



Original Article

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In vitro evaluation of the antifungal effect of vi-one mouthwash

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ABSTRACT

Candida albicans is one of the most common opportunistic fungi which cause oral cavity infections in humans. Most anti-fungal drugs posse side effects and may have an undesirable taste. Ginger is one of the oldest herbal products used in traditional medicine which has known antimicrobial effects and is used in the manufacture of Vi-One mouthwash. The present study was designed to investigate the antifungal effects of Vi-One Mouthwash. In this experimental study, the susceptibility of *Candida albicans* (PTCC 5027) to Vi-One Ginger mouthwash was evaluated in comparison with nystatin using disk diffusion method. One way analysis of variance (ANOVA) test was employed to evaluate the findings and P-values less than 0.05 were considered as significant. The data of the present study showed that the diameter of the inhibition zone in the nystatin group was 8.04 mm while, in the vi-one mouthwash group it was 1.16 mm. The difference between examined groups was significantly different (ANOVA, P-value=0.0001). According to the findings, vi-one ginger mouthwash exhibited a very weak antifungal effect in comparison with nystatin in the laboratory environment.

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Keywords

Candida Albicans,
Nystatin,
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INTRODUCTION

The occurrence of serious infections caused by yeast, exclusively species of *Candida*, has increased significantly during the past decade. Oral candidiasis typically affects immunosuppressed patients, such as transplant, cancer, and acquired immunodeficiency syndrome (AIDS) patients, and also elderly people wearing dentures [1]. Diabetes is the most common metabolic disease, which can cause different lesions in the oral mucosa. Candidiasis is one of the most common types of lesions especially in uncontrolled diabetic patients [2]. Traditionally, oral candidiasis is classified into acute and chronic forms, and the signs and symptoms are dependent upon the type.

There are topical and systemic antifungal agents that may be identified to control oral candidiasis, but, the development of resistance is an emerging trend that may threaten their clinical effectiveness [3].

Due to the increased incidence of fungal infections and anti-fungal drug resistance, many studies have been accomplished to find the antifungal properties of plants [4].

In addition, the utilization of plants in the native cultures of developing countries are numerous and diverse. For many people they still form an important economic basis and are used in medicine. Safety and lower side effects of many herbal extracts have also suggested them as sources of new pharmaceuticals [5].

lately, many plant extracts have shown to have therapeutic values with respect to oral diseases [6]. *Ginger (Zingiber officinale)* is one of the medical plant that having antimicrobial effects against various human pathogens. It has been shown to possess promising inhibitory effect on many of the oral microorganisms [7].

Commercial mouthwashes with antifungal agents have

many advantages for use as preventives or adjunctive therapy, such as fewer side effects, safety, and easily obtainable [1]. In this study, the antifungal effect of Vi-One ginger mouthwash on *Candida albicans* was investigated.

MATERIALS AND METHODS

The antimicrobial activity was done by agar disc diffusion assay by measuring the zone of inhibition against the antimicrobial activity was done by disc diffusion assay by measuring the zone of inhibition. The disk-diffusion assay was performed according to the Clinical and Laboratory Standards Institute guideline CLSI [8].

The fungi used in this study, *Candida albicans* (PTCC 5027), was purchased from Iranian microbial collection (Tehran, Iran). This was grown on Sabourauds dextrose agar (Merck, Germany).

Nystatin suspension (100,000 IU/mL, Jaber Ebne Hayyan, Iran) was used as the positive control group in this

study. Different concentrations of nystatin were used. Discs impregnated with physiologic serum and allowed to air dry served as negative control.

Vi-on mouthwashes, nystatin, and sterile physiological serum solution were dispersed, and five of the blank disks were immersed in each of the above solutions and placed at room temperature for 24 hours.

The disks were placed on Sabourauds dextrose agar plates with the help of sterile forceps. These plates were incubated at 30°C.

