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Original Article

The Antifungal Effect of *Thymus Vulgaris* on Isolated *Candida Albicans* from the Surface of Removable Orthodontic Appliances

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Abstract

Background and Aim: Orthodontic appliances can impair the balance of oral microflora and increase the load of certain microorganisms in the oral cavity. *Chlorhexidine* (*CHX*) is the gold-standard antimicrobial agent for plaque control. However, its long-term use induces adverse effects. The present study seeks to assess the effect of the essential oil of *Thymus vulgaris* on isolated *Candida albicans* (*C. albicans*) from removable orthodontic appliances.

Materials and Methods: In this study, thirty-two dental students were requested to use removable orthodontic appliances for 7 days. Subsequently, the acrylic base of the appliance was checked for the isolation of *C. albicans*. Furthermore, the effect of *Thymus Vulgaris* essential oil on isolated *C. albicans* were evaluated using the disc diffusion method, and then its minimum inhibitory concentration (MIC) was determined.

Results: The results of this study indicated that the essential oil of *Thymus vulgaris* was significantly more effective than CHX in the elimination of isolated *C. albicans* from the surface of orthodontic appliances ($P<0.05$). Moreover, its MIC was calculated to be 15.6 µL/mL.

Conclusion: The essential oil of *Thymus vulgaris* has favorable antimicrobial activity against *C. albicans* greater than that of CHX.

Keywords: Removable acrylic orthodontic appliance, *Thymus Vulgaris* essential oil, *Candida albicans*, Chlorhexidine mouthwash

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Introduction

Orthodontic treatments are performed to correct skeletal jaw discrepancies, dental crowding and orofacial myofunctional disorders. Due to the rising demand for facial esthetics, orthodontic treatment is gaining increasing popularity worldwide (1). Orthodontic appliances are used to correct dental and skeletal discrepancies in orthodontic treatments. The type of appliance may vary depending on the patient's

age, complexity of the treatment, and severity of the problem. In general, orthodontic treatments are divided into two main categories of fixed and removable orthodontics. In fixed orthodontics, orthodontic brackets are bonded to the teeth, and the patient cannot detach them from the teeth. In removable orthodontics, however, the patient can remove the appliance and place it again in the oral cavity. Removable orthodontic appliances are used to correct the relationship and position of the jaws as well as the jaw discrepancies. Moreover, they are used for simple tooth movements, and also to maintain the treatment results, and prevent relapses following the completion of fixed orthodontic treatment (2). The majority of patients need to use the removable orthodontic appliance 24 hours a day except the time of eating. The long-term 24-hour use of removable orthodontic appliances is imperative for an ideal outcome. Nevertheless, this pattern of use enhances the accumulation, growth and proliferation of microorganisms on the surface of removable orthodontic appliance (3). The oral environment provides suitable conditions for the colonization of various microorganisms (4). In a healthy oral cavity, microorganisms are in balance with the host. Any change in the normal condition of the oral cavity impairs this balance, and might cause illness (5). Orthodontic appliances can cause such alterations in the oral cavity (6). A removable orthodontic appliance creates a new retentive surface for the adhesion of oral microorganisms, and forms microbial biofilm on teeth surfaces and components of the appliance such as clasps, spring and acrylic base (7). Microbial plaque includes a microbial biofilm that forms on the surface of teeth, dental restorations, dental prostheses and orthodontic appliances. Indeed, microbial biofilm includes various microorganisms firmly adhering to each other and to the surface (8). Microorganisms are located in a matrix of proteins and polysaccharides (9). Orthodontic appliances alter the oral microflora and raise the load of pathogenic microorganisms such as *Streptococcus mutans*, lactobacilli and *Candida albicans* (*C. albicans*) in unstimulated saliva of patients (10-12).

C. albicans is known as a significant opportunistic pathogen because of its capability to adhere to various surfaces, create drug-resistant biofilms, and release proteinases and toxin (13). The significance of keeping removable orthodontic appliances *Candida*-free has been reported in a number of studies (14, 15). Oral candidiasis is associated with morbidity significantly, as it can induce chronic pain or discomfort upon mastication, and therefore restricting nutrition intake (16) The determination of the oral microbial changes in patients during orthodontic treatment is important, because some cases experienced long treatment period and the clinicians should maintain patients' oral health.

