Article published online: 2022-06-14

Original Article

Studies on Use of Heat in the Aqueous Extraction of Miswak

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Abstract

Introduction: The antimicrobial properties of Miswak (*Salvadora persica*) are well documented, and the use of its extracts in the formulation of toothpastes and mouth rinses are well established. Most of the literature agrees that the organic extracts are more effective than aqueous extracts. **Aims:** The aim of the study was to prepare aqueous Miswak extracts using three different methods. The difference between the methods is the amount of heating used for their preparation. Furthermore, to evaluate stability during storage of such extracts, their pH, and their antimicrobial activity. **Materials and Methods:** Miswak extracts were prepared by maceration, infusion, and decoction methods, followed by evaluation of their extraction efficiency, stability, pH, viscosity, and antimicrobial activity. **Results:** A correlation was found between pH of extracts and their viscosity. The pH of extract increases to 6.5 when extensive heat was used during preparation, which is close to the normal pH of saliva and oral cavity. The accompanied increase in viscosity was an indication of increased extraction efficiency. Suppression of freezing point confirmed such observation for extracts prepared by decoction. The only positive antibacterial activity was observed for decoction extract, but it was less than that of chlorhexiden. For minimum inhibitory concentration estimation, it was found that almost 50% w/v of the extract must be used to provide the minimum microbial inhibitory effect. **Conclusions:** Miswak components appear to be thermostable ingredients, and the method of decoction can produce stable and effective Miswak extract.

Keywords: Antimicrobial activity, aqueous-heat extraction, miswak

Introduction

Miswak (*Salvadora persica)* possesses various biological properties including significant antibacterial and antifungal effects.[1,2] Because of its antimicrobial effects, Miswak extract use in mouth rinses and toothpastes is highly recommended.[3] The World Health Organization has recommended the use of Miswak chewing sticks as an effective mean to improve oral hygiene.[4]

Several studies were based on nonaqueous extracts with the use of organic solvents for the process of extraction of the active constituents of Miswak.[5,6] A comparison of the alcoholic and aqueous extracts of Miswak revealed that the alcoholic extract had more potent antimicrobial activity than did the aqueous extract.[7]

The following work is aimed at determination of a suitable method for the aqueous extraction of the active principles of Miswak, using water as the extracting liquid, with the aid of heat, and the evaluation of the antimicrobial activity of such extracts. Furthermore, other physical properties of the prepared extracts will be studied including pH, stability, effect of cold storage, and viscosity.

Materials and Methods

Miswak was obtained from local supplier (Bashasha store, Benghazi), culture media used include plate-count agar (for bacterial cultures, Himedia, India). All other chemicals and reagents were of analytical standard.

Preparation of Miswak powder

The dried roots of Miswak where converted into a fine powder by cutting into small pieces and placed in a cutter mill (Molinix, France) for 5 min.[8,9] After which, the resulted

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Quick Response Code:

Website: journal.limu.edu.ly

DOI: 10.4103/liuj.liuj_72_21

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How to cite this article: Sharkasi M, Elsharif M, Sughir A, El-Mahdi I. Studies on the use of heat in the aqueous extraction of Miswak. Libyan Int Med Univ J 2021;6:55-60.

Received: 31-05-2021 **Revised:** 15-07-2021
Accepted: 25-07-2021 **Published:** 05-01-2022

material is collected and stored in well-closed plastic containers.

Extraction

The active constituents of Miswak where extracted using decoction, maceration, or infusion techniques, employing purified water as the extraction liquid, as described below:

Decoction

Amix of 50-g of miswak powder was placed in 900 ml of purified water in a beaker and allowed to boil in a water bath for 2 h, and then the beaker was placed on a stage mechanical shaker for 24 h period.

Maceration

A mix of 50 g of Miswak powder was placed in 900 ml of purified water in a beaker, and then placed on a mechanical shaker for 24 h period.

Infusion

A mix of 50 g of Miswak powder was placed in 900 ml of preboiled purified water, in a beaker, and then placed on a mechanical shaker for 24 h period.

Separation of extract and storage

All three extracts were coarse filtered through cotton plug, then re-filtered across a filter paper, volume adjusted to 900 ml and stored in a refrigerator. Furthermore, stability of representative samples of the three extracts was observed at room temperature and after freezing.

pH and viscosity measurements

The pH of the extracts was measured using a pH-meter (Hanna instruments, 8519N, Singapore). The capillary tube method was used for viscosity determination of samples from the different extracts. The flow times in an Ostwald viscometer were then determined at room temperature. The data where then subjected to estimation of relative viscosity using the following calculations:[10]

Relative viscosity = $(\eta_{rel}) = F_1/F_0$

Where F_1 is the flow time of solution and F_0 is the flow time of solvent.

