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Antifungal Effect of Ginger Essential Oil Spray on *Candida albicans* Adhering to Self-Cure Acrylic Plates

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A B S T R A C T

Objectives: The present study aimed to evaluate the efficacy of ginger essential oil spray for elimination of *Candida albicans* (*C. albicans*) adhering to self-cure acrylic plates.

Materials and Methods: In this experimental study, 120 self-cure acrylic discs were contaminated with *C. albicans* and randomly divided into four main groups: exposure to ginger essential oil, nystatin (positive control), distilled water (negative control), and no exposure. The minimum inhibitory concentration (MIC) of ginger oil and nystatin was determined by the microdilution test. The stability of *C. albicans* was determined by culturing the samples of treated acrylic plates and comparing the mean number of remaining colonies. Data were analyzed using the Kruskal-Wallis test followed by Dunn test with Bonferroni correction. P<0.05 was considered significant

Results: The MIC of ginger essential oil and nystatin was found to be 1560μg/mL and 4μg/mL, respectively. The differences between the mean count of *C. albicans* colonies before (10175±10730.25) and after the exposure to ginger essential oil (542.86±464.81) and nystatin (257.14±247.67) was statistically significant (P<0.001). The mean number of *C. albicans* colonies after spraying with nystatin was not significantly different compared with ginger essential oil (P=0.204). The efficacy of nystatin and ginger essential oil at each time was significantly more than distilled water (P<0.001). At 10 and 15min, there was no significant difference between nystatin and ginger essential oil groups (P=0.05).

Conclusion: Ginger essential oil spray was found to be a simple and effective method for elimination of *C. albicans* adhering to acrylic discs.

Keywords: Acrylic Resins; *Candida albicans*; Ginger; Oils, Volatile

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INTRODUCTION

Prolonged use of intraoral acrylic appliances can increase the number of *Candida albicans* (*C. albicans*) colonies on the tooth surface and oral mucosa. An increase in the number of these colonies may lead to oral candidiasis. It can cause mucous membrane lesions and bad breath, and may irritate the patients so much

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that they stop using the appliance regularly. These lesions are especially common in patients with predisposing conditions such as diabetes mellitus, immunosuppressive diseases, long-term use of antibiotics and corticosteroids, etc., who are using intraoral acrylic appliances [1,2].

The most effective way to prevent the growth of pathogens in the oral cavity is to maintain oral hygiene and clean the acrylic denture plates [3]. The most conventional method of cleaning the acrylic plates is the mechanical method, and above all cleaning with a toothbrush and water. However, since water and toothbrushes are more effective in removing large debris and have a limited effect on the biofilm on the surface of acrylic plates, it is best to use special disinfectants to remove such biofilms. Furthermore, disinfectants are easier to use compared with mechanical methods [4,5]. There are several common chemical methods for this purpose, including immersion in chlorhexidine, sodium hypochlorite, and cleaning tablets. However, because of certain drawbacks of chemical solutions such as high costs and their tendency to roughen the surface, cause discoloration, or confer an unpleasant odor to the appliance. There is a growing demand for herbal solutions that could be used instead of chemical substances. Indeed, some herbal remedies are not only cheaper but also have fewer side effects than some of the existing chemical solutions [6-9]. Over the years, several non-toxic herbal disinfectants have been used in the production of commercial oral hygiene products. Ginger is one of the most popular herbs in this field [9,10]. Ginger has a long history of medicinal use and is known to have an excellent antioxidant, anti-inflammatory, and anti-emetic properties. It also has blood pressure and sugar-regulating effects [11]. The antifungal effects of ginger essential oil have also been confirmed in the literature [12,13]. Ginger also has several applications in the field of dentistry for treatment of gingivitis, relieving the pain of aphthous ulcers, treating oral dryness, and application as intra-canal medicament, and is generally considered a safe medicinal plant [14-19]. Two proven methods for disinfecting the dental appliances are immersion in the disinfectant and spraying with the disinfectant solutions [20]. Immersion is the common method of disinfecting the acrylic appliances, but this method may result in water sorption and subsequent undesirable changes in the structure of acrylic resin. Spraying, however, does not have this problem and offers other advantages such as speed and ease of use, requiring a lower amount of solution, and being more cost-effective [21]. There is lack of data about the effectiveness of herbal essential oils in disinfecting acrylic appliances by the spraying method. Also, the appropriate exposure time should be tested. Therefore, this study aimed to investigate the effect of ginger essential oil spray on the biofilm of *C. albicans* adhering to self-cure acrylic resins.

