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Comparison of the *in vitro* Effect of Chemical and Herbal Mouthwashes on *Candida albicans*

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Background: During the recent decades research has focused to find scientific evidence for the effects of herbal medicines. Researchers are interested in herbal remedies for medication and aim to substitute herbal material instead of chemical formula with limited side effects for human being.
Objectives: The aim of the current study was to compare the *in vitro* effect of herbal and chemical mouthwashes against *Candida albicans*.
Materials and Methods: In this research, we used a standard strain of *C. albicans*, PTCC 5027. The suspension was made by a fresh culture of *C. albicans* (24 hours) and the optical density (turbidity equating to a McFarland standard of 0.5) was read at 530 nm. The *C. albicans* suspension was cultured on Sabouraud dextrose agar plate. Next, two wells were filled with mouthwashes and after incubation at 30°C for 24 hours, the inhibition zone was measured. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of mouthwashes were determined. Data were analyzed using the SPSS software, independent T-tests and one-sided variance analysis (ANOVA-one way).
Results: Based on these findings on agar diffusion with (P = 0.764), MIC and MFC tests (P = 0.879), there were no significant differences between the antifungal effect of herbal and chemical mouthwashes.
Conclusions: This study showed that, chemical mouthwashes acted better than herbal mouthwashes and among different chemical mouthwashes, Oral B was most effective.

Keywords: Mouthwash; *Candida albicans*; Minimum Inhibitory Concentration

1. Background

Candida albicans is considered as part of the normal flora of the mouth, which doesn't cause disease but under unfavorable local conditions, such as poor oral hygiene (1), wearing of denture (2) and general predisposing factors such as high carbohydrate diet, smoking (3), diabetes (4) malnutrition, leukemia, chemotherapy, genetic disorders (5), long term antibiotic therapy (6), corticosteroid therapy (7), senility (8), radiotherapy (9) and cancer (10)can cause candidiasis. Using mouthwashes is a common way to controlling the *C. albicans* population in the mouth, which are used widely in dentistry (11, 12). Mouthwashes have been recommended for the prevention and control of oral diseases, especially for the control of oral microorganisms (13). They usually contain water and some active components such as antibiotics, antifungal and anti-inflammatory substances. Some mouthwashes have been found to enhance the removal process and elimination of microorganisms (14).

Nowadays, different types of medicine have been used for the treatment of oral problems. Most of them are chemical and have many side effects; consequently researchers are interested in herbal remedies for medication and aim to substitute herbal material instead of chemical formula with limited side effects for human beings (15). Different mouthwashes are available in the Iranian market but there is no information about their efficiency differences. Among different herbal and chemical mouthwashes, which are available in the Iranian market (Table 1), chlorhexidine and persicac have been more considered. Chlorhexidine was introduced as an antiseptic agent with activity against different organisms such as bacteria, viruses and different types of fungi including *C. albicans,* which causes oral candidiasis (16). Different studies on chlorhexidine have demonstrated its potential to prevent oral complications, such as the occurrence of chronic or opportunistic infections (17).

In order to overcome the effects of chemical drugs, the World Health Organization (WHO) has advised researchers to investigate the possible use of natural products such as herb and plant extracts (18). One of the agents considered as an alternative to chlorhexidine is a plant called *Salvadora persica* or "Miswak". *S. persica* herbal

mouthwash contains three medicinal plants, *S. persica*, Yarrow and Mint. Furthermore, WHO encourages the use of chewing *S. persica* sticks (Miswak) as an effective oral hygiene procedure (19, 20). *S. persica*, a very popular plant in the Middle East, contains a number of identified antimicrobial and other prophylactic components including volatile oils, flavonoids, alkaloids, steroids, terpenoids, saponins and carbohydrates (18, 21).