Finally, we measured the diameter of inhibition zones (IZ) in millimeter after 48 hours. We placed three disks for each concentration in a 9 cm plate and calculated the mean of inhibition zones. We analyzed the data using one-way ANOVA and Tukey test. P value less than 0.05 was considered statistically significant.

RESULTS

Initially, to assess the effects of Vi-One ginger

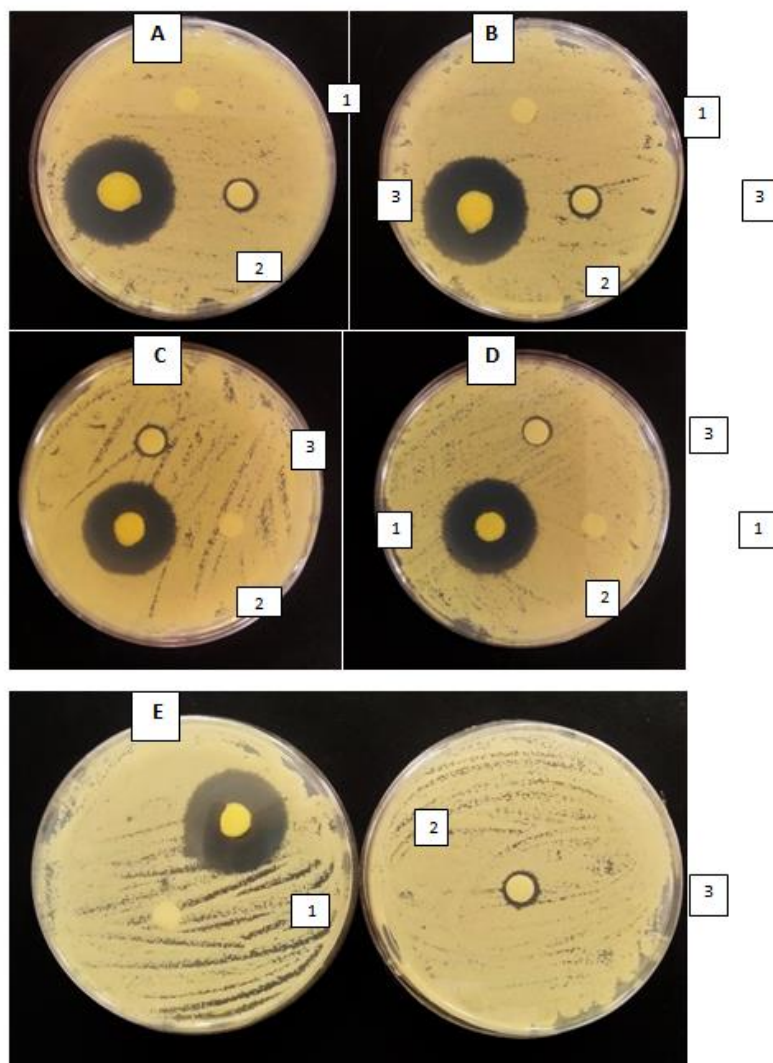


Figure 1. Zone of inhibition of Vi-one, nystatin and physiology serum

mouthwash on *C. albicans* growth, the disk diffusion test was employed. The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the discs indicated the presence of antimicrobial activity (Fig. 1).

Table 1 shows the Average zone of inhibition of physiological serum, nystatin and Vi-One ginger mouthwash against *C. albicans* respectively. The Vi-One ginger mouthwash was not effective in inhibiting the growth of *Candida albicans* as much as the positive control. The positive control (nystatin) produced significantly sized inhibition zones with *Candida albicans*, while the negative control produce

no observable zones. The inhibitory zone of nystatin was reduced while decreasing the concentration (Fig. 1). Tukey's test showed that the inhibitory zone of Vi-one is almost equivalent to the nystatin inhibition zone with a dilution of 1/256 (Table 2).

There was a significant difference between the vi-one mouthwash and nystatin in their growth inhibition against *Candida albicans*.

