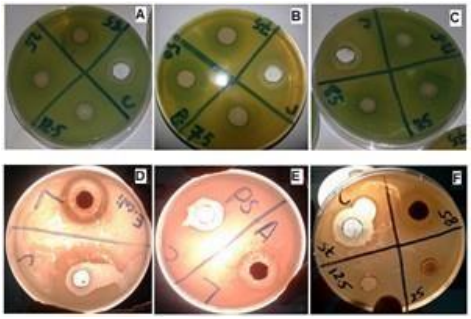

Figure 3: Antimicrobial activity zone of inhibitions: A, B, and C for *S. persica* extract; against *E. coli*, *P. aeruginosa* and *S. aureus* respectively; D, E and F for *S. persica* lozenges against *E. coli*, *P. aeruginosa* and *S. aureus* respectively.

This study confirmed the beneficial effect of *S. persica* on oral health that may due to the presence of important compounds, which have antimicrobial effect. Fluoride was reported to inhibit the growth of bacteria [21,26]. Tannins were found to reduce gingivitis [18,19], silica and chloride removing stains and helpful in tooth whitening [20,23,27], and vitamin C help in tissue healing and repairing [21]. Moreover, the essential oils beside antimicrobial activity have bitter taste which stimulates the flow of saliva and acts as a buffering agent [22,23].

CONCLUSION

Results of the current study revealed the formulation of *S. persica* aqueous extract lozenges were successful prepared with good quality. *S. persica* aqueous extract and *S. persica* aqueous extract lozenges were exhibited potent antibacterial and antifungal activities against clinical isolated oral pathogens. This study draws attention toward an easy and time saving formulation process of *S. persica* lozenges as suitable dosage form that control oral and throat infections with rapid onset of action, economic and good patient compliance.

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DISCLOSURE OF INTEREST

The authors declare that they have no competing interest.

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REFERENCES

1. Razak PA, Richard KM, Thankachan RP, Hafiz KA, Kumar KN, Sameer KM. Geriatric oral health: A review article. J Int Oral Health. 2014; 6(6):110-116.
2. Hakeem KR, Abdul WM, Hussain MM, Razvi SSI. Oral Hygiene for Healthy Life. In: Oral Health and Herbal Medicine. SpringerBriefs in Public Health. Springer Cham. 2019; 5-6.
3. GBD. 2017 Disease and Injury Incidence and Prevalence Collaborators, Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: A systematic analysis for the Global Burden of Disease Study. Lancet 2018; 392(10159):1789-1858.
4. Alsubaie AS. Oral health-related behaviors and dental pain among children in Saudi Arabia. J Int Oral Health 2019; 11:1-7.
5. Par M, Badovinac A, Plancak D. Oral hygiene is an important factor for prevention of ventilator-Associated pneumonia. Acta Clin Croat. 2014; 53(1):72-78.
6. Tada A, Hanada N. Opportunistic respiratory pathogens in the oral cavity of the elderly. FEMS Immunol Med Microbiol. 2010; 60(1):1-17.
7. Palombo EA. Traditional medicinal plant extracts and natural products with activity against oral bacteria: Potential application in the prevention and treatment of oral diseases. Evid Based Complement Alternat Med. 2011; 2011:1-15.
8. Dias DA, Urban S, Roessner U. A historical overview of natural products in drug discovery. Metabo. 2012; 2(2):303-336.
9. Sofrata A, Santangelo EM, Azeem M, Borg-Karlson AK, Gustafsson A, Pütsep K. Benzyl isothiocyanate: A major component from the roots of *Salvadora Persica* is highly active against Gram-negative bacteria. PLoS One. 2011; 6(8):e23045.
10. Ahmad H, Rajagopal K. *Salvadora Persica L.* (Meswak) in dental hygiene. Saudi J Dent Res. 2013; (5):130-134.
11. Abhary M, Al-Hazmi A. Antibacterial activity of Miswak (*Salvadora Persica L.*) extracts on oral hygiene. J Taibah Univ Sci. 2016; 10(4):513-520.
12. Noumi E, Snoussi M, Hajlaoui H, Valentin E, Bakhrouf A. Antifungal properties of *Salvadora Persica* and *Juglans regia L.* extracts against oral Candida strains. Eur J Clin Microbiol Infect Dis. 2010; 29:81.
13. Taha MYM. Antiviral Effect of Ethanolic Extract of *Salvadora Persica* (Siwak) on Herpes Simplex Virus Infection. Al–Rafidain Dent J. 2008; 8(1):50-55.
14. Ahmad H, Rajagopal K. Biological Activities of *Salvadora Persica* L. (Meswak). Med Aromat Plants. 2013; 2:4.
15. El Rabey HA, Almutairi FM, Al-Sieni AI, Al-Seeni MN, Al-Duais MA, Sakran MI, et al. The antioxidant, antidiabetic and antilipidemic activity of *Salvadora Persica* twig in alloxan diabetic male rats. IJBB. 2017; 54:314-322.
16. Edwards WP. The science of sugar confectionery. Cambridge: The Royal Society of Chemistry. 2001.
17. Limb M, Connor A, Pickford M, Church A, Mamman R, Reader S, et al. Scintigraphy can be used to compare delivery of sore throat formulations. Int J Clin Pract. 2009; 63(4):606-612.
18. Gazi M, Davies T, Al-Bagieh N, Cox S. The immediate- and medium-term effects of Meswak on the composition of mixed saliva. J Clin Periodontol. 1992; 19 (2):113-117.
19. Chaurasia A, Patil R, Nagar A. Miswak in oral cavity-An update. J Oral Biol Craniofac Res. 2013; 3(2):98-101.

