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Comparison of the Effect of Ginger and Nystatin Mouthwash on the Growth of Candida Albicans under in Vitro Conditions

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ABSTRACT

Background and Objective: Candidiasis is one of the most common diseases in the oral cavity. Due to the side effects of chemical mouthwashes, the use of antifungal herbs has increased significantly. Therefore, this study was performed to evaluate the effect of Ginger (Vi-one) mouthwash on the growth of Candida albicans under in vitro conditions.

Methods: In this experimental in vitro study, 32 special solid culture media were prepared. 15 special solid culture media containing discs and wells impregnated with ginger mouthwash (Vi-one) and 15 special solid culture media with discs and wells of nystatin extract were prepared. Moreover, two blank sterile cultures with disks and wells containing no mouthwash or extract were prepared. These plates were placed in microaerophilic conditions with the presence of carbon dioxide and were transferred to the incubator at 37°C for 48 hours. After 48 hours, the zone of inhibition was determined using standard methods.

Findings: Agar well diffusion method revealed that the zone of inhibition for ginger mouthwash equaled 23.6±0.33mm and for Nystatin equaled 28.2±1.2. Agar disc diffusion method revealed that zone of inhibition for ginger mouthwash equaled 18.3±1 and for Nystatin equaled 28.3±0.1 ($p<0.0001$).

Conclusion: Based on the results of this study, it seems that ginger mouthwash (Vi-one) compared to nystatin extract can be effective against the growth of Candida albicans under in vitro conditions.

Keywords: *Nystatin, Ginger Mouthwash, Candida Albicans*.

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Introduction

The mouth contains about 500 types of microorganisms called microflora and Candida Albicans is one of the most common microorganisms found in microflora (1-3). Candida Albicans is a fungus and diploid yeast. This fungus grows on the skin, mouth, gastrointestinal tract and genital organs. Disturbance of the environmental balance of the oral's microflora causes indiscriminate growth of this fungus, which in some cases leads to stomatitis candidiasis (4). Stomatitis candidiasis or oral thrush occurs in the form of gray white bumps on the mucosa of the mouth and tongue, and with the separation of these lesions, hemorrhagic areas are created (5). Clinical course of the disease can be acute, subacute, chronic or sporadic and involves infection of almost every part of the body (3). So far, several chemical methods have been introduced to eliminate Candida, such as Nystatin. With the prevalence of fungal infections and following increase in the use of antifungal drugs, there has been a significant elevation in the resistance of Candida species to antifungal compounds (6). On the other hand, consumption of chemical mouthwash such as nystatin can result in various complications (7, 8). Medicinal plants including ginger have recently been used in candidiasis treatment (6, 9). Considering its lower price, availability and fewer side effects of these plants, there has been an increase in the use of medicinal compounds and herbal medicines among people (1).

Ginger is obtained from the yellow plant with purple streaks with the scientific name Zingiber officinale. The most prominent medicinal properties of ginger include antibacterial and anti-fungal properties. Hence using ginger as a mouthwash due to ease of use and availability, suggests promising effects against multiple oral microorganisms, especially Candida Albicans (10). Some studies have been conducted to identify the effect of ginger mouthwash on microorganisms' growth, which report different results. For instance, Rashidi Maybodi et al. (11) showed that Vi-one ginger mouthwash has a very weak antifungal effect in comparison with nystatin under in vitro conditions. On the other hand, Aghazadeh et al. (8) proved that 0.625 mg/mL and 5 mg/mL concentrations of ginger extract have an extensive inhibitory potential against Candida Albicans in laboratory research.

Despite studies on the effects of herbs on dental health and also considering the medicinal effects of ginger, few studies have been performed on the effect of ginger mouthwash on inhibiting the growth of Candida albicans in vitro, which report a mild effect (12). Therefore, the present study was performed to investigate the effect of ginger mouthwash on inhibition of Candida albicans growth in vitro in Shahid Beheshti University of Medical Sciences.

