



Miswak starch-based gel as topical preparation for potential management of infective ulceration

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ABSTRACT

Salvadora persica or Miswak is known for its antibacterial activities towards various bacteria species, including several oral pathogens. Although Miswak has many therapeutic potentials, the delivery medium is vital to ensure that the desired effects can be delivered to the target site. Ideally, starch is a biopolymer that exhibits muco-adhesive property, suitable as topical delivery material. Hence, this study was done to develop starch-based gel for Miswak antibacterial compound delivery system. In this study, Miswak hexane extract was incorporated into starch-based gels and stored at 4°C and room temperature. Miswak gel was tested for its antibacterial activity, and its stability was monitored by visual observation. As a result, the mean inhibitory zones of Miswak gels were found to be 22.67 ± 3.79 mm (4°C) and 20.67 ± 2.08 mm (room temperature) at day-1. The inhibitory zone decreased after 30 days with 13.00 ± 2.52 mm (4°C), and 12.00 ± 1.00 mm (room temperature). It was observed that the gels were stable where there were no colour changes, no phase separation and degradation, visualised up to day-60. In conclusion, the starch-based gel is a suitable delivery system for Miswak extract, thus as potential management for infective ulceration.



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INTRODUCTION

Salvadora persica (*S. persica*), known locally as toothbrush tree, is a member of the Salvadoraceae family. It is a small evergreen tree with soft, whitish, yellow wood. The *S. persica* tree, which is 3 m in height and 30 cm in diameter, has thick succulent small leaves. New stem branches are green to grey-

ish while old branches are dark brown. It has aromatic roots, as well as warm and tangy taste. It has many local names in different geographical regions, such as Miswak or Arak in the Arab world (Noumi *et al.*, 2011).

Miswak has known for its antibacterial activities towards various bacteria species including several oral pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus mutans* and *Candida albicans* (Al-Bagieh *et al.*, 1994; Al-Bayaty *et al.*, 2013; Noumi *et al.*, 2011). The antibacterial properties are attributed by the presence of its active compound such as Salvadorian and trimethylamine, which are shown to inhibit *Streptococcus mutans* growth with an inhibition zone of 8 mm. Another study investigated the presence of antimicrobial agents in Miswak extracts based on their polarity in different solvents. The results showed that Miswak contains more than one type of antimicrobial agent that inhibits the growth of both Gram-

positive and Gram-negative bacteria. It was found that the zone of inhibition for hexane and ethanolic extracts (500 μg) measured against *Escherichia coli* was 9 mm and 10 mm respectively, *Staphylococcus aureus* (9 mm Vs 26 mm), *Lactobacillus acidophilus* (9 mm Vs 9 mm), *Streptococcus mutans* (19 mm Vs 35 mm) and *Pseudomonas aeruginosa* (0 mm Vs 16 mm) (Alamri et al., 2018). These therapeutic properties of Miswak can be further developed as a potential alternative treatment for infected wound or ulceration.

An open wound or ulcer is a favourable niche for microbial colonisation, contaminated by pathogens found in the surrounding environment, i.e. endogenous microbes living in the mucous membranes, and by the microflora available on the adjacent skin. The colonisation of pathogenic bacteria at the wound site is reportedly associated with wound chronicity where *Staphylococcus*, *Pseudomonas*, *Peptoniphilus*, *Enterobacter*, *Stenotrophomonas*, *Finegoldia*, and *Serratia* were often found to be most frequently involved (Rahim et al., 2017).

One of the common treatments for infected wounds is medicated wound dressing which incorporated several types of antimicrobial agents comprises antibiotics (e.g. tetracycline, ciprofloxacin, gentamicin and sulfadiazine) (Choi et al., 2013; S. et al., 2016), nanoparticles (e.g. silver nanoparticles) (Augustine et al., 2016) and natural products (e.g. honey, essential oils and chitosan) (Qu et al., 2019; Suganya et al., 2011) essentially.