On the other hand, *Chlorhexidine* (*CHX*) is a gold standard chemical agent used for disinfecting removable orthodontic appliances (17). However, the long-term use of chemical agents has several side effects (18, 19). Nowadays, *Thymus vulgaris* is identified as a medicinal plant with antimicrobial activity and low toxicity (20, 21). Nevertheless, the antifungal effect of *Thymus vulgaris* on *C. albicans* isolated from the surface of removable orthodontic appliances has not been examined. The study seeks to investigate the effect of the essential oil of *Thymus vulgaris* on isolated *C. albicans* from removable orthodontic appliances.

Materials and Methods

Sampling

A total of 120 fourth, fifth and sixth-year dental students from Islamic Azad University, Shiraz Branch, were invited to enroll in this clinical trial. The ethical approval required to conduct the study was granted by the Medical Ethics Committee, Shiraz Branch, Islamic Azad university, Iran (No: 16310201951011). Before the enrollment, all students who were willing to participate in the study were interviewed, and their medical and dental history, oral and dental health status, occlusion, dental alignment, and oral hygiene were evaluated. Dental students with severe dental crowding, extensive diastema or periodontal disease, those using antimicrobial mouthwashes, those reporting antibiotic intake in the past 3 months, and subjects with poor oral hygiene were excluded. After applying the eligibility criteria, a total of 32 dental students were enrolled (12). All the participants signed written informed consent forms prior to their participation in the study.

A tray (Taksaan, Iran) was used to make an impression. Prior to impression making, the tray was autoclave-sterilized while the plastic bowl and the spatula were disinfected with 1% sodium hypochlorite for 6 minutes. Impressions were made using alginate impression materials (Hydrogum 5, Zhermack clinical, Italy). According to the manufacturer's instructions, 14 g of alginate powder was mixed with 30 mL of water. The impressions were made of the maxilla and disinfected with 1% sodium hypochlorite. Subsequently, the impressions were poured with dental stone. A 0.7 mm stainless steel wire was used for the fabrication of clasps. An auto-polymerizing acrylic resin was used to fabricate the acrylic base of the appliance. A simple removable orthodontic appliance was fabricated for the maxilla with Adams clasps on the first molars and first premolars of both quadrants. The acrylic base of the appliance was extended to the canine teeth in order not to affect esthetics. The appliances were disinfected using Deconex (Solarsit, Borer Chemie, Switzerland), and then were delivered to participants. The participants were requested to use the orthodontic appliance for 7 days (24 hours a day) and only remove it when eating. This action was carried out to simulate the typical use of orthodontic appliance by patients (Figure 1).

The participants were requested to brush their teeth three times after meals and also brush their removable appliances once a day. One week after using the appliance, the appliances were collected from the participants and sent to a laboratory.

Microbial Culture

Since the main objective of this study was the isolation of *C. albicans* from the surface of removable orthodontic appliances, Sabouraud dextrose agar culture medium (dextrose 40gm, peptone 10gm, agar 15gm, Chloramphenicol and Gentamicin contains 50.0 mg of chloramphenicol and 5.0 mg gentamicin, distilled water 1000ml) was used for the isolation of the organism (22). Samples were collected from three areas of the appliance which were in direct contact with oral mucosa using a sterile swab wetted with sterile distilled water: (I) the center of the appliance, (II) area close to the gingiva between the right first and second premolars, and (III) the area close to the gingiva between the left first and second premolars. The collected samples were then spread-cultured on Sabouraud dextrose agar. Subsequently, the plates were coded and incubated in aerobic conditions at 37°C for 24 to 48 hours, and the growth was evaluated.

Isolation, Purification and Detection of the Isolates

Since roughly all *C. albicans* species can grow on Sabouraud dextrose agar culture medium, creamy, smooth, pasty and convex colonies were suspected to be *C. albicans*, and subjected to further tests to confirm their identity (23). Of 32 samples collected from 32 appliances and cultured, 4 cases exhibited positive culture for *C. albicans* (plates #2, 5, 8 and 9), which were subjected to further confirmatory tests (Figure 2). Culture purification, staining and observation of morphology of the colonies under a light microscope confirmed the presence of large oval shape organism.

Identification of Isolates

In order to identify the isolates, Gram staining, germ tube production, and sugar fermentation of the isolates were evaluated. Germ tube reaction is one the most significant methods of detecting *C. albicans*, which was performed in our study. For this purpose, horse serum was transferred to a sterile test tube in sterile conditions under a hood, and then the isolates were added to the serum using a sterile inoculation loop. The growth and proliferation of hyphae in horse serum after 2-4 hours of incubation at 37°C were evaluated (24). Subsequently, one drop of this solution was applied on a slide and the germ tubes were inspected under a microscope (25). Furthermore, carbohydrate fermentation test was also carried out to detect *C. albicans*. For this purpose, the yeast isolates (Mc Farland) were identified using defined liquid media containing an indicator supplemented with different carbohydrates including sucrose, lactose, glucose and maltose (13gm/1000ml). The tubes were incubated at 37°C for 2 days, and the fermentation of carbohydrates was evaluated by noticing a color shift in the medium from red to yellow (23, 26).