According to Figures A, B, C, D and E the test was performed in five replications. In all five repetitions, there was no inhibition zone around the physiologic serum disks (A1, B1, C1, D1, E1) as negative control. The zone of inhibition

Table 1. Diameters of inhibition zones (mm) of 3 groups

Groups	Average of IZ	Standard deviation
Negative control	0.0001	0.00001
Nystatin	8.04	0.15
Vi-one	1.16	0.16

P-value=0.0001

Table 2. Comparison of the mean Average of the inhibitory zone of Vi-one with different dilutions of nystatin (mm) in 48 hours

Nystatin dilution	Average of IZ	Standard deviation
1	7.9	0.23
1/2	7.2	0.21
1/4	5.9	0.11
1/8	4.9	0.08
1/16	4	0.07
1/32	3.1	0.15
1/64	3	0.07
1/128	2.2	0.22
1/256	1	0.14
Vi-one	1.1	0.16

P-value=0.0001

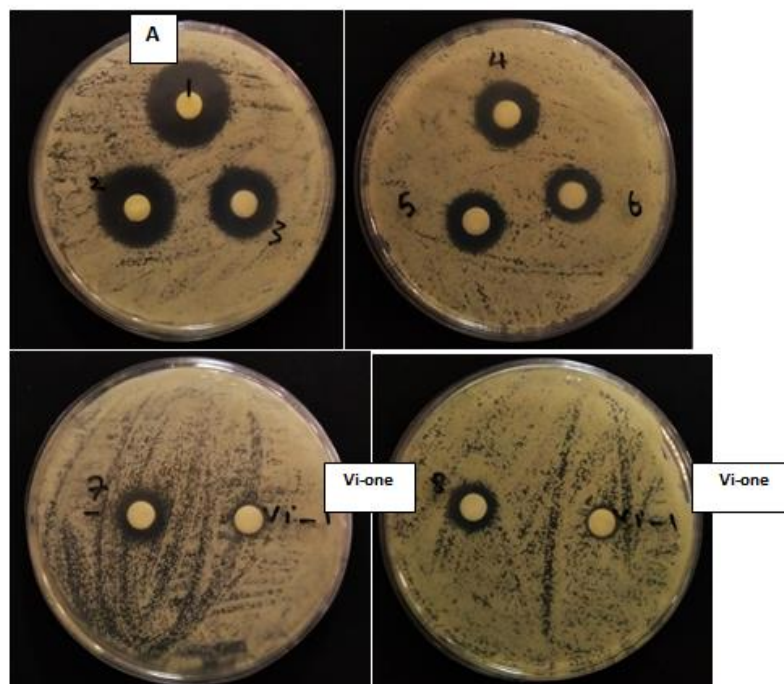


Figure 2. Zone of inhibition of nystatin mouthwashes in dilutions 1 to 256 (A1=1/2, A2=1/4, A3=1/8, A4=1/16, A5=1/32, A6=1/64, A7=1/125, A8=1/256). According to Figure the inhibitory zone of nystatin was reduced while decreasing the concentration.

around the nystatin discs (A2, B2, C2, D2, and E2) was much higher than the Vi-One mouthwash (A3, B3, C3, D3, and E3). The average diameter of inhibition zone in the nystatin group was 8.04 mm and in the vi-one mouthwash group it was 1.16 mm.

DISCUSSION

Regarding the increase of resistance in fungal pathogens, it is important that new antifungal agents be identified and developed [9].