Most dentists and physicians commonly prefer the topical application of appropriate medications for the treatment of ulcerative and inflammatory mucosal conditions. Recently, topical dosage form utilised mucoadhesive hydrogel-forming polymers, such as starch, cellulose derivatives, natural gums, polyoxyethylenes, polyacrylates and sodium alginate. These gels with good bio- or mucoadhesive properties can help to enhance the retention time of the formulation at the site of action hence improving drug delivery to achieve the desired therapeutic effect (Marques et al., 2017).

Although Miswak has many therapeutic potentials, a suitable delivery system is equally essential to make sure that this agent is appropriately delivered to the desire target site. Thus, this research aims to assess whether the starch-based gel is a suitable and compatible delivery system for Miswak extract topical preparation and to evaluate the antibacterial properties of the formulation as potential management for infective ulceration.

MATERIALS AND METHODS

Materials

The roots of *S. persica* were the product of Pakistan, purchased from a local supplier. Hexane and glycerol were purchased from R&M Chemical. Whereas citric acid and brain and heart infusion (BHI) agar were purchased from Merck.

Hexane extraction of Miswak

The roots of Miswak were collected from *S. persica* species. The roots were crushed before subjected to oven drying at 60 °C for three days. The dried roots were later ground into powder using a dry food processor. The Miswak extracts were prepared by adding 1 kg of the Miswak powder to 2000 ml of hexane in a closed container and soaked at room temperature for 48 h. The solvents were filtrated through a Whatman No.1 filter paper and dried with the solvent evaporator for two h (Alamri et al., 2018).

Determination of Miswak extracts antibacterial activity.

Antibacterial activity of Miswak extract was performed by disk diffusion assay. *Staphylococcus aureus* (*S. aureus*) was obtained from the Laboratory of Microbiology, Faculty of Dentistry, Universiti Sains Islam Malaysia (USIM).

Approximately 1×10^8 CFU/mL of bacteria densities corresponding to a 0.5 McFarland turbidity standard was used to inoculate bacteria into fresh Tryptone soy broth and further incubated at 37 °C overnight. Later, the culture was spread on brain heart infusion (BHI) with a sterile cotton swab followed by the application of 5 mm diameter paper discs (Whatmann No. 1) impregnated with 20 μl of Miswak extract at a concentration of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml. Meanwhile, 50 mg/ml of Chloramphenicol disc was used as a positive control. The diameter of zone of inhibition was measured in millimetre using Vernier calliper after incubating under 37 °C for 24 h.

Preparation of Starch-based gel

A modified method adapted from Nagaraj et al. (2017) was used to prepare the starch-based gels. The starch-based gels were prepared by mixing serial concentrations of starch (5%, 10%, 15%, 20% and 25%) with glycerol. For each 5 g of starch, 0.5 g of citric acid and 1 ml of glycerol were mixed in 100 ml of distilled water. The mixtures were stirred using a magnetic stirrer for 15 minutes to get a homogenous suspension and later heated at 90 °C for another 30 minutes. The produced starch-based gels were cooled at room temperature and

Table 1: Zone of inhibition of Miswak extracts at different concentration and Chloramphenicol antibiotic.

Microorganism	Zone of inhibition (mm)				
	Miswak extracts at different concentration				Chloramphenicol
	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	50 mg/ml
<i>S. aureus</i>	9.0	10.0	15.0	23.0	55.0

Table 2: Starch-based gel (SG) formulation.

	Starch	Citric acid	Glycerol
SG 1	5%	0.5 g	1 ml
SG 2	10%	1.0 g	2 ml
SG 3	15%	1.5 g	3 ml
SG 4	20%	2.0 g	4 ml
SG 5	25%	2.5 g	5 ml

Table 3: Zone of inhibition of Miswak gel stored at 4 °C and room temperature for the period of 60 days.