Currently, a herbal drug is defined as a remedy derived from plants (roots, leaves, flowers, fruits, seeds) and other natural sources which can be used for therapeutic purposes and its active chemical constituents provide the basis for pharmaceutical synthesis (21). Different studies have indicated that, using a mouthwash in patients with systemic infection could decrease fungal and bacterial colonization in the mouth cavity. McCourtie et al. (22) investigated the effect of chlorohexidinegluconate on *Candida* adhesion to acrylic denture and concluded that fungal colonization decreases by using chlorohexidine. A study by Pizzo et al. (23) indicated a considerable decrease in the colonization of adhesive *Candida* to epithelial cells in individuals using chlorohexidine. Nayak et al. (24) investigated the effect of chlorohexidine on dental plaque and concluded that chlorohexidine is an effective agent in decreasing dental plaque and colonization of microorganisms in plaque.

Among mouthwashes, most researches have focused on chlorhexidine against *C. albicans* and there is a lack of complete information about other mouthwashes especially herbal ones. The current study tried to evaluate the effect of the anticandidial activity of different mouthwashes, which are used in the Iranian market and attempted to find the most effective mouthwash for patients who are susceptible for candidiasis.

2. Objectives

The purpose of the current research was to compare the effect of chemical and herbal mouthwashes on *C. albicans*.

3. Materials and Methods

3.1. Strain of C. albicans

In this study, a standard strain of *C. albicans* was used, NO. PTCC 5027.The standard strain was cultivated in Sabouraud dextrose agar, SDA (Merck, Germany) and placed at 30 °C for 24 hours until activated.

3.2. Mouthwashes

Different types of mouthwashes were used for this study. Chemical mouthwashes such as Vi-one (Rojn Cosmetic Lab Co, Tabriz, Iran), Fluorine (World Health Laboratories Co, Tehran, Iran), Hexodine (World Health Laboratories Co, Tehran, Iran), Oral B (Grossgerau Co, Hessen, German), Sensodyne (GSK Co, London, UK), Foramen (Guarnizo Co, Cantabria, Spain), Epimax (Emad Pharmaceutical Co, Esfahan, Iran), Chlorohexidine (Shahre Daru Laboratories Co, Tehran, Iran), Fluoride (Shahre Daru Laboratories Co, Tehran, Iran), anti-septic Irsha (Shafa Cosmetic Laboratories Co, Tehran, Iran), anti-plaque Irsha (Shafa Cosmetic Laboratories Co, Tehran, Iran), Benzydamine (Behvazan Co, Rasht, Iran), Colgate (Kucukyali Co, Estanbol, Turkey), and herbal mouthwashes such as Matrica (Barij Essence Pharmaceutical Co, Kashan, Iran), *S. persica* (Porsina Pharmaceutical Co, Tehran, Iran), and Corpore Sano (Disna. SA Co, Barcelona, Spain) were used. The compositions of all of the mouthwashes are provided in Table 1.

3.3. Agar Diffusion

A suspension was made by physiological saline (Samen, Iran) and fresh culture of *C. albicans* (24 hours) and the OD (with turbidity equating to a McFarland standard of 0.5) was read at 530 nm wave length by a spectrophotometer (Pars Teb Novin, Iran), in which there were 2.5×10^6 CFU/mL colonies in each milliliter (optical density at 530 nm, 0.12). Next, 10 μL of the suspension was transferred to SDA medium and two wells were made with suitable distance in culture medium, filling with mouthwashes. To decrease error, the test was repeated 4 times. Plates were placed in the incubator (Behdad Medical Production, Iran) at 30 °C for 24 hours (25). Inhibition zone was measured and recorded using a Collis (Kiya Sanat Khavaran, Iran) (26). For each mouthwash, one control plate containing mouthwash and sterilized distilled water was prepared.

3.4. MIC Measurement

To determine the minimum inhibitory concentration for each mouthwash, a serial dilution was prepared according to the CLSI protocol (27). The lowest concentration of mouthwash that prevented turbidity (growth) of *C. albicans* was considered as the minimum inhibitory concentration. This method was conducted based on turbidity clearance.

3.5. Minimum Fungicidal Concentration (MFC) Measurement

To determine the minimum fungicidal concentration of fungus, 10 μL of specimens were taken from the MIC (last clear tube) and two last tubes were poured in SDA, incubating at 30 °C for 24 hours. The lowest concentration of mouthwash that prevented the growth of *C. albicans* determined by sub-culturing of the last clear MIC tube on SDA and refers to fungicidal activity. This procedure was conducted to allow more accuracy and control error. Colony counts of less than four, indicated no growth while more than four indicated growth (27).