Preparation of the Essential Oil of *Thymus vulgaris*

After extensive investigations to find *Thymus vulgaris* with higher concentrations of *thymol* and *carvacrol*, we obtained it from Ali Abad Tang cottage in Fars Province. In the present research, the plant was

identified at the Herbarium of Researches Center of Agriculture and Natural Resources of Fars province, Iran (Voucher No.: 12828). The collected plant was sent to the School of Agriculture of Shiraz University to extract its essential oil. The extracted essential oil was then analyzed using Gas chromatography/mass spectrometry (Agilent Technologies 5975C-MS,7890A, GC).

Determination of the Minimum Inhibitory Concentration (MIC) of *Thymus vulgaris* Essential Oil

Since the primary concentration of the pure essential oil was 100%, 1 mL of dimethyl sulfoxide was mixed with 1 mL of the primary essential oil. Subsequently, 1 mL of this solution was transferred to the second tube, and 1 mL of the solvent was added to it. This procedure was repeated to obtain 10 dilutions of the primary concentration. Eventually, the tubes had 1 cc of the essential oil with 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.39%, 0.19% and 0.09%, concentrations. The disc diffusion method was used in order to determine the MIC of the essential oil (27). For this purpose, 0.1 mL of the 24-hour culture of *C. albicans* (1 Mc Farland) in Sabouraud dextrose broth was collected by a pipette and was spread-cultured on a plate. Subsequently, the sterile discs were dipped at different concentrations of the essential oil and placed on the culture medium. The plates were then stored at room temperature for 30 minutes and incubated at 37°C for 24 hours. Finally, the discs which created no growth inhibition zone were considered as the MIC of *Thymus vulgaris* essential oil against *C. albicans*.

Sensitivity Testing of *C. albicans* to *Thymus vulgaris* Essential Oil, Chlorhexidine (CHX), Dimethyl Sulfoxide Solvent and Sterile Distilled Water Using Disc Diffusion Technique

Agar disc diffusion technique was used to assess the effect of *Thymus vulgaris* essential oil and CHX on *C. albicans* (22). In order to assess the inhibitory effect of *Thymus vulgaris* essential oil on *C. albicans*, initially one colony of pure *C. albicans* was collected by a sterile loop and transferred to a test tube containing Sabouraud dextrose broth and was well mixed. The tube was capped with sterile cotton pellet, and incubated at 37°C for 24 hours in order to obtain 1 McFarland standard Concentration. After the growth and proliferation of *C. albicans* species and reaching the adequate turbidity, 0.1 mL of the suspension containing *C. albicans* was collected by a pipette and swab-cultured on Sabouraud dextrose agar culture plate. Then, a series of sterile discs dipped in 0.12% *CHX*, 1.56% *Thymus vulgaris* essential oil, dimethyl sulfoxide solvent and sterile distilled water (as negative control) were placed on the plates. Finally, the plates were capped and incubated at 37°C for 24 hours (Figure 4).

Statistical Analysis

Data were analyzed using SPSS version 23 (SPSS Inc., IL, USA). Sample size was calculated according to the Cochrane's formula. The Kolmogorov-Smirnov test was applied to assess the normality of data distribution. One-way ANOVA was used to assess the impact of solutions on the diameter of growth inhibition zone. Furthermore, Tukey's post-hoc test was used to compare the inhibitory effects of the four tested solutions.

Results and Discussion

Fasting Blood Glucose Level

The results of this study indicated that all the four samples were subjected as *Candida albicans* with creamy, smooth, pasty and convex colonies. Moreover, the germ tube was observed for each isolate. Furthermore, all isolates were capable of fermenting glucose, maltose and sucrose, and caused a color shift to yellow but none of them could ferment lactose (Figure 5). The results showed that the MIC of *Thymus vulgaris* essential oil was the same for all the four *C.albicans* species isolated from the orthodontic appliances and was found to be 1.56% (6^{th} dilution). Table 1 indicates the antifungal effects of the essential oil.

