Original Article

Preparing and evaluating the anti-microbial effect of *Allium jesdianum* mouthwash on some of the most common oral microorganisms

Fereshteh Golfakhrabadi[1], Donyasadat Mansouri[2], Effat Abbasi Montazeri[3,4], Anayatollah Salimi[5], Fatemeh Babadi[6], Vahid Rakhshan[7]

[1]Department of Pharmacognosy, School of Pharmacy, Medicinal Plant Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, [2]Dental Student School of Dentistry, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, [3]Infectious and Tropical Diseases Research Center, Health Research Instiute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, [4]Department of Microbiology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, [5]Department of Pharmaceutics, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, [6]Department of Oral and Maxillofacial Medicine, School of Dentistry, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, [7]Department of Anatomy and Morphology, Azad University, Tehran, Iran

Abstract

Background: Due to the increasing resistance of bacteria to antibiotics and anti-bacterial compounds in plants, *Allium jesdianum* Boiss plant extract can be used in mouthwash compounds with its anti-microbial activity. **Methods and Materials:** The anti-bacterial and anti-fungal activity of *A. jesdianum* mouthwash was investigated on *Streptococcus mutans*, *Streptococcus sanguis*, *S. salivarius* and *Candida albicans*, and *Candida tropicalis*. To analyse the anti-microbial effect of this mouthwash, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by the broth microdilution method. **Results:** The average MIC and MBC of *A. jesdianum* mouthwash for *S. mutans* were 1.56 and 3.12 (mg/ml), respectively, for *S. salivarius*, 0.25 and 0.65 (mg/ml), and for *S. sanguis*, respectively, 0.25 and 0.65 (mg/ml). The highest MIC and MBC values were for *S. mutans*, and the MIC and MBC values were equal for *S. sanguis* and *S. salivarius*. Average MIC and MBC were determined as 2.41 and 4.16 (mg/ml) for *C. albicans* and 2.34 and 5.72 (mg/ml) for *C. tropicalis*, respectively. MIC values of mouthwash were higher for *C. albicans* and MBC values for *C. tropicalis*. **Conclusion:** Our results showed a promising anti-fungal-anti-bacterial effect of *A. jesdianum* extract. *A. jesdianum* extract may be used as an alternative to chemical mouthwashes.

Keywords: *Allium jesdianum*, anti-bacterial, anti-fungal, microorganism, mouthwash

Introduction

Mouthwashes are the most common solutions used in dentistry to prevent and control infections.[1] Chemical mouthwashes such as chlorhexidine, commonly used in dentistry today, are widely used due to their appropriate anti-bacterial power, relatively long duration of effect, and non-toxicity. Still, they also have various side effects, such as the formation of dental pigments, change in the sense of taste, burning and dryness of the mouth, swelling of the gums and adverse systemic effects if swallowed. However, the bitter taste of nystatin mouthwash and repeated use four times a day, as well as repeated preparation during the period of use, lead to patient dissatisfaction. Therefore, it seems necessary to

Address for correspondence: Dr. Fatemeh Babadi, Department of Oral and Maxillofacial Medicine, School of Dentistry, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
E-mail: babadi.fatemeh@yahoo.com

Received: 23-05-2023 **Revised:** 16-07-2023
Accepted: 21-09-2023 **Published:** 06-03-2024

Access this article online

Quick Response Code:

Website: http://journals.lww.com/JFMPC

DOI: 10.4103/jfmpc.jfmpc_855_23

How to cite this article: Golfakhrabadi F, Mansouri D, Montazeri EA, Salimi A, Babadi F, Rakhshan V. Preparing and evaluating the anti-microbial effect of *Allium jesdianum* mouthwash on some of the most common oral microorganisms. J Family Med Prim Care 2024;13:640-6.

have a mouthwash with a suitable taste, a more straightforward method of service and a practical effect.[2-4]