Microorganism	Storage period	Zone of Inhibition of Miswak gel (mm)		
		Storage condition		
		4 °C	Room temperature (~28°C)	
<i>S. aureus</i>	Day-1	22.67 ± 3.79	20.67 ± 2.08	
	Day-30	13.00 ± 2.52	12.00 ± 1.00	
	Day-60	11.33 ± 1.53	11.00 ± 2.00	



Figure 1: Miswak hexane extract produced bright orange compound with stringent odour.

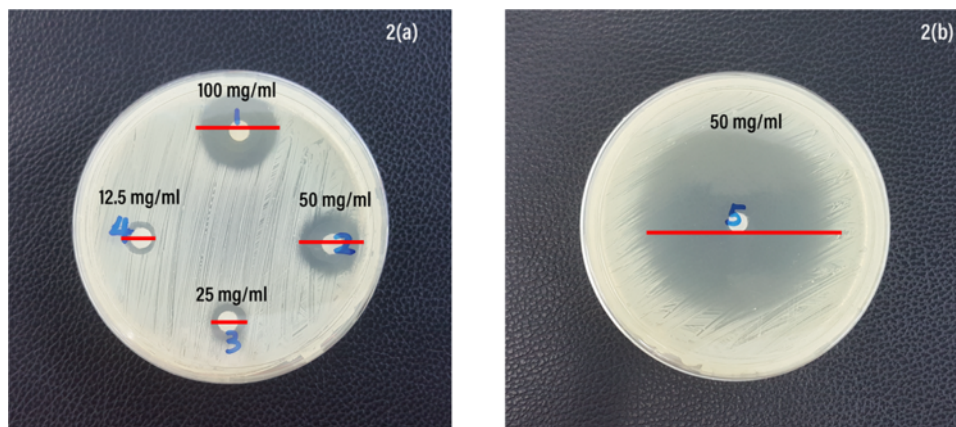


Figure 2: (a) Zone of inhibition of Miswak extract at different concentration against *S. aureus*. (b) Zone of inhibition of Chloramphenicol used as control positive.

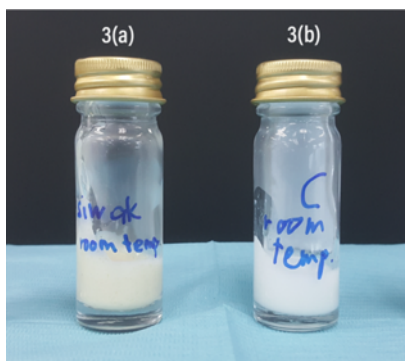


Figure 3: (a) Miswak gel (b) Chloramphenicol gel.

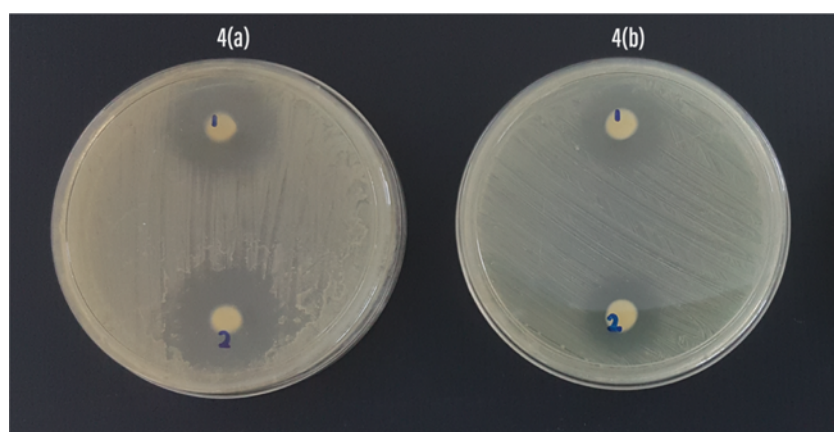


Figure 4: Zone of inhibition of Miswak gel stored at (a) 4 °C and (b) room temperature at day-1.