3.6. Statistical Analysis

The data were analyzed with the SPSS software (18^{th} edition), using independent T-test and one-sided variance analysis (one way ANOVA) with $P < 0.05$.

Table 1. Ingredients of Various Mouthwashes Tested For Anticandidial Potential

Name	Batch No.	Manufacture Date	Expiry Date	Ingredients as Listed on Packages
Oral B	KT 13	October 2010	August 2013	Aqua, glycerin, polysorbate 20, aroma, methylparaben, cetylpyridinium, chloride, sodium fluoride, sodium benzoate, propylparaben, CI42051, CI 47005.
Sensodyne	1241033	August 2011	August 2013	Sodium flouride.
Vi-one	2231	July 2012	July 2014	Deionized water, sorbitol,glycerine, ploxamer 407, polyethylene glycol, perment ethanol, menthol, sodium methyl paraben, aspartame, sodium propyl paraben, citric acid, sodium fluoride 0.05%, cetylpyridinium chloride 0.05%, CIN 42090.
Fluorine	90-F1-O1	April 2011	April 2013	Sodium flouride, cetylpyridinium chloride, zinc ions, glycerin, sorbitol, sodium fluoride.
Hexodine	C1-03	July 2012	July 2014	Aqua, glycerin, CI 42090.
Epimax	2011 EPM-11	December 2011	December 2013	Sorbitol, propylene glycol, tetra sodium pyrophosphate, citric acid, polysorbate 20, polysorbate60, chlorhexidinedigluconate, sorbic acid, menthol, sodium fluoride, sodium saccharine, dye.
Chlorhexidine	CL-06	March 2012	March 2014	Sorbitol. flavour, PEG 40 hydrogenated castor oil, alcohol, CI 16035.
Anti-Plaque Irsha	9058	December 2011	December 2014	Alcohol, glycerin, synperonic, TSPP, SLS, benzoic acid, allantoin, PVM/MA, benzoate, fluoride 0.05%, saccharin, CI 42090, CI 19140.
Anti-Septic Irsha	9022	February 2012	February 2015	Alcohol, synperonic, benzoic acid, benzoate, okaliptol, tymol, methy salicylate, mentol, aqua.
Benzydamine	BH. 89.16	December 2010	December 2013	Hydrochloride 0.15%.
Colgate	2069 CHG11B	August 2010	August 2013	Aqua, glycerin, alcohol, sorbitol, propylene glycol, polysorbate20, sodium benzoate, aroma, PVM/MA copolymer, zinc citrate, CI 42090, sodium fluoride, tetrasodium pyrophosphate, tetrapotassium pyrophosphate, sodium saccharin.
Fluoride	14	December 2011	December 2014	Sodium fluoride 0.2%.
Foramen	Ref.015	February 2012	February 2016	Sodium fluoride 0.05%, triclosan, aqua, sorbitol, glycerin, PEG-40, castoroil, aroma, cinnamal, sodium propyl paraben, sodium benzoate, sodium saccharin, CI 42090.
M.chamomilla	310038	December 2011	December 2013	Matricaria chamomilla
S. persica	09	September 2011	September 2013	Salvadora persica, Mentha spicata, Achilleamille folium.
Corpore Sano	G0019	March 2012	March 2014	Aqua, alcohol, PEG-40, hydrogenated castor oil, aroma, geraniol, sodium benzoate, sodium saccharin, Propolis extract, ethylparaben, propylparaben, methanol, citric acids, CI 19140, CI 42090.

4. Results

The mean diameter of inhibition zone by herbal and chemical mouthwashes has been indicated in Tables 2 and 3. The comparison of inhibition zone diameter by using chemical and herbal mouthwashes has been indicated in Figure 1. Oral B and *S. persica*, mouthwashes showed the highest and lowest anti-candidial activity against *C. albicans* in SDA, according to the inhibition zone diameter.