One of the current problems in treating oral and dental bacterial infections is antibiotic resistance. With the increasing resistance of bacteria to antibiotics, efforts are being made worldwide to replace them with new treatments.[5] Among the promising instances are medicinal plants highly compatible with the human body. Herbal mouthwashes have been shown to have anti-bacterial activity against oral pathogens and also relieve pain without systemic side effects.[6] Medicinal plants play a vital role in treating diseases due to their anti-microbial and anti-fungal activity against pathogens.[7] *Allium jesdianum* is a flowering plant that grows wild, belongs to the Lilaceae family, and is native to Iran. Traditionally, this plant is used to treat abdominal pain, rheumatism, vomiting, kidney stones and colds.[8,9] Recently, the results of investigating the anti-microbial effects of essential oil and aqueous, ethanolic, methanolic, and ether extracts of the *A. jesdianum* plant have shown that the extracts of this plant have anti-bacterial and anti-fungal effects.[7,10-12] Sulphide and terpenoid compounds form the main part of the essential oil of this plant, and the anti-microbial effects of this plant are probably due to the presence of these two compounds.[11,13] Aqueous and methanolic extracts of this plant have an excellent inhibitory effect on bacteria such as *Staphylococcus aureus*, *Streptococcus mutans*, Pseudomonas aeruginosa and *Escherichia coli*.[7] The anti-bacterial effects of *A. jesdianum* can make it one of the most effective ingredients in mouthwashes.

To date, no study has been conducted on preparing and evaluating the anti-bacterial effects of *A. jesdianum* in mouthwash and its impact on oral microorganisms. Therefore, after preparing *A. jesdianum* mouthwash in this study, we investigated its anti-microbial results on some of the most common oral organisms.

Materials and Methods

Ethical considerations

The Medical Ethics Committee of Jundishapur Ahvaz University approved the study (IR.AJUMS.REC.1400.491).

Place and method of collecting *A. jesdianum*

First, library studies were conducted on the distribution of *A. jesdianum* and the research conducted around it. Botanical information and distribution of plants in Iran were prepared with the help of flora books. Then, in the appropriate season, a sufficient amount of plant samples, including aerial parts, were collected from the west of Iran and analysed to identify the scientific genus and species and ensure the selection's correctness. The collected plant was kept at a room temperature of 20-28°C and away from sunlight for further studies.

Preparation of *A. jesdianum* extract

To extract the aerial part of the plant, it was dried at laboratory temperature and then crushed by a mill. To isolate and extract the most effective compounds, the ground plant was extracted three times each time for two days by maceration method with 70% ethanol. The extract was passed through a double-layer muslin cloth. Then, it was sieved using Whatman No. 1 paper and a vacuum pump device (Alwan model, Sparmax manufacturing company, Kimia Teb Tajhiz agency). The extract was concentrated by a rotary evaporator device (model LABROtA4011, Heidolph company, representative of Heidolph) at 40°C. Then, the extract was placed in two ML 300 glass cells and, using a freeze dryer (model FDCF-12006, manufactured by Operon, serial number 20091125-EO2), was dried for 24 hours. The dried powder was stored in a closed container at 4°C.

Strains and culture conditions

This laboratory study was conducted at Jundishapur University of Medical Sciences, Ahvaz (Department of Microbiology, Faculty of Medicine and Department of Pharmacognosy, Faculty of Pharmacy).

Standard strains of bacteria and fungi prepared from the American Type Culture Collection (ATCC) were used. The standard strains of *S. mutans* (PTCC1683), *Streptococcus sanguis* (PTCC1449), *Streptococcus salivarius* (PTCC1448), *Candida albicans* (ATCC 10231), and *Candida tropicalis* (ATCC20336) were prepared as lyophilised vials from the Scientific and Industrial Research Center of Iran. The lyophilised materials were inoculated into the sterile medium Tryticase Sos Broth (Merck, Germany) and incubated for 48 hours at 37°C. After the mentioned time, the bacterial and fungal suspension was cultured on the solid medium of Brain Heart Infusion Broth (Merck, Germany) for 48 hours to create a colony.

