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Comparison between Iranian and foreign mouthwashes effect against *Candida albicans* as a common fungal mouth flora

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Abstract

Introduction: *Candida albicans* has been isolated in up to 65% of healthy individuals without signs of clinical disease. Mouthwashes can be used for many preventative and therapeutic purposes. This study evaluates the effectiveness of Iranian and foreign mouthwashes against *C.albicans* as a common fungal flora in the mouth.

Materials and methods: In this research, standard strain of *C.albicans*, ATCC 10231 is used. The suspension is provided by a fresh culture of *C.albicans* (24 hours) and the OD is read in 530 nm. The *C. albicans* suspension was cultured on Sabouraud dextrose agar plate. In the next step, two wells created on SDA, filling with mouthwashes (100 µl). After incubation at 37ºC for 24 hours, the inhibition zone was measured. Minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC) of mouthwashes were determined. The data are analyzed by SPSS software, independent T- tests and one sided variance analysis (ANOVA- one way).

Results: Findings of our study showed in which agar diffusion, MIC and MFC tests, there are no significant differences between the antifungal effect of the Iranian and foreign mouthwashes on *C.albicans* (*P value*>0.05).

Discussion and conclusion: This study shows that both Iranian and foreign mouthwashes have a good effect against *C.albicans*, as a common fungal flora in the mouth.

Key words: *Candida albicans*, Fungal flora, Mouthwash

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Introduction

Oral candidiasis is the most common fungal infection in human, which is produced by different types of Candida especially *C.albicans.* It has been known as a common opportunistic infection in immunocompromised patients and more than 90% of patients suffer from HIV infection at primary and advance stages (1- 14).

The use of chemical materials is a complementary method for controlling the population of *C.albicans*. Chemical materials are described in different forms such as alkaline peroxidase, acids, enzymes, toothpaste and mouthwashes (15). Mouthwashes are a kind of washing solutions, which are used to control the population of fungi in the mouth such as *C.albicans*. Based on their special structure used for mouth and teeth hygiene, these are washing and cleaning solutions (15, 16 and 17).

Different studies indicate that, using mouthwashes could decrease fungal and bacterial colonization in the mouth cavity, Ellepola *et al*., investigated the antifungal effect of Chlorhexidine gluconate 0.2% on mucous membrane inflammation caused by Candida which resulted in decreasing the colonization (2). Machado *et al*., investigated the effect of biofilms in different species of Candida and indicated that the colonization of Candida species have been decreased (4). Finally, Vianna *et al*., indicated the evaluation of antimicrobial activity of Chlorhexidine, which causes to decrease the colonization of facultative and aerobic microorganisms such as *C. albicans* (18). Among the mouthwashes, many of the researches have been focused on chlorhexidine against *C.albicans* and there is no enough information about other mouthwashes.

Nowadays in dentistry, antifungal agents are used in the local and systemic forms. Recently, resistance to antifungal therapy has been reported specially for organisms such as *C.albicans*. In Iran drug market, there are many Iranian and foreign mouthwashes, which their efficiencies against Candida haven't been studied. The aim of this study is to compare the effect of Iranian and foreign mouthwashes against *C.albicans.*

Materials and methods

Preparation of *C. albicans*

In this study, standard strain NO. ATCC 10231 of *C. albicans* is used. Standard strain cultivated in Sabouraud dextrose agar (Merck, Germany) and placed at 37ºC for 24 hours until activated.

Preparation of mouthwashes

Many different types of mouthwashes are used in this study as follow. Iranian 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), Matrica (Barij Essence Pharmaceutical Co, Kashan, Iran), Persica (Porsina Pharmaceutical Co, Tehran, Iran), Epimax (Emad Pharmaceutical Co, Esfahan, Iran), Chlorohexidine (ShahreDaru laboratories Co, Tehran, Iran), Fluoride (ShahreDaru 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) and foreign mouthwashes include Oral B (Grossgerau Co, Hessen, German), Sensodyne (GSK Co, London, UK), Corpore Sano (Disna.S.A Co, Barcelona, Spain), Colgate (Kucukyali Co, Estanbol, Turkey) and Foramen (Guarnizo Co, Cantabria, Spain).