Amount of MIC was determined by the tube dilution test, with 10 dilution tubes. The point at which growth of *C. albicans* was inhibited was recognized as the minimum inhibitory concentration. Statistical analysis of the MIC and MFC amounts of chemical and herbal mouthwashes are shown in Tables 4 and 5. Comparison of the amounts of MIC and MFC by using chemical and herbal mouthwashes are indicated in Figure 2. Colgate and anti-plaque Irsha mouthwashes showed the highest amount of MIC and MFC against *C. albicans* (Figure 3) and Vi-one mouthwash showed the lowest amount of MIC and MFC (Figure 4). The results of the MFC were similar to MIC (Tables 4 and 5).

Table 2. Statistical Analysis, Mean Diameter and Standard Deviation of Inhibition Zone Exhibited by Chemical Mouthwashes Against *C. albicans* [a,b]

Types of Mouthwashes	Mean (mm)	SD (mm)	P value From One Way ANONA Test	Control P value
Oral B	23.25	0.65		
Sensodyne	19.87	1.32		
Vi-one	19.43	0.81		
Fluorine	18.46	1.84		
Hexodine	16.65	0.85		
Epimax	14.37	0.79	P < 0.001	P < 0.05
Chlorhexidine	14.21	0.45		
Anti-Plaque Irsha	R	R		
Anti-Septic Irsha	R	R		
Benzydamine	R	R		
Colgate	R	R		
Fluoride	R	R		
Foramen	R	R		

[a] R. means *C. albicans* resistance to these mouthwashes.
[b] The units of mean and standard deviation of inhibition zone are in millimeter (mm).

Table 3. Statistical Analysis, Mean Diameter and Standard Deviation of Inhibition Zone Exhibited by Herbal Mouthwashes Against *C. albicans* [a,b]

Types of Mouthwashes	Mean (mm)	SD (mm)	P value From One Way ANONA Test	Control P value
M.chamomilla	16.37	0.74		
S. persica	10.93	0.77	P < 0.001	P < 0.05
Corporesano	R	R		

[a] R. means *C. albicans* resistance to these mouthwashes.
[b] The units of mean and standard deviation of inhibition zone are in millimeters (mm).

Table 4. Statistical Analysis of Minimum Inhibitory Concentration and Minimum Fungicidal Concentration Exhibited by Chemical Mouthwashes Against *C. albicans* [a]

Types of Mouthwashes	MIC and MFC (mg/L)	P value From One Way ANONA Test	Control P value
Anti-Plaque Irsha	0.250		
Colgate	0.250		
Anti-Septic Irsha	0.125		
Benzydamine	0.125		
Foramen	0.062		
Fluoride	0.062	P < 0.001	P < 0.05
Epimax	0.031		
Hexodine	0.019		
Chlorhexidine	0.015		
Fluorine	0.007		
Sensodyne	0.007		
Oral B	0.003		
Vi-one	0.001		