Preparation of trypticase soy broth (TSB) culture

According to the manufacturer's instructions, 30 grams of Trypticase soy broth (TSB) powder (Merck, Germany) was completely dissolved in one litre of distilled water; then, it was heated and boiled for one minute until the powder was completely dissolved. After distribution inside the plates, it was autoclaved at 121°C for 15 minutes and finally kept in the refrigerator.

Preparation of Mueller Hinton agar culture

According to the manufacturer's instructions, 38 grams of Moller Hinton agar powder was dissolved in one litre of distilled water. After boiling and dissolving completely, it was sterilised in an autoclave at a temperature of 121°C for 15 minutes. After that, the culture medium was placed in the laboratory temperature to reach 50°C. Then, it was distributed in sterile plates next to the flame.

McFarland standard

A suspension with a concentration equal to 0.5 McFarland units (1.5 × 108 CFU/mL) was prepared using colonies and physiological serum. 1% sulfuric acid (HSO4) and 1.175% barium chloride (BaCl2) stocks were prepared. To prepare 0.5 McFarland (1.5 × 108 ml/ml), 0.05 ml of 1.75% barium chloride was mixed with 9.95 ml of 1% sulfuric acid. The optical absorbance

of the prepared solution at 625 nm wavelength should be between 0.08 and 0.13. The desired standard is stable for one month in the dark and at room temperature, and it was used as a cell suspension standard for subsequent tests and MIC determination.

Preparation of mouthwash

The effective ingredients of *A. jesdianum* mouthwash include *A. jesdianum* extract (the main material), distilled water (carrier and base), glycerin (solvent help), phosphoric acid (pH regulator), 8% ethanol, sodium benzoate (conservator), propylene glycol and sodium saccharin (sweetener). The prepared mouthwash was evaluated regarding physicochemical properties, including pH and viscosity. pH paper (McolorpHast-Merck-1.09535.0001) and pH metre (Seven multi Mettler-Toledo) were used to check pH. According to the instructions on the package, the first colour change of the pH paper indicated pH 4 and acidic conditions; until reaching the desired pH (pH 7), sodium bicarbonate was gradually added to the mouthwash. After reaching the desired pH, the viscosity of the mouthwash was checked with a viscometer (DV-IITPRO model, serial number RTP86028C, BROOKFIELD brand). The viscosity of *A. jesdianum* mouthwash is 4.6% at a torque of 40 RPM and 69 centipoises (CP).

Minimum inhibitory concentration (MIC) measurement

This study used the micro broth dilution method to determine *A. jesdianum* mouthwash. First, a stock solution with a concentration of 200 mg/ml was prepared from mouthwash. Then, using sterile distilled water, serial concentrations of herbal mouthwash were prepared in sterile microplates in wells 1 to 10. In this way, the mouthwash concentration was reduced by 2 to 1.

The bacterial suspension was prepared in a TSB medium of 1.5 × 108 (equivalent to half of McFarland) and diluted 1:100 with a TSB medium (concentration of about 106 CFU/ml). In the next step, 100 microliters of bacterial suspension and 100 microliters of *A. jesdianum* mouthwash were added to each well with twofold dilutions. Double dilutions ranged from 50 to 0.09 mg/ml were obtained, respectively. The final concentration of the bacterial suspension in each well was about 5 × 105 CFU/ml. Also, one well (well no. 11) was prepared as a negative control of inoculation, which has a TSB culture medium without bacteria or fungi and mouthwash. Another well (well no. 12) was also considered a negative control of the treatment, which only contained the suspension of bacteria or fungi, and no mouthwash was added. The microplate was kept at 37C0 for 18-24 hours in the next step. After this time, the lowest concentration of the mouthwash in which bacterial growth was not visually visible in the corresponding well, or in other words, no detectable turbidity was obtained, was considered MIC.

Determining the minimum bactericidal concentration (MBC)

To determine MBC, 20 microliters of the MIC contents well and two wells after that (higher concentration) were cultured on the Mueller Hinton Agar (MHA) plate, and after 24 hours of incubation at 37°C, the number of grown colonies was counted. The lowest concentration in which at least 3log growth reduction compared to the initial concentration (5×10^5 CFU/ml) occurred was considered as MBC.