Agar diffusion method

The suspension by physiological saline (Samen, Iran) and fresh culture of *C.albicans* was provided (24 hours), and its concentration (OD) was read at 530 nm (nanometer) wavelengths by spectrophotometer (Pars Teb Novin, Iran) in which there were 2.5×10^6 CFU/ml colonies in every milliliter**.** 10 µl of provided suspension transferred to SDA medium**.** In the next step, two wells were made with suitable distance in culture medium, filling with mouthwashes. To reduce the error, the test repeated 4 times. Plates located in the incubator (Behdad Medical Production, Iran) at 37ºC for 24 hours. Inhibition zone diameter measured and then recorded by using a Collis (Kiya Sanat Khavaran, Iran) (19). For each mouthwash, one control plate including mouthwash and sterilized distilled water was prepared.

Measurement of minimum inhibitory concentration

To determine the minimum inhibitory concentration for each mouthwash, dilution (from 1/2 to 1/1024) was prepared according to CLSI protocol. The lowest concentration of mouthwash that prevents turbidity (growth) of *C.albicans* considered being the minimum inhibitory concentration. This method was conducted based on turbidity clearance (20).

Measurement of minimum fungicidal concentration

To determine the minimum fungicidal concentration of mouthwash, 10 µl of specimens were taken from the MIC (last clear tube) and two last tubes poured in SDA, incubating at 37ºC for 24 hours. The lowest concentration of mouthwash that prevents the growth of *C.albicans* is determined by subculturing last clear MIC tube on SDA and refers to fungicidal activity. These procedures were conducted for more accuracy and controlling the error. Colony count less than 4 indicate non-growth and more than 4 refer to growth status (20).

Statistical analysis

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

Results

The mean inhibition zone diameter using Iranian and foreign mouthwashes are indicated in table 1 and 2. The comparison within inhibition zone diameter caused by Iranian and foreign mouthwashes are shown in Fig. 1.

Amount of MIC determined by tube dilution test (with 10 dilution tubes). The point at which growth of *C.albicans* was inhibited is recognized as a minimum inhibitory concentration. Statistical analysis of the Iranian and foreign mouthwashes MIC are presented in table 3 and 4. The comparison within Iranian and foreign mouthwashes MIC are shown in Fig. 2. The results of the MFC were similar to MIC (table 5 and 6 and Fig. 3).

Table 1- Statistical analysis, mean diameter and standard deviation of inhibition zone exhibited by Iranian mouthwashes against *C. albicans*

Types of mouthwash	Mean of inhibition zone (mm)	SD (mm)	P-value in ANONA-one way test
Vi- one	19.43	0.81	*P value*<0.001
Fluorine	18.46	1.84	
Hexodine	16.65	0.85	
Matrica	16.37	0.74	
Epimax	14.37	0.79	
Chlorhexidine	14.21	0.45	
Persica	10.93	0.77	
anti- plaque Irsha	R*	R	
anti-septic Irsha	R	R	
Benzydamine	R	R	
Fluoride	R	R	

*R means *C. albicans* resistance tothese mouthwashes

Table 2- Statistical analysis, mean diameter and standard deviation of inhibition zone exhibited by foreign mouthwashes against *C.albicans*

Types of mouthwash	Mean of inhibition zone (mm)	SD (mm)	P-value in ANONA-one way test
Oral B	23.25	0.65	*P value*<0.001
Sensodyne	19.87	1.32	
Colgate	R*	R	
Foramen	R	R	
Corpore Sano	R	R	

*R means *C. albicans* resistance to these mouthwashes

Table 3- Statistical analysis of minimum inhibitory concentration exhibited by Iranian mouthwashes against *C. albicans*

Types of mouthwash	MIC (mg/l)	P-value in ANONA-one way test
anti-plaque Irsha	0.250	*P value* <0.001
anti-septic Irsha	0.125	
Benzydamine	0.125	
Fluoride	0.062	
Epimax	0.031	
Hexodine	0.019	
Chlorhexidine	0.015	
Fluorine	0.007	
Vi-one	0.001	

Table 4- Statistical analysis of minimum inhibitory concentration exhibited by foreign mouthwashes against *C. albicans*