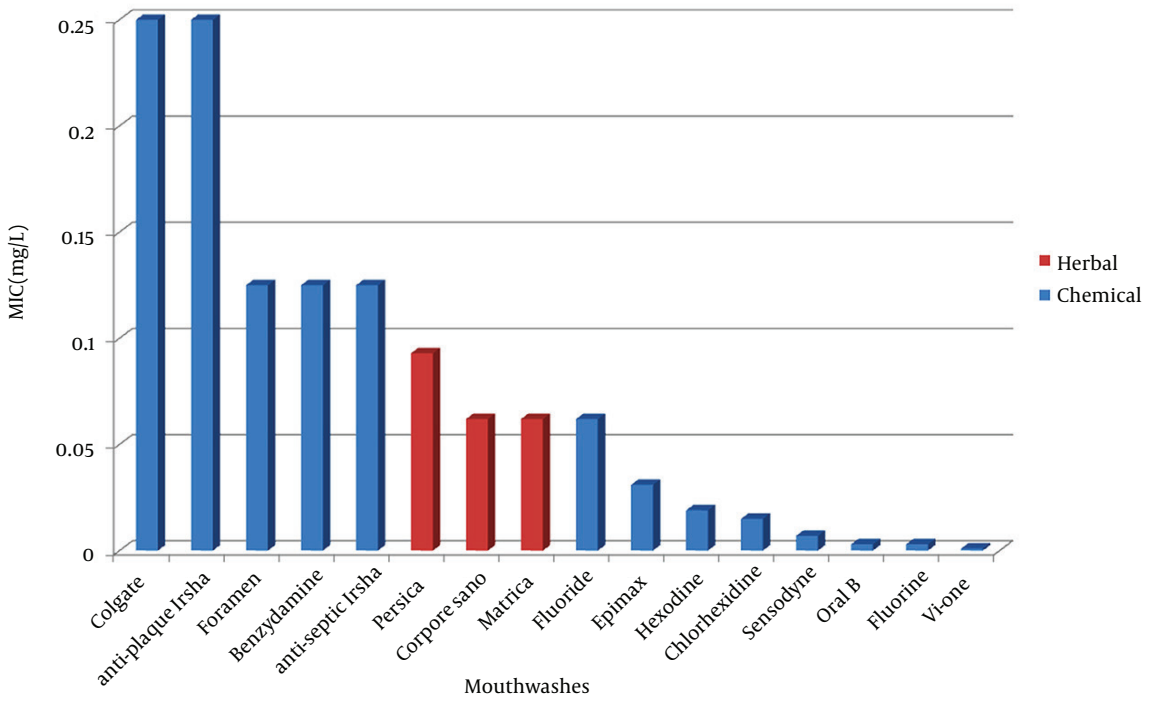
[a] The unit of MIC is in mg/L.

Table 5. Statistical Analysis of Minimum Inhibitory Concentration and Minimum Fungicidal Concentration Exhibited by Herbal Mouthwashes Against *C. albicans* [a]

Types of Mouthwashes	MIC and MFC (mg/L)	P value From One Way ANONA Test	Control P value
S. persica	0.093		
***M. chamomilla* Matrica**	0.062	P = 0.465	P < 0.05
Corporesano	0.062		

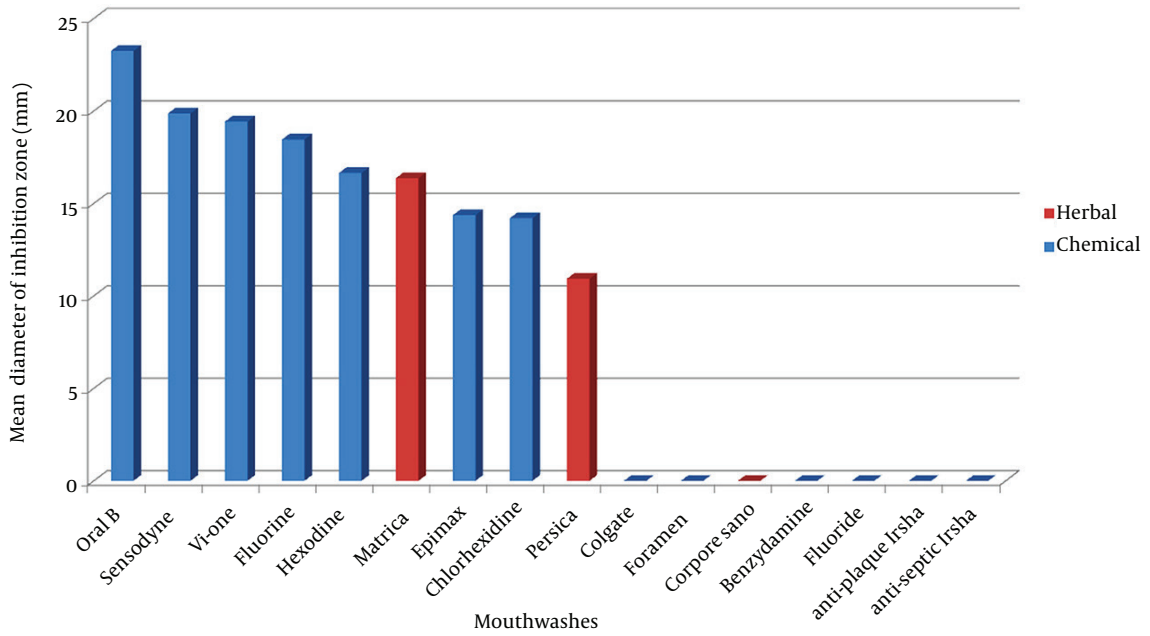
[a] The unit of MIC is in mg/L.