To ensure the accuracy of the results, each experiment was repeated six times for each microorganism.

Statistical analysis

The Shapiro–Wilk test was used to check the normality of the responses in the groups. Due to the non-establishment of the normality assumption, non-parametric Mann–Whitney and Kruskal–Wallis tests were used to compare MIC and MBC values in cases where the comparison was between two groups, the non-parametric Mann-Whitney test was used. The significance *P*-value was considered <0.05.

Results

To investigate the anti-microbial effect of *A. jesdianum* mouthwash, MIC and MBC were determined by broth microdilution method. MIC and MBC values are reported in Tables 1 and 2, respectively. The average MIC and MBC are shown in Tables 3 and 4, respectively.

Anti-bacterial effect of *A. jesdianum* mouthwash on *S. mutans, S. salivarius* and *S. sanguis* bacteria

The MIC and MBC of *A. jesdianum* mouthwash were determined as 1.56 and 3.12 mg/ml for *S. mutans*, 0.25 and 0.65 mg/ml for *S. salivarius*, and 0.25 and 0.65 mg/ml for *S. sanguis*. The mouthwash's highest MIC and MBC values were for *S. mutans*, and the MIC and MBC values were equal for *S. sanguis* and *S. salivarius*.

To compare the average MIC of *S. mutans*, *S. salivarius* and *S. sanguis,* and because of the non-establishment of the assumption of normality, the non-parametric Kruskal–Wallis test was used. There was a significant difference between the average MIC and MBC of *S. mutans*, *S. salivarius* and *S. sanguis* (sig = 0.001) [Table 5].

Anti-fungal effect of *A. jesdianum* mouthwash on *C. albicans* and *C. tropicalis* fungi

The MIC and MBC of the mouthwash were 2.41 and 4.16 mg/ml for *C. albicans* and 2.34 and 5.72 mg/ml for *C. tropicalis*, respectively. MIC of the mouthwash was higher for *C. albicans* and MBC for *C. tropicalis*.

To compare the average MIC and MBC of *C. albicans* and *C. tropicalis* and due to the non-establishment of the assumption of normality, Mann–Whitney non-parametric test was used. There was no significant difference between the mean MIC (sig = 0.789) and MBC (sig = 0.093) of *C. albicans* and *C. tropicalis* [Table 6].

Table 1: MIC values (mg/ml) for the studied microorganisms

Bacterium strain	Number of tests	Minimum inhibitory concentration [MIC] (mg/ml) of *Allium jesdianum*											
		50	25	12.5	6.25	3.125	1.5625	0.78125	0.390625	0.1953125	0.09765625	Control (-)	Control (+)
		Disk1	Disk2	Disk3	Disk4	Disk5	Disk6	Disk7	Disk8	Disk9	Disk10	Disk11	Disk12
Streptococcus mutans (PTCC 1683)	1	-	-	-	-	-	-	+	+	+	+	-	+
	2	-	-	-	-	-	-	+	+	+	+	-	+
	3	-	-	-	-	-	-	+	+	+	+	-	+
	4	-	-	-	-	-	-	+	+	+	+	-	+
	5	-	-	-	-	-	-	+	+	+	+	-	+
	6	-	-	-	-	-	-	+	+	+	+	-	+
Streptococcus sanguis (PTCC1449)	1	-	-	-	-	-	-	-	-	-	+	-	+
	2	-	-	-	-	-	-	-	-	+	+	-	+
	3	-	-	-	-	-	-	-	-	-	+	-	+
	4	-	-	-	-	-	-	-	-	+	+	-	+
	5	-	-	-	-	-	-	-	-	-	+	-	+
	6	-	-	-	-	-	-	-	-	-	+	-	+
Streptococcus salivarius (PTCC1448)	1	-	-	-	-	-	-	-	-	-	+	-	+
	2	-	-	-	-	-	-	-	-	+	+	-	+
	3	-	-	-	-	-	-	-	-	-	+	-	+
	4	-	-	-	-	-	-	-	-	+	+	-	+
	5	-	-	-	-	-	-	-	-	-	+	-	+
	6	-	-	-	-	-	-	-	-	-	+	-	+
Candida albicans (ATCC10231)	1	-	-	-	-	-	-	+	+	+	+	-	+
	2	-	-	-	-	-	+	+	+	+	+	-	+
	3	-	-	-	-	-	-	+	+	+	+	-	+
	4	-	-	-	-	-	+	+	+	+	+	-	+
	5	-	-	-	-	-	+	+	+	+	+	-	+
	6	-	-	-	-	-	-	+	+	+	+	-	+
Candida tropicalis (ATCC20336)	1	-	-	-	-	-	+	+	+	+	+	-	+
	2	-	-	-	-	-	-	+	+	+	+	-	+
	3	-	-	-	-	-	-	+	+	+	+	-	+
	4	-	-	-	-	-	+	+	+	+	+	-	+
	5	-	-	-	-	-	+	+	+	+	+	-	+
	6	-	-	-	-	-	-	+	+	+	+	-	+