The results of gas-chromatography/mass spectrometry revealed that the *Thymus vulgaris* essential oil had 43.7% thymol. Table 2 presents the main constituents of *Thymus vulgaris* essential oil. Furthermore, table 3 indicates the results of three repetitions of antimicrobial sensitivity testing by determining the diameter of the growth inhibition zone around discs dipped in 1.56% *Thymus vulgaris* essential oil, 0.12% *CHX*, dimethyl sulfoxide solvent and sterile distilled water.

One-way ANOVA (Table 4) revealed a significant

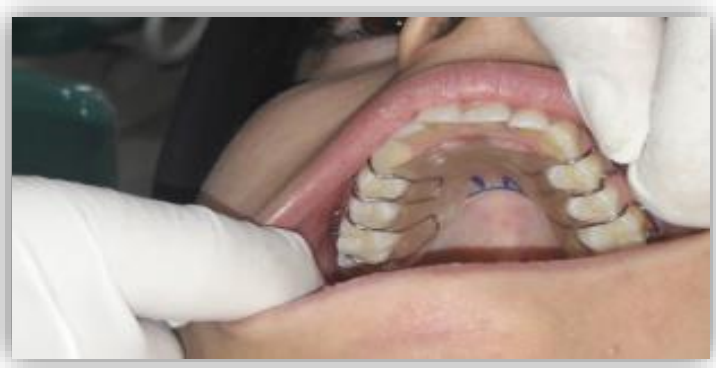

Figure 1. Placement and adjustment of removable orthodontic appliance.

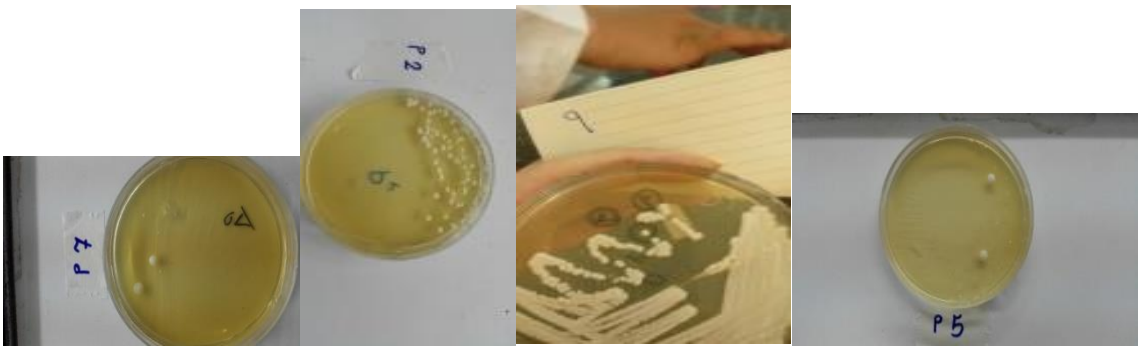

Figure 2. *C. albicans* isolates from Dentistry students.

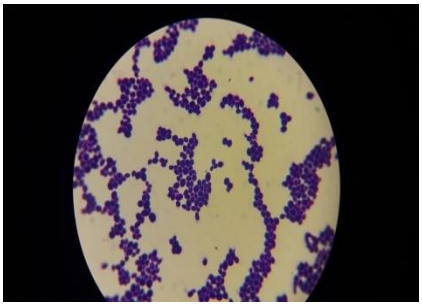

Figure 3. Presence of large oval shaped microorganisms under a light microscope.

difference in the inhibitory effect of the four tested solutions on *C. albicans* (P<0.001). Furthermore, Table 5 shows the pairwise comparisons of the solutions in this respect.

The use of removable orthodontic appliances increases the load of microorganisms such as *C. albicans* in the oral cavity and on the appliance surface (14). Many chemical and herbal products have been suggested to eliminate or decrease the count of microorganisms (28). The material used for the disinfection of orthodontic appliances must efficiently eliminate pathogenic microorganisms in a reasonably short period of time with no adverse effects on the appliance (29). *CHX* is one the most significant chemical agents used for the disinfection of removable orthodontic appliances; however, its long-term use has several side effects (18, 19).