Identification of saponines and nitrates in miswak extracts

It has been reported that some anionic components such as nitrates are naturally occurring in Miswak which are responsible on some of the antimicrobial activity against various species of bacteria.[11] Nitrates can be detected using the following 2 methods:

Method A

To a mixture of 0.1 ml of nitrobenzene and 0.2 ml sulfuric acid, a quantity of 10 mg of Miswak powder was added and allowed to stand for 5 min, cooled on ice, then slowly with stirring the following solvents were added: 5 ml of water, 5 ml of 10M NaOH, and 5 ml of acetone, shaken, and allowed to stand. If the sample contains nitrates, a violet color in the upper layer is produced.

Method B

Dissolve 15 mg of material in 0.5 ml of water, add sulfuric acid, mix and cool. Incline the tube and add 0.5 ml of 0.5M iron sulfate. If the sample contains nitrates, a brown color is produced at the interface of the two liquids. Saponines can be detected by the formation of stable foam after shaking.

Microbiological studies

Preparation of cultures

The antimicrobial activity of the three extracts was tested using various culture media using standard methods. Blood and chocolate agar were described in the literature as best media for selective growth of certain species of streptococci which is known to be one of main inhabitants of the oral cavity, examples of these bacteria are *Streptococcus pyogenes*, *Streptococcus* mutans, and *Streptococcus* faecalis.[12] The blood agar was prepared by allowing 7 g of nutrient agar to soak in a sterile beaker, the mix was then sterilized using autoclave (Prestige Medical, S 2100, UK) for 15 min at 121°C. The mix was allowed to cool at room temperature and then mixed well with 25 ml of blood sheep. The new mix was then poured into Petri dishes, aseptically, and placed in a refrigerator at 2°C–8°C until inoculation. The chocolate agar was prepared in a similar manner, but the formed mix was heated slowly to 50°C to allow for lysis of red blood cells.[13] The bacteria were inoculated from infected teeth into both blood and chocolate agar, and then the media were incubated for 24 h at 37°C in an incubator (Selecta, Germany). Different controls were prepared on frequent basis to check for aseptic conditions of the study.

Activity of Miswak extracts

The various Miswak extracts were tested for their antimicrobial activity by adding small portion of the extracts to cultured media. Chlorhexidine was used as a reference antimicrobial agent. It is considered as the gold standard when testing antimicrobial activity against oral cavity pathogens.[14]

Statistical analysis

ANOVA (one-way) was applied to the data where appropriate. The 0.01 level of significance was used at all times (Excel 8 statistical package, Microsoft, USA).[15]

Results

Rheological studies and pH measurements

Based on the relative viscosity measurements summarized in Table 1, it was evident that the decoction extract has the highest viscosity, followed by infusion and maceration, respectively. It also shows that the behavior of all extracts was a Newtonian system. Such finding was in agreement with the pH measurements. The pH of the extracts varies with the change in the extraction method. The available results, and

Table 1: Relative viscosity and pH measurements of miswak extracts prepared by the different methods

	pH	Viscosity
Decoction	6.34±0.03	1.0856±0.0036
Maceration	4.04±0.01	1.054±0.0110
Infusion	5.87±0.01	1.063±0.0060

n=5. SD: Standard deviation

the small values of the standard deviation, indicate that the decoction method of extraction is capable of extracting more of the constituents of Miswak. The application of one-way ANOVA, at 1% significance level, to the pH and viscosity data resulted in significant difference between the three methods for these two parameters ($P < 0.01$).

A relationship was observed between viscosity and pH of Miswak extract from the three methods. A representative graphical presentation to such relationship is shown in Figure 1.