[+] = microorganism growths detected; [-] =microorganism growths undetected; Control (-): disk with no microorganism, just mouthwash; Control (+): disk with no mouthwash, just microorganism

Comparison of the average MIC and MBC of all studied microorganisms

There was a significant difference between the mean MIC of *S. mutans*, *S. salivarius* and *S. sanguis* (sig = 0.000). There was also a difference between the mean MBC of *S. mutans*, *S. salivarius* and *S. sanguis* (sig = 0.000) [Table 7].

Among all studied microorganisms, the highest MIC value was related to *C. albicans*, and the lowest MIC value was associated with *S. sanguis* and *S. salivarius*. However, the highest MBC value was associated with *C. tropicalis*, and the lowest MBC value was related to *S. sanguis* and *S. salivarius*.

Discussion

In recent years, herbal agents have been used in oral care products. There is a lot of clinical evidence for using herbal products in mouthwashes.[14,15] Considering the chemical nature of many types of mouthwash, such as chlorhexidine and various side effects, such as tooth discoloration, change in the sense of taste, burning and dryness of the mouth, gum recession, and adverse systemic effects on swallowing,[16] we conducted this study to introduce a new *A. jesdianum* mouthwash and investigate its anti-fungal and anti-bacterial impact to replace chemical products.

The present study showed that *A. jesdianum* mouthwash influenced the *S. mutans*, *S. sanguis*, *S. salivarius* and *C. albicans*, and *C. tropicalis*. The highest MIC and MBC were obtained in *S. mutans*. Also, the highest MIC and MBC were related to *C. albicans* and *C. tropicalis*, respectively. As a result, our study's most significant effect of mouthwash was related to *S. sanguis* and *S. salivarius*, and a minor effect was observed on *S. mutans*. There was no significant difference in the effect of mouthwash on *C. albicans* and *C. tropicalis*. Due to the novelty of *A. jesdianum* mouthwash and the fact that no other research has been conducted on this mouthwash, we inevitably compare the findings of the present study with the literature conducted on *A. jesdianum* plant extracts and essential oils.

The anti-microbial effects of *A. jesdianum* are probably due to its presence of sulphide and terpenoid compounds.[17] Terpenoids,

Table 2: MBC values (mg/ml) for the studied microorganisms

Bacterium strain	Number of tests	Minimum Bactericidal concentration (MBC) OF Allium Jesdianum
Streptococcus Mutans (PTCC 1683)	1	3.125
	2	3.125
	3	3.125
	4	3.125
	5	3.125
	6	3.125
Streptococcus Sanguinis (PTCC 1449)	1	0.78125
	2	0.78125
	3	0.78125
	4	0.78125
	5	0.390625
	6	0.390625
Streptococcus Salivarius (PTCC 1448)	1	0.78125
	2	0.78125
	3	0.78125
	4	0.78125
	5	0.390625
	6	0.390625
Candida Albicans (ATCC 10231)	1	3.125
	2	3.125
	3	3.125
	4	6.25
	5	6.25
	6	3.125
Candida Tropicalis (ATCC 20336)	1	6.25
	2	6.25
	3	6.25
	4	6.25
	5	6.25
	6	3.125