Thymus vulgaris is a medicinal plant with antimicrobial activity (20). This plant has a favorable taste and odor, and is characterized by low toxicity (21). However, the antifungal effect of *Thymus vulgaris* on *C. albicans* isolated from the surface of

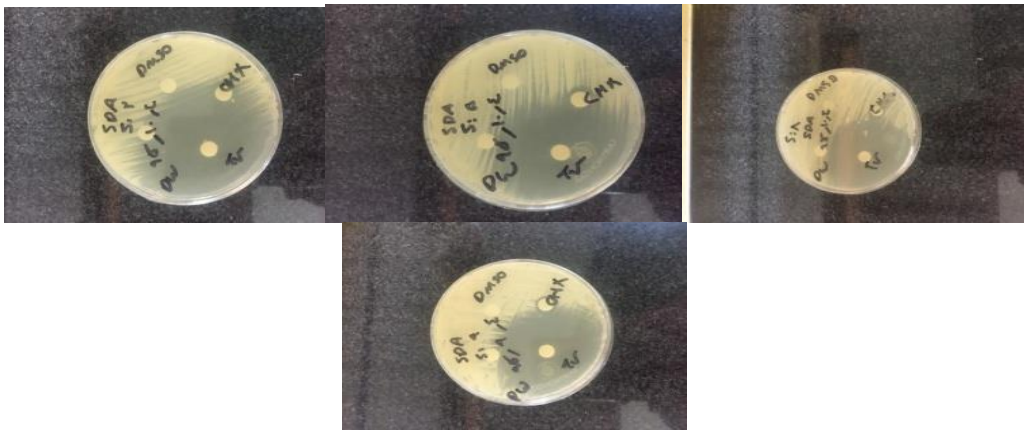

Figure 4. Sensitivity testing of *C. albicans* to *Thymus vulgaris* essential oil, chlorhexidine (CHX), dimethyl sulfoxide solvent and sterile distilled water using disc diffusion technique.

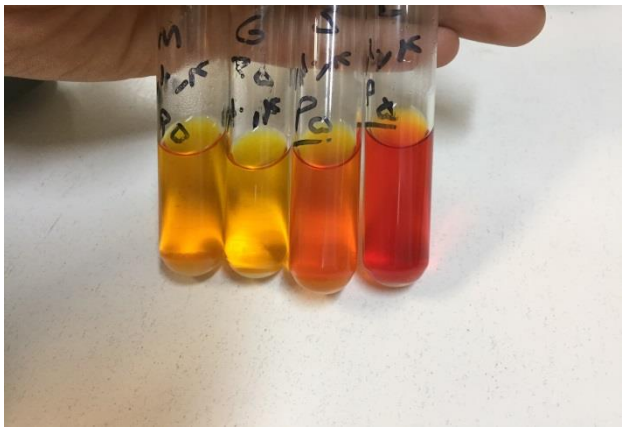

Figure 5. Capability of the isolates to ferment carbohydrates: from left to right: Maltose, glucose, sucrose and lactose.

removable orthodontic appliances has not been evaluated. Its MIC against clinical species of *C. albicans* isolated from the surface of acrylic appliances has not been determined either. Hence, this clinical trial sought to assess the antifungal effect of *Thymus vulgaris* essential oil on *C. albicans* isolated from the surface of removable orthodontic appliances in comparison with 0.12% CHX (as positive control) and sterile distilled water (as negative control). In the present study, the disinfection of appliances was performed by the spraying technique, instead of immersion, in order to prevent possible changes in the acrylic structure. Moreover, the spraying technique is more affordable and easier for the patient (11).

The MIC of *Thymus vulgaris* essential oil against *C. albicans* species isolated from the surface of removable orthodontic appliances was found to be 15.6 µL/mL in the present study. Thosar *et al.* reported the MIC of *Thymus vulgaris* essential oil to be 16 µL/mL (30). Nonetheless, they used the standard strain *C. albicans in vitro*. Jafari *et al.* reported the MIC of *Thymus vulgaris* essential oil to be 12.5 µL/mL against standard strain *C. albicans* inoculated on the surface of acrylic plates *in vitro* (31*)*.

The antimicrobial activity of plants is mainly attributed to the presence of phenolic compounds, saponin and flavonoids in their composition. Some of these compounds affect the cytoplasmic membrane or inhibit some fundamental enzymes in cell membranes of microorganisms, and exert their antimicrobial effects (32,33). *Thymol* and *Carvacrol* are among the main constituents of *Thymus vulgaris* essential oil, which are believed to be responsible for its antifungal

effects. *Carvacrol* increases the level of ATPase, and consequently inhibits enzymatic activity and non-specific permeability of bacterial cell membrane. As a result, the sensitivity of microorganisms to the penetration of foreign bodies increases (34). Hence, the difference in MIC of different essential oils of *Thymus vulgaris* could be attributed to different percentages of their constituents.