Methods

In this experimental in vitro study, 32 samples were prepared after approval by the ethics committee of Islamic Azad University, Tehran Medical Branch with the code No. D/1330/P. These 32 samples included 15 samples of ginger mouthwash, 15 samples of Nystatin extract (control group) and 2 samples of blank sterile blank discs (negative control group). Agar well diffusion and agar disk diffusion methods were used to conduct this experiment. In agar disk diffusion method, Kirby-Bauer disk diffusion susceptibility test protocol was followed. First, live cultures of Candida albicans strain (PTCC 5027) were purchased and cultured on blood agar medium. To prepare 1 liter of blood agar medium, 37 g of Hinton B agar powder was dissolved in 1 liter of distilled water and heated using heater to make the full boil smooth and clear and then was sterilized at 121°C and pressure of 15 psi for 15 minutes. Then, it was removed from the autoclave. In order to maintain sterile conditions, the medium was divided into the required test volumes inside separate test plates under the Laminar flow hood after cooling slightly. Then, to ensure that this medium was sterile, it was incubated for 24 hours at 37 C˚ and was subsequently hold in the fridge until use. After the incubation,

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about 1.5×10^8 standard strain Candida Albicans equal 0.5 McFarland Standard was suspended in 2ml of sterile normal saline and vortexed. Then, the turbidity of test suspension was visually compared with 0.5 McFarland Standard in the presence of good lighting and Wickerham card. The sterile swab was dipped in suspension and swabbed on dried plates of Sabouraud's dextrose agar to get proper culture. Each of the two appropriate ginger Vi-one mouthwash and nystatin extract impregnated disks were placed on the surface of the agar using forceps. Nystatin disks were impregnated with Nystatin suspension (100,000 IU/mL) purchased from Persian Type Culture Collection. In agar well diffusion method, Ginger Vi-one mouthwash was cultured on 15 special solid culture media containing ginger Vi-one mouthwash disk. Moreover, the other 15 special solid culture media containing nystatin extract impregnated disks were cultured with nystatin extract. 2 culture media were also prepared with wells and disks; however, no extract or mouthwash was used. 2 wells with a diameter of 7 mm were used on solid medium and the extract was poured into the well by standard loop of 1.100 ml. Once all the wells and disks were in position, the lid was replaced and these plates were placed in microaerophilic conditions with the presence of carbon dioxide and transferred to the incubator at 37°C for 48 hours. After 48 hours, zone of inhibition was measured with a ruler. The collected data were analyzed by SPSS software version 20 for parametric and non-parametric data analysis. Moreover, these data were also analyzed by T-test according to specific objectives and research hypotheses.

Results

The study was conducted on 32 samples including 15 samples in Nystatin group and 15 in ginger group. In this study, two negative controls were used. There was no inhibition zone around the discs and wells in negative control. The inhibition zones in the samples were acceptable according to 2015 CLSI standards and were recorded. The diameters of the inhibitory zone in nystatin and ginger wells are shown in Figure 1 and show that the mean diameter of the inhibition zone in ginger mouthwash is 23.6±0.33 mm with a coefficient of variation of 1.4 and in nystatin 28.2±1.2 mm with a coefficient of variation of 4.3. Therefore, this amount was 4.6 mm or 16.3% lower in the ginger mouthwash group than in the nystatin group. The difference in the diameter of the inhibition zone in the wells was statistically significant ($p<0.0001$).

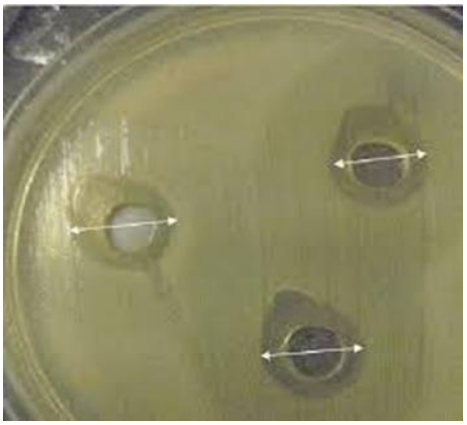


Figure 1. Inhibition zone in nystatin and ginger wells

The diameter of the inhibition zone in the discs of each group is presented in Figure 2 and shows that the diameter of the inhibition zone in the nystatin group is 28.3±0.1 mm with a coefficient of variation of 0.5 and in the ginger mouthwash group is 18.3±1 mm with a coefficient of variation of 5.6, which is 10 mm or 35% lower in the ginger group compared to nystatin, and is statistically significant ($p<0.0001$).