Table 3: Average MIC values

Groups	*n*	Mean±SD of MIC	Minimum	Maximum
Streptococcus mutans	6	1.56200±0.000000	1.562	1.562
Streptococcus sanguis	6	0.25667±0.103280	0.190	0.390
Streptococcus salivarius	6	0.25667±0.103280	0.190	0.390
Candida albicans	6	2.41633±0.949381	1.562	3.125
Candida tropicalis	6	2.34350±0.856090	1.562	3.125

alkaloids and phenolic compounds are components that can react with enzymes and proteins of the microbial membrane and disrupt the path of protons to the outside of the cell, leading to cell death or inhibiting the enzymes needed for the synthesis of amino acids.[18] In addition, the inhibitory effects of plant extracts can be related to their hydrophobic properties, which lead to destruction and changes in their permeability by reacting with cell membrane proteins and mitochondria.[19,20] The presence of bioactive compounds with antioxidant and anti-microbial activity in the extract of the *A. jesdianum* plant has been confirmed in the study of Hojjati. Based on these results, the high antioxidant activity of aqueous and methanol extracts of *A. jesdianum* indicates the potential use of this plant extract as a natural antioxidant. This plant extract can destroy or prevent the growth of pathogenic microorganisms. Therefore, the bioactive extract of the *A. jesdianum* plant can be used as a natural preservative and alternative to chemical antibiotics to prevent the growth of harmful microorganisms, treat infectious diseases and increase the safety of food products.[21] Similar to our study, in Gholami *et al*.'s study on the effect of methanolic and aqueous extracts of the *A. jesdianum* plant on several pathogenic bacteria, the anti-bacterial effect of these extracts on *S. mutans* bacteria has been confirmed. However, it was shown that methanolic and aqueous extracts of *A. jesdianum* affected all Gram-positive and Gram-negative bacteria except *Enterococcus faecalis*.[7] Unlike Amiri's study, the aqueous extract showed a better effect than the methanolic extract because it inhibited the growth of bacteria at a lower MIC. Differences in results can be due to the type of bacterial species and differences in the kind of compounds available in various extraction methods.

In the past, studies have been conducted on the anti-fungal activity of *A. jesdianum* plant extract and its effect on *C. albicans*, which was also proven in the present study. In the study by Naeini *et al*., the hydroalcoholic extract of *A. jesdianum* inhibited the growth of *C. albicans* and the effectiveness of its aqueous extract on the survival of macrophages and the production of nitric oxide (NO) by macrophages was evaluated. Similar to our results in this study, the anti-fungal activity of *A. jesdianum* plant extract against *C. albicans* was confirmed. This extract inhibited the growth of *C. albicans* and also killed it.[22] The results of Shahrokh *et al*.'s study also confirm the anti-fungal effect of *A. jesdianum* extract on all vaginal isolates sensitive and resistant to fluconazole of Candida glabrata.[12] In general, the most medicinal effects of *A. jesdianum* have been attributed to organic sulfur compounds.[10] These compounds reduced the expression of the HWP1 gene, which leads to the inhibition of biofilm formation by *C. albicans* and justifies the inhibitory effects of the hydroalcoholic extract of *Allium iesdianum* on *C. albicans*.[23,24]

Considering that *Allium iesdianum* mouthwash was produced for the first time in this study, it was only investigated in a laboratory environment. Its effect in the oral environment may differ due to the presence of saliva and related factors. Therefore, it seems necessary to conduct clinical studies to confirm the results of this study.

However, in the present study, only the anti-microbial effects of *A. jesdianum* mouthwash were investigated. Other characteristics of the mouthwash, such as taste and smell, the ability to change the colour of the teeth, cause burning and dryness in the mouth, its acceptability among consumers, expiration date and the duration of effectiveness, etc., have not been investigated, for this reason, it is recommended to conduct other studies to investigate these cases.