These results indicated that the antifungal activity of *Thymus vulgaris* essential oil was significantly greater than that of *CHX* (positive control). Moreover, sterile distilled water and dimethyl sulfoxide solvent had no antifungal effect on *C. albicans* species; while Oshagh *et al.* indicated that 25 µL/mL concentration of *Zataria multiflora* essential oil could effectively eliminate *C. albicans* accumulated on the surface of removable orthodontic appliances after one week (12). Nevertheless, 25 µL/mL concentration of *Zataria*

Table 1: Determination of MIC of *Thymus vulgaris* essential oil (the diameter of each disc was 7 mm, the diameter of growth inhibition zones is reported in millimeters).

Dilution number	Percentage of essential oil	inhibition zone appliance #2	inhibition zone appliance #5	inhibition zone appliance #8	inhibition zone appliance #9
1	50	60	60	60	50
2	25	50	45	45	45
3	12.5	30	40	35	40
4	6.25	13	12	18	15
5	3.125	10	9	10	12
6	1.56	8	8	8	8
7	0.78	0	0	0	0
8	0.39	0	0	0	0
9	0.19	0	0	0	0
10	0.097	0	0	0	0

Table 2: Main constituents of *Thymus vulgaris* essential oil using GCMass.

Constituents	Percentage (%)
Alpha-Phellandrene	1.30
Alpha-Pinene	5.65
Camphene	1.88
Beta-Pinene	0.42
Beta-Myrcene	1.35
Alpha-Terpinene	0.76
Cymene	16.96
Gamma-Terpinene	4.52
Cis-Sabinenehydrate	1.46
Camphor	1.16
Borneol	4.33
Alpha-fenchyl acetate	0.94
Thymol methyl ether	1.77
Thymol	43.7

Table 3: Mean diameter of growth inhibition zone (three repetitions, in millimeters).

Appliance number	Chlorhexidine	Sterile distilled water	Dimethyl sulfoxide	*Thymus vulgaris* essential oil
2	6.33±5.51	0	2.67±4.62	47.33±2.31
5	8.67±1.15	0	0	45.33±5.03
8	8±0.00	0	0	55.67±3.79
9	8.67±1.15	0	2.34±4.04	40.67±8.32

multiflora essential oil could not replace *CHX* for this purpose since *CHX* was significantly more effective in reduction of *C. albicans* colonies. The low antimicrobial efficacy of *Zataria multiflora* essential oil in their study might be due to the low percentage of Carvacrol and thymol in its composition, although they did not report the constituents of *Zataria multiflora* essential oil evaluated in their study.

The ability of *CHX* to bind non-specifically to protein and phospholipid moieties of the wall structure alters the surface tension of the cell wall structure and increase *C. albicans* cell permeability, and eventually might result in cell wall leakage and cell death (35, 36). However, the efficacy of *CHX* as an antimicrobial agent is affected by its exposure time (37). It had been found that Immersing acrylic appliances in *CHX* could remove 95.5 % viable yeast cells of *C. albicans* (38). In the present study, the lower antifungal activity of *CHX* compared to *Thymus vulgaris* might be related to the limited exposure time of *CHX* because of using the spraying technique, instead of immersion. Immersion of acrylic components in disinfecting solutions, which is capable of increasing exposure time in comparison to sparing, could lead to water sorption and adverse changes in the structure of acrylic resin (11).

Conclusion

The present study revealed that *C. albicans* species could be isolated from the surface of removable orthodontic appliances after one week of use. *Thymus vulgaris* had strong antifungal activity against *C. albicans* isolated from the surface of removable

Table 4: Comparison of diameter of growth inhibition zone around the four solutions.

Solution	Mean and standard deviation (mm)	P value
Chlorhexidine	8.58±0.90	<0.001*
Sterile distilled water	0.00	
Dimethyl sulfoxide	0.00	
Thymus vulgaris essential oil	47.25±7.27	

*Highly significant

Table 5: Pairwise comparison of diameter of growth inhibition zone around the four solutions.

Solutions	Mean difference	P value
Thymus vulgaris essential oil Vs chlorhexidine	38.67	<0.001*
Thymus vulgaris essential oil Vs sterile distilled water	47.25	<0.001*
Thymus vulgaris essential oil Vs dimethyl sulfoxide	47.25	<0.001*
Chlorhexidine Vs sterile distilled water	8.58	<0.001*
Chlorhexidine Vs dimethyl sulfoxide	8.58	<0.001*
Sterile distilled water Vs dimethyl sulfoxide	0.00	1.00

*Highly significant

orthodontic appliances with a MIC of 15.6 µL/mL. Hence, it can be used as an alternative to *CHX* in order to eliminate *C. albicans* from the surface of appliances.