MATERIALS AND METHODS

Preparation of acrylic discs:

In this experimental study, 120 self-cure acrylic discs with 15mm diameter and 5 mm thickness were prepared by mixing self-cure acrylic powder and the monomeric liquid (Acropars; Marlic Medical Industries Co., Tehran, Iran) as follows: The inner surface of the plastic molds was washed with water and dried and then covered with a thin layer of petroleum jelly. The acrylic powder and the monomer were poured into the molds in small amounts. After 15min, the discs were removed, and their surfaces were polished with 200, 600, and 1000-grit sandpapers (Matador, Melle, Germany). the acrylic discs were then immersed in saline (0.85% NaCl) for 48 h to remove the extra monomer. The discs were air-dried and then sterilized under UV-light for 24h. The other side was also exposed to UV-light for 24h. The rest of the process was done entirely under a sterile hood and next to the flame.

Preparation of yeast suspension:

The standard isolates of *C. albicans* (ATCC-10231) which is pathogenic to humans [22] were obtained from the Iranian Research Organization for Science and Technology and cultured in plates containing Sabouraud dextrose agar and chloramphenicol (Himedia,

Mumbai, India). The plates were incubated at 35°C for 48h. Pure single colonies collected from the fungal culture medium were used to prepare *C. albicans* suspensions with 1×10^{6} - 5×10^{6} cells/mL of saline. The final fungal suspension was prepared with a concentration of 0.5×10^{3}-2.5×10^{3} cells/mL by diluting the solution with twice that concentration (*i.e.*, 1×10^{3} –5×10^{3} cells/mL).

Determination of minimum inhibitory concentration (MIC) of ginger essential oil and nystatin by the microdilution method:

The MIC of ginger essential oil (concentration of 50000µg/mL) (Imen-Pakhsh-Daru, Mashhad, Iran) produced by the supercritical carbon-dioxide technique, and nystatin (Sigma, Steinheim, Germany) was determined as instructed by CLSI-M27-A3 using the microdilution method [23]. The steps of this process were as follows:

The microdilution procedure was performed using a 96-well plate with sterile round-bottom sealable wells and ginger essential oil at 0.1-50% (0.05mg/mL -25mg/mL) concentrations. The first 10 rows of the wells were used for dilution and the last 2 rows were used for positive and negative controls. The positive control wells contained 100µL of the medium (1640 RPMI, Biowest, Nuaille, France) and 100µL of the fungal suspension. The negative control wells contained 200µL of RPMI medium (this was the only well with 200µL of RPMI medium as all other wells contained only 100µL of this medium). For dilution, 100µL of 100% ginger essential oil (50000µg/mL) was poured into the first and second rows of wells. Then, 100µL of the contents of the second row were transferred to the third row wells and this process was repeated until the tenth row. Then, 100µL of the fungal suspension was added to each well (except the negative control). After 48h of incubation at 35°C, the wells were visually examined. The MIC was determined by comparing the turbidity of the wells based on the growth in the positive control (containing culture medium and fungi) and lack of growth in the negative control (containing culture medium only) wells. The same procedure was used to determine the MIC of nystatin.

Exposure of acrylic discs to fungal suspension and counting the grown colonies:

Each sample was placed in a test tube containing 6mL of *C. albicans* suspension (6-7×10^{6} CFUs/ml) and incubated at 37°C for 72h and then removed and washed with sterile distilled water (handling of the samples was done using sterile forceps). Twelve samples were randomly selected and rinsed with sterile distilled water 3 times each time for 1min. To separate the yeasts adhering to these samples, they were transferred to a Falcon tube containing 1mL of sterile saline and heavily mixed by a vortex machine at 800 rpm (Dena Gene Tajhiz, Tehran, Iran) for 5min. To determine the colony count per 1mL of the suspension (CFUs/mL), 10µL of the obtained suspension along with 90µL of sterile saline was cultured on Sabouraud dextrose agar and chloramphenicol and incubated for 48h at 37°C. The number of adhering colonies was determined based on the number of colonies grown on the plate per 1mL of the suspension.