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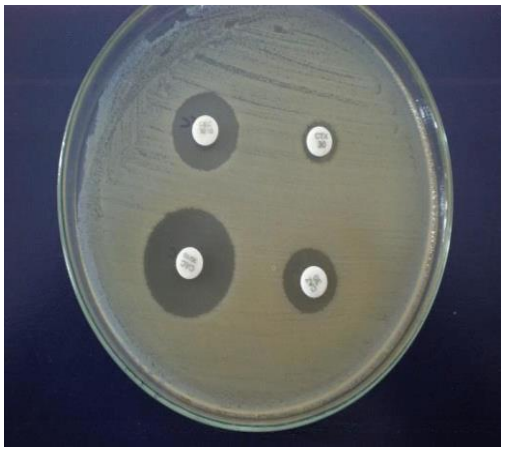


Figure 2. Inhibition zone in nystatin and ginger disks

Discussion

This research indicated that ginger mouthwash is effective on inhibition zone diameter of Candida Albicans under in vitro conditions. However, the diameter of Candida inhibition zone in well method and in disk method in ginger group was lower than nystatin group. Multiple studies have been conducted to assess the efficacy of ginger against Candida Albicans. In 2018, Dr. Lee (13) conducted a study to show that 6-Gingerol with concentration of 10 μg/ml effectively reduces the formation of Candida Albicans biofilm. Another study was performed in 2020 by Dr. Khalaf (14) to investigate the effect of alcoholic ginger extract on Candida Albicans isolated from the mouth of 120 patients of Al-Hussein Hospital in Karbala. The results showed that 50, 100, 150 μg/ml of pure extract caused 18-, 21- and 25-mm inhibition of growth and the MIC score was 25 mm. Therefore, this study suggested that the alcoholic extract of ginger can be used as an anti-Candida albicans treatment. Atai et al. (7) investigated the inhibitory effect of ginger extract on Candida ATCC10231 and PTCC 5027 in an experimental study. The results showed ginger extract was effective in limiting the growth of Candida Albicans. In addition, there was a significant difference between the ginger extract and Nystatin in inhibition of Candida.

Despite all of these studies confirming positive effects of ginger extract against Candida Albicans, only a few studies have been performed to indicate the efficacy of ginger mouthwash. Moreover, these few studies report contradictory results. Eslami et al. (4) examined the effect of ginger mouthwash for the treatment of stomatitis denture in 30 patients with type 2 denture stomatitis. The results showed that during 20 days of study, ginger mouthwash significantly decreased the length and width of erythema. Therefore, ginger mouthwash can clinically be effective against Candida Albicans. On the other hand, a study was conducted by Rashidi Maybodi (11) to investigate the effect of ginger Vi-one mouthwash on Candida Albicans PTCC 5027 species in comparison with nystatin. The inhibition zone diameter of Nystatin group was 8.04 mm and Vi-one mouthwash was 1.16 mm and the results were statistically significant. This study had the same result as the present study and confirmed the small effect of ginger on Candida.

Ginger mouthwash is an herbal ingredient which not only lacks harmful side effects but also has a suitable antifungal effect compared to common antifungal drugs. Ginger mouthwash contains a lot of antioxidants, which means that ginger has anti-cancer and anti-inflammatory properties. Moreover, ginger is saliva stimulant and has antibacterial and antifungal activity which is helpful in the treatment of fungal lesions. Therefore, ginger mouthwash is widely recommended for people with fungal lesions in the mouth as well as those with dentures (6).

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It seems that using ginger mouthwash (Vi-one) compared to nystatin extract can be effective against the growth of Candida Albicans under in vitro conditions and with further clinical studies, it can be introduced as a definite treatment in near future.

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