Acknowledgment

None.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Steinberg D, Eyal S. Initial biofilm formation of Streptococcus sobrinus on various orthodontics appliances. J Oral Rehabil. 2004;31(11):1041-5.
2. Littlewood SJ, Tait AG, Mandall NA, Lewis DH. The role of removable appliances in contemporary orthodontics. Br Dent J. 2001;191(6):304-6.
3. Houston WJB, Isaacson KG. Orthodontic Treatment with Removable Appliances. 2 nd ed. Bristol: John Wright & Sons Ltd; 1980.
4. Lara-Carrillo E, Montiel-Bastida NM, Sánchez-Pérez L, Alanís-Tavira J. Effect of orthodontic treatment on saliva, plaque and the levels of Streptococcus mutans and Lactobacillus. Med Oral Patol Oral Cir Bucal. 2010;15(6):e924-9.
5. Menzaghi N, Saletta M, Garattini G, Brambilla E, Strohmenger L. Changes in the yeast oral flora in patients in orthodontic treatment. Prev Assist Dent. 1991;17:26-30.
6. Anhoury P, Nathanson D, Hughes CV, Socransky S, Feres M, Chou LL. Microbial profile on metallic and ceramic bracket materials. Angle Orthod. 2002;72(4):338-43.
7. Batoni G, Pardini M, Giannotti A, Ota F, Giuca MR, Gabriele M, et al. Effect of removable orthodontic appliances on oral colonization by mutans streptococci in children. Eur J Oral Sci. 2001;109(6):388-92.
8. Chertus V, Lon R. Dental Plaque Classification, formation, and identification. International Journal of Medical Dentistry. 2013;3:139-143.
9. Nobbs AH, Lamont RJ, Jenkinson HF. Streptococcus adherence and colonization. Microbiol Mol Biol Rev. 2009;73(3):407-50.
10. Kundu R1, Tripathi AM, Jaiswal JN, Ghoshal U, Palit M, KhandujaS. Effect of fixed space maintainers and removable appliances on oral microflora in children: An in vivo study J Indian Soc Pedod Prev Dent. 2016;34(1):3-9.
11. Lessa FC, Enoki C, Ito IY, Faria G, Matsumoto MA, Nelson-Filho P. In-vivo evaluation of the bacterial contamination and disinfection of acrylic baseplates of removable orthodontic appliances. Am J Orthod Dentofacial Orthop. 2007;131(6):11-7.
12. Oshagh M, NazariDashlibrum Y, EbrahimiSaravi M, Bazargani A, Evaluation of Chlorhexidine and Zataria Multiflora Essential Oil in Removing Streptococcus Viridans and Candida from the Surface of Removable Orthodontic Appliances: a randomized clinical trial. J Mazandaran Univ Med Sci. 2014;23(110):191-99.
13. Garcia-Cuesta C, Sarrion-Perez MG, Bagan JV. Current treatment of oral candidiasis: a literature review. J Clin Exp Dent. 2014;6(5):576-82.
14. Kundu R, Tripathi AM, Jaiswal JN, Ghoshal U, Palit M, Khanduja S. Effect of fixed space maintainers and removable appliances on oral microflora in children: an in vivo study. J Indian Soc Pedod Prev Dent. 2016;34(1):3-9.
15. Arendorf T, Addy M. Candidal carriage and plaque distribution before, during and after removable orthodontic appliance therapy. J Clin Periodontol. 1985;12(5):360-8.
16. Sherman R. G., Prusinski L., Ravenel M. C., Joralmon R. A. Oral candidosis. Quintessence Int. 2002;33:521-32.
17. Moshrefi A. Chlorhexidine. J West Soc Periodontol Periodontal Abstr. 2002;50(1):5-9.
18. Bishara Se, Damon PL, Olsen ME, Jakobsen JR. Effect of applying chlorhexidine antibacterial agent on the shear bond strength of orthodontic brackets. Angle Orthodontist. 1996;66(4):313-16.
19. Jafari AA, Falah-Tafti A, Lotfi-Kamran MH, Zahraeii A, Kazemi A. Vinegar as a removing agent of candida albicans from acrylic resin plates. Jundishapur J Microbiol. 2012;5(2):388-92.