Conclusion

A. jesdianum mouthwash prevents the growth of *S. sanguis*, *S. salivarius*, *C. albicans* and *C. tropicalis* and has a minor effect

Table 4: Average MBC values

Groups	*n*	Mean±SD of MBC	Minimum	Maximum
Streptococcus mutans	6	3.12500±0.000000	3.125	3.125
Streptococcus sanguis	6	0.65000±0.201395	0.390	0.780
Streptococcus salivarius	6	0.65000±0.201395	0.390	0.780
Candida albicans	6	4.16667±1.613743	3.125	6.250
Candida tropicalis	6	5.72917±1.275776	3.125	6.250

Table 5: The results of the Kruskal-Wallis test to compare the average MIC and MBC of *Streptococcus mutans*, *Streptococcus salivarius* and *Streptococcus sanguis*

Kruskal-Wallis test statistic		
y. MBC	**y. MIC**	**Chi-square**
13.114	13.114	Z
2	2	df
		Asymp. Sig

Table 6: The results of the Mann-Whitney test to compare the average MIC and MBC of *Candida albicans* and *Candida tropicalis*

Mann-Whitney test statistic		
y. MBC	**y. MIC**	**Chi-square**
-1.682	-0.267	Z
0.093	0.789	Asymp. Sig (2-tailed)

Table 7: The results of the Kruskal-Wallis test to compare the average MIC and MBC of all studied microorganisms

Kruskal-Wallis test statistic		
y. MBC	**y. MIC**	**Chi-square**
25.183	24.103	
4	4	df
0.000	0.000	Asymp. Sig

on *S. mutans*. *A. jesdianum* showed suitable anti-bacterial and anti-fungal activities, which is recommended as an alternative mouthwash and combination therapy for treating oral and dental infections.

Key messages and recommendations

The present study showed the anti-fungal and anti-bacterial potential of *A. jesdianum* mouthwash against the growth of *S. mutans*, *S. sanguis*, *S. salivarius* and *C. albicans*, and *C. tropicalis*. We recommend using this mouthwash to maintain oral and dental hygiene.

Acknowledgement

The authors thank the Vice-Chancellor of Research and Medicinal Plant Research Centre, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Authors' contribution

Fereshteh Golfakhrabadi: Methodology, Data curation, Formal analysis, Effat Abbasi Montazeri: Methodology, Data curation. Fatemeh Babadi: Methodology, Data curation, Formal analysis, Writing – original draft. Donyasadat Mansouri: Methodology, Data curation, Formal analysis, Writing – original draft. Anayatollah Salimi: Methodology, Writing – original draft. Vahid Rakhshan: Data analysis.

Ethical considerations

This study was approved by the ethics committee of Ahvaz Jundishapur University of Medical Sciences (code: IR. AJUMS. REC.1400.491).

Financial support and sponsorship

The current research was supported by Grant (No. MPRC-0023), Research Vice-Chancellor and Medicinal plant research centre, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Conflicts of interest

There are no conflicts of interest.