Figure 1. Comparison of Mean of Inhibition Zone Diameter of Chemical and Herbal Mouthwashes Against *C. albicans* by the Agar Diffusion Method



The unit of mean of inhibition zone is in millimeters (mm).

Figure 2. Comparison of the Minimum Inhibitory Concentration and Minimum Fungicidal Concentration of Chemical and Herbal Mouthwashes Against *C. albicans*

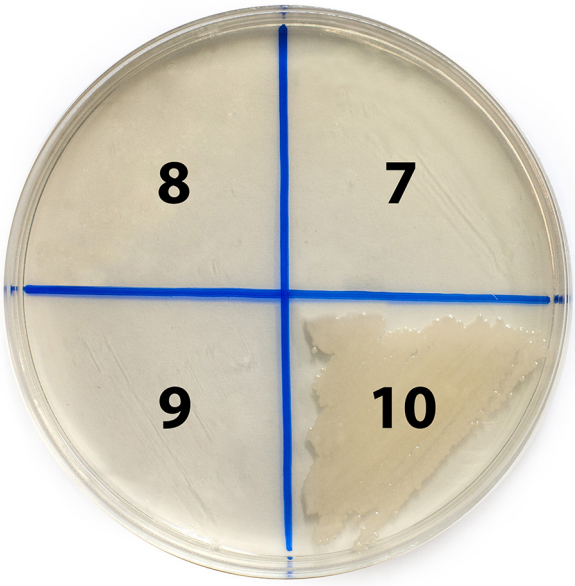


The unit of MIC is in mg/L.

Figure 3. Minimum Fungicidal Concentration of Colgate Mouthwash

The numbers are based on the numbers of the MIC tubes. The second zone indicates the minimum fungicidal concentrations of Colgate mouthwash against *C. albicans*.

Figure 4. Minimum Fungicidal Concentration of Vi-one Mouthwash



The numbers are based on the numbers of the MIC tubes. The ninth zone indicated the minimum fungicidal concentrations of Vi-one mouthwash against C. *albicans*.

5. Discussion

The use of antiseptic mouthwashes as a component of a complete oral hygiene regimen. Mouthwashes are a convenient and accepted method of oral hygiene (28). In the current study, *C. albicans* indicated the most and the least sensitivity to Oral B and *S. persica*, respectively. Among different mouthwashes, *C. albicans* was resistant against fluoride, benzydamine, Colgate, Foramen, anti-septic Irsha and anti-plaque Irsha.

Giuliana et al. (29) investigated the *in vitro* antifungal effect of mouthwashes containing antimicrobial factor against *Candida* species and indicated that, chlorohexidine has antifungal properties and is effective against *C. albicans*. Also, in the current study, chlorohexidine was indicated to be effective against *C. albicans*, but in comparison with other mouthwashes, it showed a lower effect. Bajaj and Tandon (30) evaluated the effect of chlorohexidine on dental plaque, gum inflammation and microbial growth and indicated that microbial growth was decreased after the application of this substance. The current study investigated the effect of chlorohexidine against *C. albicans* and indicated that it has a lower effect in comparison with other mouthwashes, especially Oral B.