Grouping of the samples and spraying:

The remaining 108 samples were divided into nine groups of 12:

Groups 1, 2, 3: 5, 10, and 15min of exposure to ginger essential oil at MIC

Groups 4, 5, 6: 5, 10, and 15min of exposure to nystatin at MIC

Groups 7, 8, 9: 5, 10 and 15min of exposure to distilled water

The nystatin solution, ginger essential oil, and distilled water were poured in sterile glass spray bottles. In each group, the acrylic discs were sprayed 5 times on each side from 5cm distance such that their surface was completely wet. After spraying, each disc was placed in a separate sterile sealed container, which contained a sterile gauze dipped in sterile distilled water, and kept at room temperature for the desired exposure time. Then, the steps described in the previous section (rinsing with distilled water) were followed to determine the number of colonies adhering to the acrylic plates.

Statistical analysis:

Data were analyzed by SPSS and non-parametric tests because of the non-normal distribution of data. Data were analyzed using

the Kruskal-Wallis test followed by the Dunn test with Bonferroni correction. Statistical significance was set at P<0.05.

RESULTS

The MIC of ginger essential oil was found to be 3.12% (v/v) that was equivalent to 1560µg/mL. The MIC of nystatin was found to be 4µg/mL. The mean number of *C. albicans* colonies was 10175 CFUs/mL before the exposure, and 542.86, 257.14 and 2866.67 CFUs/mL after the exposure to ginger essential oil and nystatin at their MIC and distilled water, respectively (Table 1).

Table 1. Number of colonies (CFUs/mL) in the groups

Groups	Mean	SD	Median	P*
No exposure	10175	10730.25	5400	<0.001
GEO (1560 µg/mL)	542.86	464.81	400	
Nystatin (4µg/mL)	257.14	247.67	200	
Distilled water	2866.67	2607.02	1700	

*Based on the Kruskal-Wallis test
SD: standard deviation; GEO: ginger essential oil

Based on the Dunn test, differences between the mean number of colonies before the exposure and after exposure to nystatin and ginger essential oil were significant (P<0.001), but this difference was not significant for distilled water (P=0.521). The mean number of colonies showed no significant difference after spraying with nystatin compared with ginger essential oil (P>0.05).

The number of colonies after spraying with distilled water was significantly more than that after spraying with nystatin and ginger essential oil (P<0.001).

Nystatin had the strongest disinfecting effect and removed 98% of all *C. albicans* colonies adhering to acrylic discs. However, ginger essential oil also showed desirable disinfecting power by removing 95% of *C. albicans* colonies. As shown in Table 2, the number of *C. albicans* colonies in ginger essential oil, nystatin, and distilled water groups showed no significant differences by the exposure time (P>0.05).

As shown in Figures 1 to 3, pairwise comparison of the subgroups with the same exposure times demonstrated that nystatin was significantly superior to ginger essential oil after 5min of exposure (P=0.035); whereas, this difference was not statistically significant after 10 and 15min of exposure (P>0.05).

DISCUSSION

This study investigated the efficacy of ginger essential oil spray for elimination of *C. albicans* colonies adhering to acrylic discs.

In the present study, the MIC of ginger essential oil against *C. albicans* was measured to be 1560µg/mL. The MIC of ginger essential oil was reported to be 2500µg/mL by Sharifzadeh and Shokri [23]. They used *C. albicans* isolated from an HIV-positive patient with oral candidiasis and produced the ginger essential oil by the distillation method.

Table 2. Number of colonies (CFUs/mL) in the groups by the exposure time

Exposure time	Groups		
	GEO (1560µg/mL)	Nystatin (4µg/mL)	Distilled water
5 minutes	727.27±525.53	200±180.9	3925±3176.36
10 minutes	508.33±501.73	258.33±296.82	2641.67±2536.8
15 minutes	408.33±331.54	318.18±260.07	2033.33±1751.01
P*	0.19	0.52	0.2

*Based on the Kruskal-Wallis test
GEO: ginger essential oil

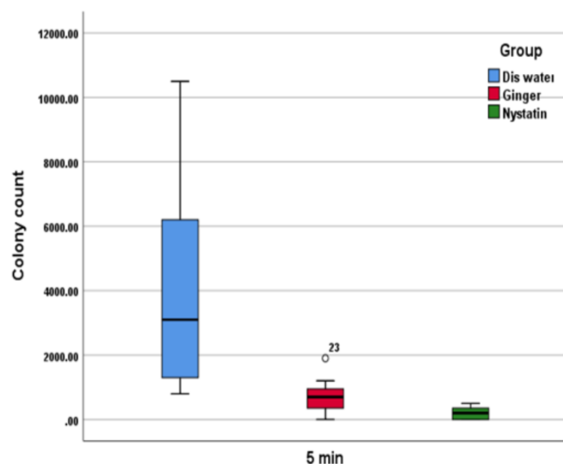

Fig.1. Number of *Candida* colonies after 5 minutes of exposure to the solutions (Dis: distilled)