References

1. Talebi S, Sabokbar A, Riazipour M, Saffari M. Comparison of the *in vitro* effect of chemical and herbal mouthwashes on Candida albicans. Jundishapur J Microbiol 2014;7.
2. Newman M, Takei H, Klokkevold P, Carranza F. Clinical features of gingivitis. Clinical Periodontology. 10th ed. Philadelphia: WB Saunders; 2006. p. 362.
3. Belting CM, Haberfelde GC, Juhl LK. Spread of organisms from dental air rotor. J Am Dent Assoc 1964;68:648-51.
4. Atai Z, Ansari M, Mousavi A, Mirzaei A. In-vitro study of antifungal effects of selected herbal extracts on standard and wild strains of Candida albicans. J Iranian Dent Assoc 2007;19:91-7.
5. Talei GR, Meshkatalsadat MH, Mosavi SZ. Antibacterial activity native medicinal plants extracts in Lorestan, Iran. J Gorgan Univ Med Sci 2008;10:31-5.
6. Banu JN, Gayathri V. Preparation of antibacterial herbal mouthwash against oral pathogens. Int J Curr Microbiol App Sci 2016;5:205-21.
7. Gholami A, Arabestani MR, Ahmadi M. Evaluation of antibacterial activity of aqueous and methanol extracts of *Allium jesdianum* plant on a number of pathogenic bacteria resistant to antibiotics. Pajouhan Sci J 2016;14:18-26.
8. Zarrei M, Wilkin P, Ingrouille MJ, Chase MW. Gagea robusta (Liliaceae), a new species from Flora Iranica area. Kew Bulletin 2010;65:327-36.
9. Shafizadeh F. Medicinal Plants of Lorestan province. Tehran: Hayian Publisher; 2002.
10. Ghasemi Pirbalouti A. Phytochemical and bioactivity diversity in the extracts from bulbs and leaves of different populations of *Allium jesdianum*, a valuable underutilized vegetable. Acta Sci. Pol. Hortorum Cultus 2019;18.
11. Amiri H. Chemical composition and antibacterial activity of the essential oil of *Allium jesdianum* Boiss. & Buhse from Iran. J Med Plants 2007;6:39-44.

12. Shahrokh S, Vahedi G, Khosravi A-R, Mahzounieh M, Ebrahimi A, Sharifzadeh A, *et al. In vitro* antifungal activity of aqueous-ethanolic extract of *Allium jesdianum* against fluconazole-susceptible and-resistant human vaginal Candida glabrata isolates. J Herbmed Pharmacol 2017;6:165-70.
13. Petropoulos SA, Di Gioia F, Polyzos N, Tzortzakis N. Natural antioxidants, health effects and bioactive properties of wild Allium species. Curr Pharm Design 2020;26:1816-37.
14. Tafazoli A, Moghadam ET. Camellia sinensis mouthwashes in oral care: A systematic review. J Dent 2020;21:249.
15. Amin M, Babadi F, Baghipour N, Sadeghi-Nejad B. Evaluation of the effect of Jaftex herbal mouthwash on the growth of Candida albicans and Candida tropicalis. J Family Med Prim Care 2021;10:3815.
16. Guerra F, Pasqualotto D, Rinaldo F, Mazur M, Corridore D, Nofroni I, *et al.* Therapeutic efficacy of chlorhexidine-based mouthwashes and its adverse events: Performance-related evaluation of mouthwashes added with Anti-Discoloration System and cetylpyridinium chloride. Int J Dent Hyg 2019;17:229-36.
17. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999;12:564-82.
18. Low WL, Kenward K, Britland ST, Amin MC, Martin C. Essential oils and metal ions as alternative antimicrobial agents: A focus on tea tree oil and silver. Int Wound J 2017;14:369-84.
19. Möller MN, Cuevasanta E, Orrico F, Lopez AC, Thomson L, Denicola A. Diffusion and transport of reactive species across cell membranes. Bioactive Lipids Health Dis 2019:3-19.
20. Perez MJ, Briz O. Bile-acid-induced cell injury and protection. World J Gastroenterol 2009;15:1677.
21. Hojjati M, Alizadeh Behbahani B. Evaluation of the effect of aqueous and methanolic extraction methods on the antioxidant and antimicrobial characteristics of *Allium jesdianum* extract: *In vitro* study. Iranian Food Sci Technol Res J 2021;17:83-91.
22. Naeini A, Yaraee R, Shokri H. Antifungal and immunomodulatory activity of *Allium jesdianum* Boiss extracts. J Herbmed Pharmacol 2019;9:75-80.
23. Mikaili P, Maadirad S, Moloudizargari M, Aghajanshakeri S, Sarahroodi S. Therapeutic uses and pharmacological properties of garlic, shallot, and their biologically active compounds. Iran J Basic Med Sci 2013;16:1031.
24. Khodavandi A, Alizadeh F, Namvar F, Rosfarizan M, Chong P. Anti-candida potential of allium ascalonicum Linn: Antibiofilm activity and biomolecular mechanism of action. J Pure Appl Microbiol 2014;8:349-56.