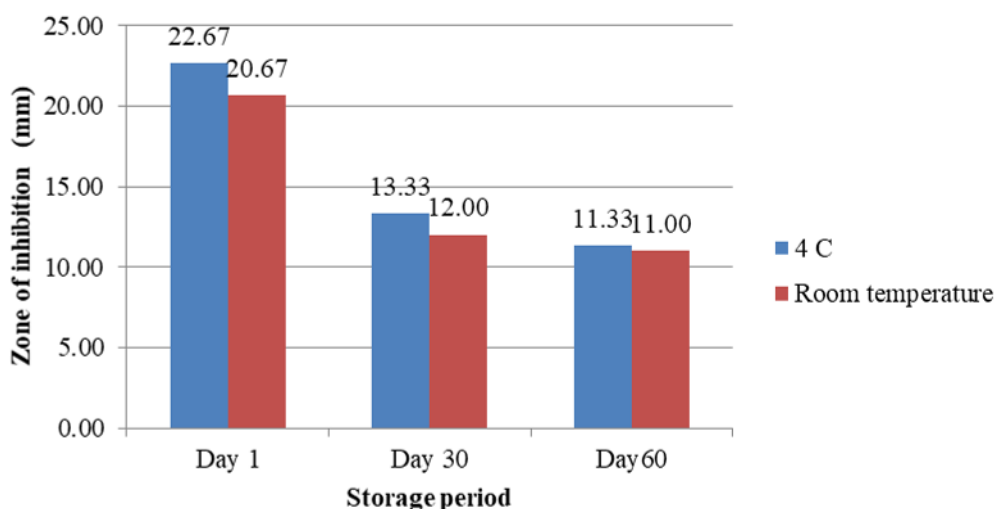


Figure 5: Comparison of Miswak gel zone of inhibition stored at 4 °C and room temperature.

kept in the refrigerator until further use (Nagaraj *et al*, 2017). The best formulation of starch-based gel will be further used to be incorporated with Miswak extract.

Preparation of Miswak gel

Miswak gel was prepared by incorporating Miswak extract into starch-based gels. 0.5 g of Miswak extract was dissolved in 1 ml alcohol and was added to 10 g of starch-based gels. The gel was mixed thor-

oughly to produce Miswak gel with 50 mg/g of Miswak concentration. A control positive was prepared in the same manner but using 0.25 g chloramphenicol.

Determination of Miswak gel antibacterial activity

A modified protocol was applied to evaluate the antibacterial activity of the Miswak gel. Instead of using Whatmann paper as disc, the gel was directly



Figure 6: Physical appearance of Miswak gel stored at 4 °C and room temperature at day-1, day-30 and day-60.

placed onto the BHI agar cultured with *S. aureus*. The concentration was calculated based on the formulation of the Miswak gel, which was 50 mg/g (Miswak extracts/starch gel).

Stability study of Miswak gel

Stability study of Miswak gel is evaluated by visual observation of its physical appearance for day-1, day-30 and day-60 at two different conditions; room temperature and 4 °C. The formulation was observed for any presence of colour changes, degradation and phase separation.

RESULTS AND DISCUSSION

Miswak hexane extract

11.06 g of extract was produced from 1 kg of Miswak powder. The extract appeared as a bright orange colour with a stringent odour (Figure 1). This extract was kept at room temperature until further use.

Antibacterial activity of Miswak extract

Relatively all Miswak extract of different concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml) exhibited antibacterial activity against *S. aureus* (Figure 2). The zone of inhibition was notable at a concentration of 100 mg/ml with 23 mm of the inhibitory zone. As the concentration of Miswak extracts decreased, the inhibitory zone contracted, as shown in Table 1. Meanwhile, Chloram-

phenicol that was used as control positive has high sensitivity towards *S. aureus* with an inhibitory zone of 55 mm (Figure 2).