Stability of Miswak extracts

The stability of the different extracts was monitored throughout this work both in cold and room temperature conditions for a minimum of 3 months. Fungal growth was observed within 1 week for maceration and infusion extracts, while the decoction extract maintained its stability throughout the experimental work in cold or room temperature conditions. Such an observation is in agreement with the literature.[16]

After respective samples of the three extracts were frozen for 24 h, it was observed that the decoction sample maintained its normal state without conversion to the solid state, while the maceration and infusion extracts are converted to the solid state. This is an indication to suppression of freezing point due to the high number of constituents available in the decoction extract. It is well known that the change in the freezing point of aqueous solution is an indication of change in concentration of its constituents.[10]

Presence of saponine and nitrate in Miswak extracts

When representative samples of Miswak extract were shaken in a beaker, the decoction extract produced large, persistent foam, while the foams of the infusion and maceration extract produced small and less persistent foam. This indicates that the saponine content of decoction extract is higher than its content in the other extracts. The deep violet upper layer produced in Method A, and the brown color produced at the interface of the two liquids in Method B indicate that miswak contains nitrates.[17]

Microbiological studies

Confirmation of the presence of streptococcus viridans

The presence of streptococcus viridians (VGS) was confirmed by finding visible colonies in blood agar and green colonies on chocolate agar 48 h after inoculation with the test material.[18]

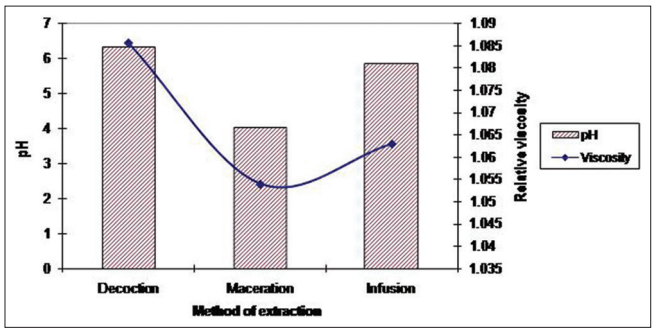

Figure 1: The relationship between the method of extraction of miswak, pH of extract and the relative viscosity

Antimicrobial activity of miswak extracts

A summary to the results obtained by disc plate method to evaluate the efficiency of extraction method is summarized in Table 2. It can be seen that both the maceration and infusion extracts possess no antimicrobial activity. While the decoction extract exhibited some activity, which was less than the reference value of chlorhexidine. The results of this test were not significant enough; consequently, the ditch method was performed.

A summary to the results obtained by ditch plate method to test the efficiency of extraction method is summarized in Table 3. The obtained results were in agreement with the previous experiment using the plate method.

Based on the results, it was concluded that extraction by decoction is the best method to obtain the active constituents of *S. Persica.*

Determination of Miswak minimum inhibitory concentration

The results for the measurement of minimum inhibitory concentration (MIC) using the dilution method are presented in Table 4. It was observed that the increase in Miswak extract concentration in the medium resulted in inhibition of growth. Complete inhibition of bacterial growth was observed at the concentration of 53% v/v when 20% Miswak extract was used.

Discussion

Qualitative studies on the different extracts showed that all three extracts succeeded in the extraction of some of the active principles of Miswak. The results confirmed the presence of saponins and nitrates in all three types of extracts. The viscosity and pH measurements were in good agreement for the three extracts, and a correlation between the two parameters was developed. The viscosity measurements revealed that the highest relative viscosity was achieved by the decoction method and that the behavior of the systems is Newtonian.[10,19] Newtonian behavior usually describes a system which resists the change in viscosity upon application of stress.[20] In addition, the differences in viscosities of the three extracts are a direct indication of differences in concentrations of active constituents.[21] The pH measurements of Miswak extracts by the different used methods revealed that the decoction method produced an extract with the highest pH and the closest to the pH of biological fluids in the oral cavity.[22] As a general rule in pharmaceuticals intended for the oral cavity, they should possess pH close to the pH of saliva.[23] The average pH of saliva is 6.5.[24,25] Thus, the decoction method is primarily favored for direct use in the oral cavity.

Stability of liquid dosage forms is always an important aspect of their design[26,27] and additives such as preservatives and antioxidants are essential to incorporate in such systems to maintain stability.[28] It was found that both maceration and infusion extracts could not prevent the fungal growth after storage in cold or room temperature conditions. The decoction extract was found to be stable in all storage conditions, which

Table 2: Diameters of inhibition zone for different miswak extracts

Disc number	Type of extraction	Diameter of inhibition zone (mm)
1	Maceration	0
2	Infusion	0
3	Decoction	2
4	Chlorohixidine	7

Table 3: Diameter of inhibition zone for different miswak extracts

Ditch number	Type of extraction	Diameter of inhibition zone (mm)
1	Maceration	0
2	Infusion	0
3	Decoction	5
4	Chlorohixidine	10

Table 4: Effect of miswak volume extracted by decoction method in blood agar on microbial growth in blood agar