20. Almas K, Al-Lafi T. The Natural Toothbrush, World Health Forum. 1995; 16(2):206-210.
21. Almas K. Miswak (chewing stick) and its role in oral health. Postgraduate Dent. 1993; 3:214-218.
22. Alali F, Hudaib M, Aburjai T, Khairallah K, Al-Hadidi N. GC-MS Analysis and Antimicrobial Activity of the Essential Oil from the Stem of the Jordanian Toothbrush Tree *Salvadora Persica*. Pharm Biol. 2005; 42(8):577-580.
23. Halawany HS. A review on miswak (*Salvadora Persica*) and its effect on various aspects of oral health. Saudi Dent J. 2012; 24(2):63-69.
24. Al-Dosari A, Kafrawy A, Standish S. The effect of benzylisothiocyanate on epithelial changes induced by trauma and DMBA in the hamster tongue. Saud Dent J. 1992; 4(1):4-10.
25. Al-Bagieh N. Anti-Herpes Simplex Virus type I activity of benzylisothiocyanate. Biomed Lett. 1992; 47:67-70.
26. Hattab FN. Meswak: The natural toothbrush. J Clin Dent. 1997; 8(5):125-129.
27. Farooqui MI, Srivastava JG. The Toothbrush Tree (*Salvadora Persica*). J Crude Drug Res. 1968; 8:1297-1299.
28. Abo Al-Samh D. *In vitro* study of the antimicrobial activity and toxicity of the miswak extract as an endodontic irrigation solution. King Saud University, Riyadh, 1995.
29. Khan FAl, Iqbal H, Shahid F, Majed A, Muhammad A, Inayat UR. Phytochemical screening of some Pakistanian Medicinal Plants. Middle-East J Sci Res. 2011; 8(3):575-578.
30. Allen LV. The Art, Science, and Technology of Pharmaceutical Compounding (5th Edn.) American Pharmacists Association. 2016.
31. Peters D. Medicated Lozenges. In: Lieberman HA, Lachman L, Schwartz JB (2nd edn), Pharmaceutical Dosage Forms: Tablets. New York: Marcel Dekker, Inc., 2005; 419-577.
32. Kini R, Rathnanand M, Kamath D. Investigating the suitability of Isomalt and liquid glucose as sugar substitute in the formulation of Salbutamol sulfate hard candy lozenge. J Chem Pharm Res. 2011; (4):69- 75.
33. United States Pharmacopoeia and National Formulary USP 40-NF 35. United States Pharmacopoeial Convention Inc.: Rockville, 2017.
34. Tietz K, Gutknecht SI, Klein S. Bioequivalence of locally acting lozenges: Evaluation of critical *in vivo* parameters and first steps towards a bio-predictive *in vitro* test method. Eur J Pharm Biopharm. 2018; 123:71-83.
35. Zain ME, Awaad AS, Al-Outhman MR, El-Meligy RM. Antimicrobial activities of Saudi Arabian desert plants. Phytopharmacol. 2012; 2(1):106-113.
36. Tiwari SB, Rajabi-Siahboomi AR. Extended-release oral drug delivery technologies: Monolithic matrix systems. Methods Mol Biol. 2008; 437:217-243.
37. Abdoun AS, Alenizi R. Formulation and evaluation of metronidazole lozenges for oral thrush. J Innov Pharm Biol Sci. 2019; 6:5-10.
38. Al-Bayati FA, Sulaiman KD. *In vitro* antimicrobial activity of *Salvadora Persica L.* extracts against some isolated oral pathogens in Iraq. Turk J Biol. 2008; 32:57-62