This finding was in accordance to the previous study done by Alamri *et al.* (2018) where Miswak hexane extract exhibit potent antibacterial properties towards Gram-positive bacteria like *S. aureus* (Alamri *et al.*, 2018). The extracts were reportedly contained, as primary parts, benzeneacetonitrile (71.47%), 4-aminocarbonyl-5-fluoro-1- α -D-ribofuranosyl-imidazole (10.99%), and benzyl isothiocyanate (5.05%) (EL-Hefny *et al.*, 2017).

Miswak gel formulation

Starch-based gels (SG) were prepared by mixing serial concentrations of starch with glycerol, as shown in Table 2. It was found that SG1, SG2 and SG3 only formed a cloudy liquid and failed to solidify after 24 hours cooling at room temperature. Meanwhile, SG5 formed a very hard gel. Only SG4 formed a semi-solid gel which was ideal as a topical gel. SG4 was then selected to be incorporated with Miswak extract to produce Miswak gel. The incorporation of Miswak extract into starch hydrogel produced a homogenous, creamy whitish colour gel (Figure 3) and was formulated at a concentration of 50 mg/g (Miswak extracts/starch gel). This gel was later referred to as Miswak gel (MG). Miswak gel was stored at two different conditions; 4 °C and room temperature for stability study. Chloramphenicol was also incorporated into starch gel and used as a

positive control (Figure 3).

Antibacterial activity of Miswak gel

This research aims to evaluate whether the starch-based gel is a suitable delivery system for Miswak extract as a topical preparation. Therefore, the antibacterial activity of Miswak gel was assessed for two months. 0.5 g of Miswak gel (equivalent to 25 mg/ml of Miswak extract) was placed onto BHI agar, cultured with *S. aureus* (Figure 4). It was observed that both Miswak gel (stored at 4°C and room temperature) exhibited antibacterial activity tested at day-1, day-30 and day-60.

The zone of inhibition for Miswak gel stored at 4 °C and room temperature were almost similar, suggesting that the antibacterial activity was not affected by storage condition (Figure 5). The inhibitory zone of Miswak gel stored at 4 °C and room temperature were 22.67 ± 3.79 mm and 20.67 ± 2.08 respectively on day-1. At day-30, the zone of inhibition decreased for Miswak gel stored at both conditions, as shown in Table 3. However, the inhibitory zone slightly changed at Day-60.

This finding indicates that the antibacterial activity of Miswak extract was affected by the starch formulation. Hexane has a 0-polarity index according to Snyder's polarity index; thus, nonpolar essential and volatile oils that are hydrophobic are more likely to be dissolved in hexane (Abhary and Al-Hazmi, 2016). Meanwhile, the starch gel is a water-based formulation. The prolong storage may have caused the degradation of starch gel which might interfere with the antibacterial activity of Miswak chemical compound. The other possibility was that the presence of hydroxyl group in the water-based formulation could have interacted or may cause hydrolysis of the Miswak nonpolar compound thus deterring its antibacterial activity (Wathoni et al., 2018). It is suggested that a proper additive or preservative could be added to overcome this issue and to prolong the shelf life of Miswak gel preparation.

Stability study of Miswak gel

The stability of Miswak gel was observed for any colour changes, phase separation or degradation on day-1, day-30 and day-60. Visually, Miswak gels remained stable for the storage period of 60 days, where there were no colour changes, no phase separation and also no degradation observed (Figure 6). However, any physicochemical changes need to be assessed further using FTIR and viscosity of the formulation itself, which will be carried out in the near future.

CONCLUSIONS

Miswak extract has been successfully incorporated into a starch-based gel, and the formulation was found to be suitable as a delivery system for topical preparation. Miswak gel was found to be positive against *S. aureus*, and its antibacterial activity can be observed up to 60 days. Miswak gel was also visually stable where there were no colour changes, no phase separation and degradation noted from day-1 to day-60. Therefore, it is concluded that starch-based gel was suitable for Miswak extract delivery system, and this promising material as potential management for infective ulceration remains physically stable for up to two months.

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Conflict Of Interest

The authors declare that there is no conflict of interest for this study.

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