Meiller et al. (31) investigated the antifungal effect of 0.2% chlorohexidine against *C. albicans*. There is less information about chlorohexidine and its effect on *C. albicans* in comparison with the other mouthwashes. Chlorhexidine (CHX) is the most active agent, due to its wide spectrum of antimicrobial activity against a wide variety of organisms, including *C. albicans* (17). The mode of action of this substance is not entirely understood, but it is known that it acts as a fungicide and has a fungistatic function, leading to the coagulation of nucleoproteins and changes in cell walls allowing the possible escape of cytoplasmic components through the plasmalemma (17, 32). However, for oral use as a mouthwash, chlorhexidine has been reported to have a number of side effects, such as unpleasant taste, staining of teeth and tongue, gingival desquamation, taste disturbance and painful mucosa (18, 33). Chlorhexidine is not the first choice of drug for the treatment of *Candida* infections. However, the increase in the number of opportunistic infections caused by fungi, mainly in HIV infected individuals, and the great number of strains that have become resistant to the common antifungals has encouraged new research in relation to alternative treatments of such infections, among which is the use of chlorhexidine (32).

Among herbal mouthwashes examined by the agar diffusion method, Matrica and *S. persica* had the most and the least effect on *C. albicans,* respectively and Corpore Sano didn't have any effect on *C. albicans*. Almas (34) investigated the antibacterial effect of *S. persica* extract (50%) in Persica mouthwash and chlorohexidine against oral microbes and *C. albicans* and concluded that, chlorohexidine mouthwash has more antimicrobial effect in comparison with extract of toothbrush plant and Persica has less antibacterial activity in comparison with chlorohexidine. It is important to note that Persica couldn't change the oral microbial and *C. albicans* level. In the current study, the results were similar to previous studies

and Persica indicated less activity than chlorohexidine and Matrica against *C. albicans* with an average inhibition zone diameter of 10.93 mm and there was a significant difference between Persica and chlorohexidine anticandidial activity as indicated by the Tukey-HSD test ($P < 0.001$).

Alali and Al-Lafi (35) reported that there is considerable amounts of antibacterial agent in Persica leaves, which has activity against all types of oral bacteria and is comparable with different types of antiseptics. In the current study, Persica indicated mild anticandidial activity. Several studies have been conducted on Persica compounds and there isn't enough information about other mouthwashes. Based on our findings, there was no significant difference between herbal and chemical mouthwashes using independent T-test ($P = 0.764$). Using the agar diffusion method and according to the one-way ANOVA test, there was a significant difference within the group of chemical and herbal mouthwashes ($P< 0.001$). In the group of chemical mouthwashes, Oral B indicated a significant difference with other mouthwashes and in the group of herbal mouthwashes Matrica indicated a significant difference with other mouthwashes as indicated by the Tukey-HSD test ($P < 0.001$).

Investigation of minimum inhibitory concentration and minimum fungicidal concentration in the group of chemical mouthwashes, revealed that Colgate and antiplaque Irsha had the least and Vi-one had the most activity with an average concentration of 0.250 mg/L and 0.001 mg/L, respectively. In the group of herbal mouthwashes, also *S. persica* had the least activity (0.093 mg/L) and Matrica and Corpore Sano had the most activity (0.006 mg/L). The MIC and MFC results were the same and all mouthwashes had a minimum fungicidal concentration at the same level as the minimum inhibitory concentration. There was no significant difference between chemical and herbal mouthwashes using independent T-test ($P = 0.879$). al-Bagieh et al. (36) evaluated the antifungal activity of Persica extract and concluded that Persica concentrations more than 15% has a fungistatic effect on *C. albicans*.

In the current study, Persica a concentration of 0.093 mg/L indicated fungistatic activity against *C. albicans*. Guggenheim and Meier (37) indicated that, chlorohexidine could significantly decrease the number of microorganisms in biofilm. In the current study chlorohexidine effectively decreased candidial growth (MIC 0.015 mg/L). Using the MIC and MFC methods and according to the one-way ANOVA test, there was a significant difference within the group of chemical mouthwashes ($P < 0.001$) and there was no significant difference within the group of herbal mouthwashes ($P = 0.465$).

In conclusion, as our study showed, among chemical and herbal mouthwashes, Oral B is a better chemical mouthwash. These mouthwashes can be used for infection treatment, rather than antibiotics, to decrease antibiotic resistance. Furthermore, clinical studies are needed to confirm the efficiency of *in vivo* application.

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Authors' Contributions

All of the authors contributed to the development of the protocol, data abstraction, and preparation of the manuscript.

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