Types of mouthwash	MIC (mg/l)	P-value in ANONA-one way test
Colgate	0.250	*P value*<0.001
Persica	0.093	
Matrica	0.062	
Corpore Sano	0.062	
Foramen	0.062	
Sensodyne	0.007	
Oral B	0.003	

Table 5- Statistical analysis of minimum fungicidal concentration exhibited by Iranian mouthwashes against *C. albicans*

Types of mouthwashes	MFC (mg/l)	P-value in ANONA-one way test
anti-plaque Irsha	0.250	*P value*<0.001
anti-septic Irsha	0.125	
Benzydamine	0.125	
Fluoride	0.062	
Epimax	0.031	
Hexodine	0.019	
Chlorhexidine	0.015	
Fluorine	0.007	
Vi-one	0.001	

Table 6- Statistical analysis of minimum fungicidal concentration exhibited by foreign mouthwashes against *C. albicans*

Types of mouthwashes	MFC (mg/l)	P-value in ANONA-one way test
Colgate	0.250	*P value*<0.001
Persica	0.093	
Matrica	0.062	
Corpore Sano	0.062	
Foramen	0.062	
Sensodyne	0.007	
Oral B	0.003	

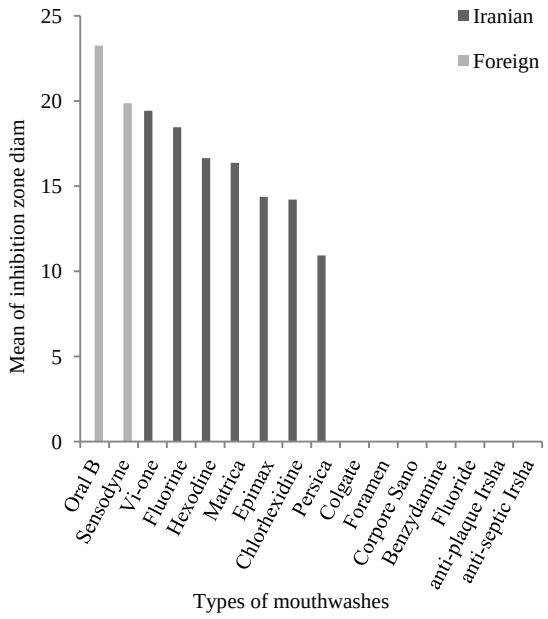

Fig. 1- Comparison the inhibition zone diameter within Iranian and foreign mouthwashes against *C. albicans*in agar diffusion method

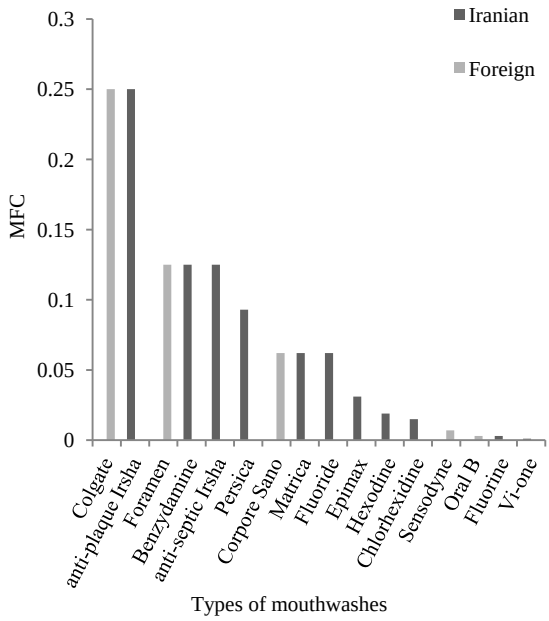

Fig. 2- Comparison the minimum inhibitory concentration within Iranian and foreign mouthwashes against *C. albicans* in MIC method