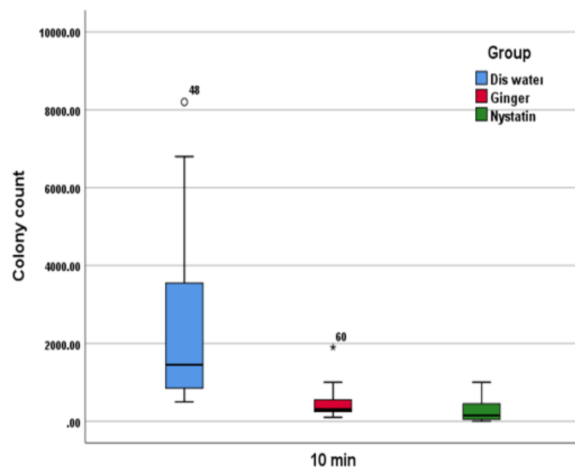

Fig. 2. Number of *Candida* colonies after 10 minutes of exposure to the solutions (Dis: distilled)

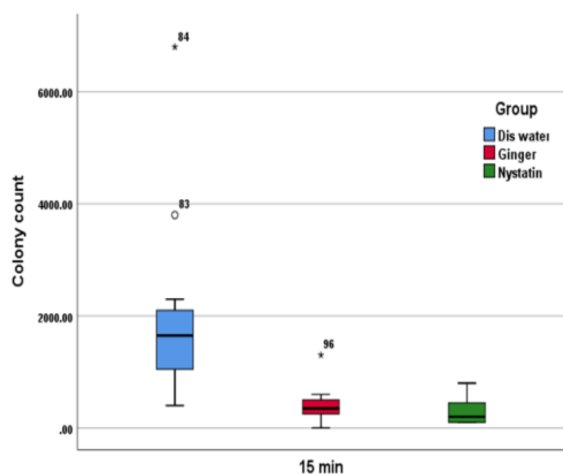

Fig. 3. Number of *Candida* colonies after 15 minutes of exposure to the solutions (Dis: distilled)

Aghazadeh et al. [24] reported the MIC value of the ethanolic extract of ginger against *C. albicans* (ATCC-10231) to be 1000µg/mL. Agarwal et al. [25] investigated the anti-*Candida* effects of 30 herbal essential oils and reported a MIC of 3% (v/v) for ginger essential oil. In their study, the ginger essential oil was produced by the distillation method and *C. albicans* was isolated from clinical specimens. In a study by Atai et al, [26] the MIC for the ethanolic extract of ginger against *C. albicans* (ATCC-10231) was measured to be 2000µg/mL. Different MIC values were reported in the literature can be due to differences in the type of medium or fungal strain, type of essential oil or extract, or the extraction method.

It should be noted that this study was performed on ginger essential oil produced by the supercritical carbon-dioxide technique and on the standard stain of *C. albicans* (ATCC-10231). The supercritical carbon-dioxide technique has multiple advantages over other extraction methods; most importantly, it has a relatively short processing time, does not need high-temperature heating (which helps preserve the physical and chemical properties of the substance), is environment friendly, allows the adjustment of temperature and pressure to separate the extract components and produce products with high quality and purity. However, it involves high-pressure processing and requires expensive equipment [27].

The findings of the present study showed that both ginger essential oil and nystatin caused significantly greater reductions in the number of *C. albicans* colonies than distilled water. But, nystatin was superior to ginger essential oil in decreasing the colony count, which was in agreement with the results of Atai et al, [26] and Uzama et al [28].

The MIC of ginger essential oil was measured to be considerably higher than that of nystatin (1560 vs. 4µg/mL). According to the literature, the antimicrobial activity of products is classified as strong, moderate and weak for MIC values up to 500µg/mL, between 500 to 1500µg/mL and above 1500µg/mL, respectively [29]. Regarding this classification,

the ginger essential oil in the present study showed a relatively moderate antifungal activity against *C. albicans* (ATCC-10231). Interestingly, spraying with this essential oil reduced a considerable portion of colonies adhering to the discs (95%) and had an acceptable antifungal effect. The authors think that the droplet pressure caused by spraying on the discs may facilitate the removal of microbial colonies.