Petri dish number	Volume of blood-agar added to the petri dish (ml)	Volume of miswak extract added to the petri dish (ml)	Bacterial growth
1	15	0	+++
2	13	2	++
3	11	4	+
4	9	6	+/–
5	7	8	–
6	5	10	–
7	3	12	–
8	1	14	–

implies that the known antifungal activity of Miswak and the components responsible on this action were extracted by a high percentage, and its concentration is present in abundance during extraction and possibly above the MIC.[29] Following freezing of the different extracts, it was observed that suppression in the freezing point has occurred for the decoction extract. Suppression was observed as the liquid state was maintained during freeze storage.[30] This was not the case during infusion or maceration. In these two extracts, the solution converted to the solid state at freezing temperature.

Microbiological studies revealed that the extracts prepared by maceration and infusion could not provide any antimicrobial activity using either disc diffusion method or the ditch method. Moreover, only the extracts prepared with decoction method showed some antimicrobial activity. This is in good agreement with previous studies which showed that the alcoholic/organic solvent extracts are more potent than extracts based on water.[2] Miswak decoction extract at a concentration above 50% of the volume of microbial cultures provided complete inhibition of microbial growth. In addition, this particular extract appears to be self-preserving and preservative-free formulation.[31] These findings also suggest that the active principles of Miswak require heat extraction and these constituents are thermostable. The antimicrobial effect is probably due to the active constituents of Miswak which include chloride, trimethylamine, alkaloid resin, nitrate, thiocyanate, and sulfur compounds.[17,32]

Conclusions

Based on this work, Miswak aqueous extracts were found to exhibit antibacterial activity. This activity will significantly depend on the method of extraction used. The highest activity can be obtained using heat as a mean of extraction of active constituents. These results also indicate that some of the active constituents of Miswak are thermo-stable components. Based on these findings, it is possible to utilize and design a stable mouth wash based on aqueous extraction of Miswak with promising antibacterial activity with possible fewer side effects.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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ملخص المقال باللغة العربية

دراسات حول استخدام الحرارة في الاستخلاص المائي للسواك

المؤلفون

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مقدمة: تم توثيق خصائص السواك (سلفادورا بيرسيكا) المضادة للميكروبات بشكل جيد، كما استخدمت مستخلصاته في صياغة معاجين الأسنان وغسول الفم. اتفقت معظم الأبحاث على أن المستخلصات العضوية للسواك أكثر فعالية من المستخلصات المائية.

الأهداف: الهدف من هذه الدراسة هو تحضير مستخلصات السواك المائية باستخدام ثلاث طرق مختلفة. الفرق بين الطرق هو كمية التسخين المستخدمة في تحضيرها. علاوة على ذلك تم تقييم الثبوتية أثناء تخزين هذه المستخلصات، ودرجة الحموضة، والتأثير المضاد للميكروبات.

المواد والطرق: تم تحضير مستخلصات السواك من خلال طرق النقع، والتسريب، والاستخراج بالإغلاء. تم تقييم كفاءة المستخلصات وثبوتيتها، ودرجة الحموضة واللزوجة، وكذلك النشاط المضاد للميكروبات.

النتائج: وجد ارتباط ما بين الأس الهيدروجيني للمستخلصات ولزوجتها. يزداد الرقم الهيدروجيني للمستخلص إلى 6.5 عند استخدام الحرارة الشديدة أثناء التحضير، وهو قريب من الرقم الهيدروجيني الطبيعي للعاب وتجويف الفم. كانت الزيادة المصاحبة في اللزوجة مؤشرا على زيادة كفاءة المستخرج. أكد قمع نقطة التجمد هذه الملاحظة بالنسبة للمستخلصات المحضرة بواسطة الاستخراج بالإغلاء. لوحظ النشاط الإيجابي الوحيد المضاد للبكتيريا في مستخلص الاستخراج بالإغلاء، لكنه كان أقل فاعلية من الكلورهيكسيدين. لتقدير التركيز المثبط الأدنى للبكتيريا، وجد أن ما يقرب من 50٪ وزن/حجم من المستخلص يجب استخدامها لتوفير الحد الأدنى من التأثير المثبط للميكروبات.

الاستنتاجات: يبدو أن مكونات السواك هي مكونات تتأثر بالحرارة، وطريقة الاستخراج بالإغلاء تنتج مستخلص السواك المستقر والفعال.

الكلمات المفتاحية: النشاط المضاد للميكروبات، الاستخلاص الحراري المائي، المسواك.