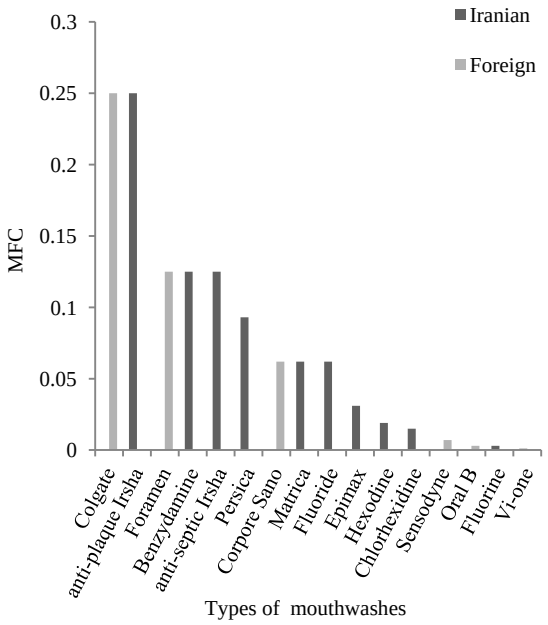

Fig. 3- Comparison the minimum fungicidal concentration within Iranian and foreign mouthwashes against *C.albicans*in MFC method

Discussion and conclusion

C.albicans is a commensal organism that can be isolated from the majority of healthy individuals (21). However, in certain susceptible individuals *C.albicans* can become pathogenic leading to the mucocutaneous infection; oral candidiasis. *C.albicans* is a fungal organism that is part of the normal oral microbial flora in approximately 50% of healthy individuals (22 and 23). *Drugs* such as broad spectrum antibiotics alter the local oral flora and lead to a suitable environment for proliferation of Candida (5). Today, in dentistry, in addition to systemic antifungal agents, the local forms (mouthwash) are frequently used. In recent years, increasing resistance to antifungal agents has been observed and the concern about organisms such as *C. albicans* has been created.

In the present study, among foreign mouthwashes, Oral B and Sensodyne showed the most and the least activity against *C.albicans* respectively. *C.albicans* resistant to foreign mouthwashes include Colgate, Foramen and Corpore Sano. On the other hand, among Iranian mouthwashes, Vi-one and Persica showed the most and the least activity against *C.albicans* and among Iranian mouthwashes, including anti-septic Irsha, Benzydamine and anti-plaque Irsha also indicated resistance to *C.albicans*. Based on our findings, there is no significant difference between Iranian and foreign mouthwashes using independent T-test. Barasch *et al*., investigated the effect of Chlorhexidine in preventing and treatment of oral candidiasis in children with HIV (24). Lanzos *et al*., also investigated the effect of Chlorhexidine mouthwash for decreasing oral diseases in individuals which contain a cancer and under radiotherapy. The investigation conducted on 70 patients and showed Chlorhexidine has effective activity for decreasing oral diseases (25).

In agar diffusion method, according to ANOVA-one way test, there is a significant difference within the group of Iranian and foreign mouthwashes (*P value*<0.001). It's important that there was a significant difference between Vi-one and all other Iranian mouthwashes except Fluorine. Also among foreign mouthwashes, there was a significant difference between Oral B and all other foreign mouthwashes. Machado *et al*., investigated the antifungal activity of Chlorhexidine on different species of *C.albicans* and concluded that it has decreased the colony count of *C.albicans* 79 to 99% (26). Soares *et al*., investigated the effect of Chlorhexidine on oral mucositis and microbiological analysis in children which have acute lymphoblastic leukemia, indicated that the number of pathogenic agents, such as *C. albicans* has been decreased 35.3% (27). In the present study, as the same as other investigations, Chlorhexidine inhibited the growth of *C.albicans in vitro* and inhibition zone (the mean of 14.21 mm) has been showed. In spite of former studies, which investigated the clinical manifestation of using Chlorhexidine, current study compared the *in vitro* effect of Iranian and foreign mouthwashes against *C. albicans*. Giardino *et al*., compared the antimicrobial activity of sodium hypochlorite and Chlorhexidine and concluded, there was significant difference between the antimicrobial effect of sodium hypochlorite and Chlorhexidine statistically and sodium hypochlorite was more effective (28). In the present study, also there was a significant difference between all mouthwashes and Chlorhexidine except Epimax (*P value* <0.001). It's notable that, Sensodyne, Oral B, Matrica, Persica, Fluorine, Hexodine had more effect in comparison with Chlorhexidine and there was a significant difference between Chlorhexidine and mentioned mouthwashes.