20. Ahmad A, Khan A, Akhtar F, Yousuf S, Xess I, Khan LA, Manzoor N. Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against Candida. Eur J Clin Microbiol Infect Dis. 2011;30(1):41-50
21. Chitsazi M, Shirmohammadi A, Balayi E. Effect of herbal and chemical mouth-rinses on periodontal indices; comparison of Matrica, Persica and Chlorhexidine. J Dent. 2007;8(4):54-60.
22. ArbabiklatiF,SHerzaee M, Poorzamani M, Dabiri S. Inhibitory Effects of Plant Extracts Containing Thyme, Clove and Cinnamon Compared to Nystatin On Candida albicans (in vitro). J Res Dent Sci. 2012;8(4):175-9.
23. Chakrabarti A, Shivaprakash M, Ghosh A. Standard Operating procedures, Mycology Laboratories. Indian Council of Medical Research. 2016:5-47.
24. Byadarahally Raju S, Rajappa S. Isolation and identification of Candida from the oral cavity. Review Article. International Scholarly Research Network. 2011; 2011:487921.
25. Chandrabhan D, Hemlata R, Renu B, Pradeep V. Isolation of Dental Caries Bacteria from Dental Plaque and Effect of Tooth Pastes on Acidogenic Bacteria. Journal of Medical Microbiology. 2012;2:65-69.
26. Kali A, Srirangaraj S, Charles PM. A cost-effective carbohydrate fermentation test for yeast using microtiter plate. Indian J Med Microbiol. 2015 Apr-Jun; 33(2):293-5.
27. Yücesoy M, Guldaş NS, Yuluğ N. Disk diffusion method for fluconazole susceptibility testing of Can dida albicans strains. J Chemother. 2001;13(2):161-6.
28. Haas AN, Pannuti CM, Andrade AK, Escobar EC, Almeida ER, Costa FO, Cortelli JR, Cortelli SC, Rode SD, Pedrazzi V, Oppermann RV .Mouthwashes for the control of supragingival biofilm and gingivitis in orthodontic patients: evidence-based recommendations for clinicians. Braz Oral Res. 2014;28(spe):1-8.
29. Pavarina AC, Machado AL, Giampaolo ET, Vergani CE. Effects of chemical disinfectants on the transverse strength of denture base acrylic resins. J Oral Rehabil. 2003;30(11):1085-89.
30. Thosar N, Basak S, Bahadure RN, RajurkarM. Antimicrobial efficacy of five essential oils against oral pathogens: An in vitro study. Eur J Dent. 2013;7:71-7.
31. Jafari AA, Falah Tafti A, Hoseiny SM, Kazemi A. Antifungal Effect of Zataria multiflora Essence on Experimentally ContaminatedAcryl Resin Plates with Candida albicans. Iran Red Crescent Med J. 2015;17(1):e16552.
32. Shahnaz H, Hifza A, Bushra K, Khan JI. Lipid studies of Cuminum Cyminum fixed oil. Pak J Bot. 2004;36(2):395-401.
33. Shahsavari N, Barzegar M, Sahari MA, Naghdibadi H. Antioxidant activity and chemical characterization of essential oil of Buniumpersicum. Plant Foods Hum Nutr 2008;63(4):183-8.
34. Sharifian M, Bolhari B, Nosrat A, Aligholi M. The effect of

carvacerol on Enterococcus faecalis as an intracanal medicament-Invitro study. J Dent Med. 2009;1(22):35-40. (Persian)
35. Veerman EC, Nazmi K, Van't Hof W, Bolscher JG, Den Hertog AL, Nieuw Amerongen AV. Reactive oxygen species play no role in the candidacidal activity of the salivary antimicrobial peptide histatin 5. Biochemistry Journal. 2004;381:447–52.
36. Pusateri CR, Monaco EA, Edgerton M. Sensitivity of Candida albicans biofilm cells grown on denture acrylic to antifungal proteins and chlorhexidine. Archives of Oral Biology. 2009; 54:588–94.
37. Fathilah AR, Himratul-Aznita WH, Fatheen AR, Suriani KR. The antifungal properties of chlorhexidine digluconate and cetylpyr inidiniumchloride on oral Candida. J Dent. 2012;40(7):609-15.
38. Ghazal ARA, Idris G, Hajeer MY, Alawer K, Cannon RD. Efficacy of removing Candida.albicans from orthodontic acrylic ba ses: an in vitro study. BMC Oral Health. 2019;19(1):71.