Although the antifungal activity of nystatin was considerably higher than that of ginger essential oil, there was no significant difference between *C. albicans* colonies after spraying with nystatin compared with ginger essential oil.

One limitation of this study was the difficulty of using the commercial nystatin available on the market (100000 units/mL) which resulted in a foamy suspension that could not be sprayed. However, the commercial nystatin contains several additives, which means that the MIC determined for this product would not be the same as that of pure nystatin. Thus, this study was performed using a laboratory type nystatin.

There are several ways to assess the potential of solutions in cleaning the microbial plaque from the acrylic resins. In this study, this assessment was performed experimentally by creating *C. albicans* biofilm on acrylic resin fragments and measuring the number of viable *C. albicans* cells before and after disinfection, which is more accurate than other methods such as staining and weighing [30].

The results of this study showed that in addition to merits such as high speed, ease of use, needing smaller amounts of disinfectant and cost-effectiveness, the spraying method is highly practical and has good efficacy in removing *C. albicans* colonies. Thus, given the adverse effects of the immersion method on the acrylic resin structure, spraying can be considered as an excellent alternative method for disinfecting dental appliances [21].

Further studies with a larger sample size for comparison with the immersion method are recommended to investigate its effect at different times.

CONCLUSION

Overall, given the significant reduction in the number of *C. albicans* colonies after spraying of acrylic surfaces with ginger essential oil, it may be useful for development of disinfectant sprays for prevention of *C. albicans* growth.

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CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Hibino K, Wong RW, Hägg U, Samaranayake LP. The effects of orthodontic appliances on Candida in the human mouth. Int J Paediatr Dent. 2009 Sep;19(5):301-8.
2. Scully C, Porter S. ABC of oral health. Swellings and red, white, and pigmented lesions. BMJ. 2000 Jul 22;321(7255):225-8.
3. Silva PM, Acosta EJ, Jacobina M, Pinto Lde R, Porto VC. Effect of repeated immersion solution cycles on the color stability of denture tooth acrylic resins. J Appl Oral Sci. 2011 Nov-Dec;19(6):623-7.
4. Lee HE, Li CY, Chang HW, Yang YH, Wu JH. Effects of different denture cleaning methods to remove Candida albicans from acrylic resin denture based material. J Dent Sci. 2011 Dec 1;6(4):216-20.
5. Yildirim-Bicer AZ, Peker I, Akca G, Celik I. In vitro antifungal evaluation of seven different disinfectants on acrylic resins. Biomed Res Int. 2014;2014:519098.
6. Paranhos Hde F, Peracini A, Pisani MX, Oliveira Vde C, de Souza RF, Silva-Lovato CH. Color stability, surface roughness and flexural strength of an acrylic resin submitted to simulated overnight immersion in denture cleansers. Braz Dent J. 2013;24(2):152-6.
7. Pisani MX, Silva CH, Paranhos HD, Souza RF, Macedo AP. The effect of experimental denture