Previous studies have confirmed that effective antimicrobials can be identified in consultation with natural remedies [10]. The various therapeutic efficacy of ginger has been reported which consist anti-emetic activity, anti-ulcer, antipyretic, antiplatelet, antioxidant and anti-inflammatory activity. The antifungal and antibacterial activity of ginger has been attributed to gingerol and [6] shagelol derived from the ethanolic extracts of ginger [6]. In this study the vi-one ginger mouthwash was not comparable to nystatin (Gold standard), and only a brief effect was seen in comparison with the negative control. This result is comparable with some other studies [11, 12] suggesting that the *Ginger extract* has weak antifungal properties. In the study of Zaman-zad et al., The antibacterial properties of raw aquatic and ethanolic extracts of *Allium cepa* (onion) and *Zingiber officinale* (ginger) were investigated against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* isolated from vaginal specimens. The Antimicrobial activity of raw extract, alcohol, cold water and hot water of ginger on *Candida albicans* was investigated using the disk diffusion method. Crude extracts of ginger on *Candida albicans* had a weak effect, and three other ginger extracts did not prevent *Candida albicans* growth. The findings of this study indicated that the extracting solvent had an effect on the degree of antimicrobial activity of the extracts. Alcohol, because of its organic nature, dissolves organic compounds better than water. According to the manufacturers' claims, which this mouthwash is alcohol-free, the cause of not affecting may be attributed to its inappropriate extract solvent [13]. In a study by Alizadeh, the effect of ginger mouthwash were evaluated by clinical trial on patients with denture stomatitis, the results were contradictory with our study. The details of ginger mouthwash, such as extraction method, water solvent or alcohol, etc., have not been described in this study. The difference between the results of two tests may be due to the different preparation of mouthwashes. also This discrepancy may be explained by the differences in the methodology of experiments.[14] Another study by Eslami et al., aimed at comparing the effectiveness of Vi-one and nystatin mouthwashes. This study was performed as a double-blind clinical trial On 30 patients with denture stomatitis and the efficacy of both types of mouthwash was equally expressed. The discrepancy between the results of two studies may be explained by the various local and systemic factors, such as the essence of the abstergent of the mouthwash. That property can reduces microbial load and is likely to be effective in reducing the tissue erythema

in patients. The presence of substances other than ginger derivatives in the formulation of the vi-one mouthwash, such as vitamin E (which also has a distinct anti-inflammatory effect), can be another reason to justify the different results of these two studies. Finally the difference in the method of two experiments can be the other reason for this contradiction [15]. In a *in_vitro* study, Atai Z, demonstrated that ethanolic ginger extract has significant antifungal properties, but its inhibitory effects against *candida albicans* are fewer than nystatin [3]. According to previous studies, the fact that the main antibacterial material in the Medicinal Plants is susceptible to the warmth. Therefore, all spice preparations lose antibacterial activity at about 100 ° C for 20 minutes. So The probability of losing antifungal properties of ginger in the process of extraction and mouthwash preparation is probable [16]. The unidimensional concentration of effective antifungal compounds and the loss of anti-fungal properties of ginger in the process of extraction and preparation of Vi-One mouthwash [12, 13, 17-19], can be the other possible reasons for the inconsistency of the results of our study with other studies.

Perhaps another reason for the unacceptable effect of the ginger mouthwash in the Iranian market, called vi-one, can be attributed to the lack of ginger and its effective compounds, or its low concentration. The mouthwash compounds were not analyzed in this study. It was documented only on the claim of the mouthwash brochure on the use of ginger antifungal effects.

CONCLUSION

Our study focused on the effect of Vi-One ginger mouthwash on the *Candida Albicans* and showed that it does not have significant anticandidal properties. In this study The Vi-One ginger mouthwash has no antifungal properties comparable to nystatin as the gold standard for anti-Candida treatment. Average of the inhibitory zone of Vi-one was equivalent to nystatin concentration of 1/256. As with other studies, this study also has its limitations as it is an *in vitro* study. According to the results of this study and other research, Vi-One ginger antifungal mouthwash, has very low effect on inhibition of growth of *Candida albicans* in the *in vitro* environment and the positive effect in studies conducted in clinical trials. We suggest that in later studies, the effect of mouthwash on the morphological structure of this fungus should be considered. Because the pathogenicity of *Candida albicans* depends on the morphological structure of this fungus.

CONFLICTS OF INTEREST

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article

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