Aspalli *et al*., investigated antiplaque and antigingivitis effect of mouthwash in treatment of plaque induced gingivitis and showed mouthwash is effective in treatment of plaque induced gingivitis and can be effectively used as an adjunct to mechanical therapy with lesser side-effects (29).

In determination of MIC and MFC, in the group of Iranian mouthwashes, the most and the least minimum inhibitory concentration belong to anti-plaque Irsha and Vi-one respectively (0.250 mg/l, 0.001 mg/l). In the group of foreign mouthwashes, the most and the least minimum inhibitory concentration belong to Colgate and Oral B respectively (0.250 mg/l, 0.003 mg/l). Based on independent T-test, in the current investigation, between Iranian and foreign mouthwashes, there was no significant difference. The results of MFC were similar to MIC and all mouthwashes indicated the minimum fungicidal concentration same as minimum inhibitory concentration. There was no significant difference between Iranian and foreign mouthwashes, using independent T-test. Botelho *et al*., indicated the Chlorhexidine in minimum MIC (0.25-8 µg/ml) also showed anti streptococcal activity (30). Jarvinen *et al*., also indicated MIC of Chlorhexidine which was effective against *Streptococcus mutans* (31).

In the current study, MIC of Chlorhexidine (0.015 mg/l) was also active against *C. albicans*. In MIC and MFC methods, according to ANOVA-one way test, there was a significant difference within the group of Iranian and foreign mouthwashes, there was a significant difference within the group of Iranian mouthwashes and the group of foreign mouthwashes (*P value* <0.001).

These mouthwashes can be used for infection treatments rather than antibiotics to decrease antibiotic resistance. Furthermore, clinical studies are needed to confirm the efficiency of *in vivo* application.

Our study showed, both Iranian and foreign mouthwashes had a good effect against *C.albicans*, as a common fungal flora in the mouth.

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مقایسه آزمایشگاهی اثر دهانشویه‌های ایرانی و خارجی بر علیه کاندیدا آلبیکنس به‌عنوان فلور قارچی شایع در دهان

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چکیده

مقدمه: *کاندیدا آلبیکنس* در ۶۵ درصد از افراد سالم بدون علایم بالینی جدا شده است. دهانشویه‌ها برای بسیاری از اهداف پیشگیرانه و درمانی استفاده می‌شوند. مطالعه حاضر با هدف بررسی تأثیر دهانشویه‌های ایرانی و خارجی بر *کاندیدا آلبیکنس* به عنوان فلور قارچی رایج در دهان انجام شد.

مواد و روش‌ها: در این مطالعه، ازسوش استاندارد *کاندیدا آلبیکنس* ATCC ۱۰۲۳۱ استفاده شد. سپس از کشت تازه *کاندیدا آلبیکنس* (۲۴ ساعته)، سوسپانسیون تهیه و OD آن در ۵۳۰ نانومتر خوانده شد. سوسپانسیون *کاندیدا آلبیکنس* در محیط سابورودکستروزآگارکشت داده شد. سپس، دو چاهک در محیط کشت ایجاد، در داخل آن‌ها دهانشویه ریخته (۱۰۰ میکرولیتر) و در دمای ۳۷ درجه سانتی‌گراد به مدت۲۴ ساعت انکوبه شد. سپس، هاله عدم رشد اندازه گیری شد. حداقل غلظت مهارکنندگی رشد (MIC) و حداقل غلظت کشندگی (MFC) دهانشویه‌ها نیز مشخص شد. داده‌ها با استفاده از نرم افزار آماری SPSS، آزمون‌های آماری T مستقل و تحلیل واریانس یک طرفه بررسی شد.

نتایج: نتایج مربوط به روش آگار دیفیوژن، MIC و MFC، اختلاف معناداری بین اثرات ضد قارچی دهانشویه‌های ایرانی و خارجی نشان ندادند (*Pvalue*>۰/۰۵).

بحث و نتیجه‌گیری: این مطالعه نشان داد که هر دو دهانشویه ایرانی و خارجی اثر خوبی دربرابر کاندیدا آلبیکنس به عنوان فلور قارچی شایع در دهان داشتند.

واژه‌های کلیدی: *کاندیدا آلبیکنس*، فلور قارچی، دهانشویه

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* نویسنده مسؤول مکاتبات

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