cleanser solution Ricinus communis on acrylic resin properties. Mater Res. 2010;13:369-73.
8. Amin F, Iqbal S, Azizuddin S, Afridi FI. Effect of denture cleansers on the color stability of heat cure acrylic resin. J Coll Physicians Surg Pak. 2014 Nov;24(11):787-90.
9. Alizadeh Z, Babaee N, Moghadamnia AA, Kazemi S, Sefidgar SA, Mortazavi N. Comparison of the effect of 2% ginger mouthwash and nystatin mouthwash on denture stomatitis. Int J Adv Biotechnol Res. 2016 Apr 1;7:1152-7.
10. Eslami H, Pakroo S, Maleki TE, Sadeghi N, Fakhrzadeh V. Is ginger (Zingiber officinale) mouthwash a convenient therapeutic for denture stomatitis?. Adv Biosci Clin Med. 2015 Jul 1;3(3):17-23.
11. Ali BH, Blunden G, Tanira MO, Nemmar A. Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): a review of recent research. Food Chem Toxicol. 2008 Feb;46(2):409-20.
12. Jain J, Tikare S, Mahuli A. Antifungal activity of ginger extract on Candida albicans: An in-vitro study. J Dent Sci Res. 2011;2(2):18-21.
13. Murugesan A, Sivapathasundharam B. Inhibitory eff ects of ginger extract on Candida albicans, Staphylococcus aureus and Lactobacillus acidophilus. Int Dent Med J Adv Res. 2016;2(1):1-5.
14. Haghpanah P, Moghadamnia AA, Zarghami A, Motallebnejad M. Muco-bioadhesive containing ginger officinal e extract in the management of recurrent aphthous stomatitis: A randomized clinical study. Caspian J Intern Med. 2015 Winter;6(1):3-8.
15. Chamani G, Zarei MR, Mehrabani M, Taghiabadi Y. Evaluation of effects of Zingiber officinale on salivation in rats. Acta Med Iran. 2011;49(6):336-40.
16. Patel RV, Thaker VT, Patel VK. Antimicrobial activity of ginger and honey on isolates of extracted carious teeth during orthodontic treatment. Asian Pac J Trop Biomed. 2011 Sep 1 (Suppl);1(1):S58-S61.
17. Maekawa LE, Valera MC, Oliveira LD, Carvalho CA, Camargo CH, Jorge AO. Effect of Zingiber officinale and propolis on microorganisms and endotoxins in root canals. J Appl Oral Sci. 2013 Jan-Feb;21(1):25-31.
18. Mahyari S, Mahyari B, Emami SA, Malaekeh-Nikouei B, Jahanbakhsh SP, Sahebkar A, et al. Evaluation of the efficacy of a polyherbal mouthwash containing Zingiber officinale, Rosmarinus officinalis and Calendula officinalis extracts in patients with gingivitis: A randomized double-blind placebo-controlled trial. Complement Ther Clin Pract. 2016 Feb;22:93-8.
19. Rashmi KJ, Tiwari R. Pharmacotherapeutic properties of ginger and its use in diseases of the oral cavity: A narrative review. J Adv Oral Res. 2016 May;7(2):1-6.
20. Infection control recommendations for the dental office and the dental laboratory. ADA Council on Scientific Affairs and ADA Council on Dental Practice. J Am Dent Assoc. 1996 May;127(5):672-80.
21. Oshagh M, Nazari Dashlibrun Y, Ebrahimi Saravi M, Bazargani A. Evaluation of Chlorhexidine and Zatariamultiflora essential oil in removing streptococcus viridans and candida from the surface of removable orthodontic appliances: A randomized clinical trial. J Mazandaran Univ of Med Sci. 2014 Mar 10;23(110):191-9.
22. Naeini A, Naseri M, Kamalinejad M, Khoshzaban F, Rajabian T, Nami H, et al. Study on Anti_candida Effects of Essential Oil and Extracts of Iranian Medicinal Plants, In vitro. J Med Plants. 2011 May 10;10(38):163-72.
23. Sharifzadeh A, Shokri H. Antifungal activity of essential oils from Iranian plants against fluconazole-resistant and fluconazole-susceptible Candida albicans. Avicenna J Phytomed. 2016 Mar-Apr;6(2):215-22.
24. Aghazadeh M, Zahedi Bialvaei A, Aghazadeh M, Kabiri F, Saliani N, Yousefi M, et al. Survey of the Antibiofilm and Antimicrobial Effects of Zingiber officinale (in Vitro Study). Jundishapur J Microbiol. 2016 Feb 7;9(2):e30167.
25. Agarwal V, Lal P, Pruthi V. Prevention of Candida albicans biofilm by plant oils. Mycopathologia. 2008 Jan;165(1):13-9.
26. Atai Z, Atapour M, Mohseni M. Inhibitory effect of ginger extract on Candida albicans. Am J App Sci. 2009;6(6):1067-9.
27. Skupien JA, Valentini F, Boscato N, Pereira-Cenci T. Prevention and treatment of Candida colonization on denture liners: a systematic review. J Prosthet Dent. 2013 Nov;110(5):356-62.
28. Uzama D, Ahmadu R, David MB, Thomas SA. Comparative study on the antifungal activities of aqueous Extracts of the rhizomes of ginger and the leaves of Eucalyptus saligna and Polyalthia longifolia. Res J Eng App Sci. 2012;1:51-3.
29. Ferreira MR, Santiago RR, Langassner SM, Mello JC, Svidzinsk TI, Soares LA. Antifungal activity of medicinal plants from Northeastern Brazil. J Med Plants Res. 2013 Oct;7(40):3008-13.
30. Dashtianeh M, Vatanara AR, Rezazadeh S. Supercritical carbon dioxide; an efficient solvent for herbal extraction. J Med Plants. 2017;16(Suppl. 11):1-1