Future Dental Journal

Volume 4 | Issue 2 Article 33

2018

Is immersion in mint oil or apple vinegar solution a valid antifungal approach for acrylic soft liners?

Mehtab H. Deyab
mehtab3184@gmail.com

Basma EL. Awady
basma.elawady@kasralainy.edu.eg

Nahed G. Bakir
nahed.bakir@dentistry.cu.edu.eg

Future Dental Journal 4 (2018) 302–307

Contents lists available at ScienceDirect

Future Dental Journal

journal homepage: www.elsevier.com/locate/fdj

Is immersion in mint oil or apple vinegar solution a valid antifungal approach for acrylic soft liners?

Mehtab H. Deyab[a,*], Basma EL. Awady[b], Nahed G. Bakir[c]

[a] Biomaterials Department, Faculty of Dentistry, Cairo University, Egypt
[b] Department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University, Egypt
[c] Biomaterials Department, Faculty of Dentistry, Cairo University, Egypt

ARTICLE INFO

Keywords:
Soft liner
Immersion
Mint
Apple vinegar
Antifungal

ABSTRACT

Objectives: In-vitro assessment of the validity of immersion in mint oil or apple vinegar solutions as antifungal approach for acrylic soft liners.
Materials and methods: Sixty disc-shaped specimens: 9 mm in diameter and 2 mm in length, and sixty cylinders: 12.5 mm in diameter and 20 mm in length of Vertex-Dental Heat-cured acrylic soft liner were prepared for antifungal activity and resilience measurements respectively. Specimens were divided into three groups; twenty in each, for immersion in mint oil, apple vinegar and distilled water (control). The groups were divided into four subgroups, five in each, for the different immersion periods: one day, one week, three weeks and six weeks. For each group, the daily immersion protocol was 8 h of immersion in the testing solution followed by 16 h in artificial saliva. This was repeated for each immersion period. Antifungal activity was assessed using disc diffusion method by measuring the inhibition zone for each disc twice: after 24 and 48 h incubation. Modulus of resilience was determined using a universal testing machine, where a stress-strain curve was obtained for each specimen and the area under the elastic portion of the curve was calculated.
Results: A significantly higher antifungal activity was revealed following immersion in mint oil compared to apple vinegar solution. The immersion period was a significant variable for the antifungal activity measured after 24 h following immersion in either solution whereas it was an insignificant variable for the antifungal activity measured after 48 h following immersion in apple vinegar solution. A significant reduction in the antifungal activity was noted as the incubation period was increased from 24 to 48 h except after six weeks immersion in apple vinegar solution. Modulus of resilience of the acrylic soft liner was adversely affected by immersion in mint oil solution for more than one day and in apple vinegar solution for more than one week.
Conclusions: Mint oil and Apple vinegar represent possible natural antifungal immersion solutions for acrylic soft liner provided that the immersion protocol is implemented properly.

1. Introduction

The main challenge in prosthetic dentistry is developing a biocompatible prosthetic material that is durable in the oral environment. Wearing dentures for a long time leads to resorption of alveolar and basal bones, thinning of oral mucosa and deterioration of salivary flow as well as oral perception. As the oral tissue becomes more fragile, old patients will become unable to cope with hard dentures. Therefore, using denture soft lining materials to moderate the effects of forces of occlusion on the supporting tissue is highly recommended [1,2].

Denture stomatitis, known as denture sore mouth, is related to an inflammatory lesion of the mucosa following the use of complete or partial removable prostheses with or without soft liner in approximately 60% of denture wearers. Poor oral hygiene, ill-fitting dentures and using denture liners are the most common contributing factors of denture stomatitis. Candida species; in particular Candida Albicans, one of the human oral microbial flora, is the main cause of developing such infection. The ability of Candida to adhere to mucosal tissues as well as to acrylic denture surfaces where it produces proteolytic enzymes facilitates penetration of Candida into the tissues and switching of the yeast to hyphae form. The reduced physiological cleaning properties of the tongue in denture wearers resulting from the reduced salivary flow, will also create a suitable environment for microbial survival and colonization in the oral cavity [3,4].

Peer review under responsibility of Faculty of Oral & Dental Medicine, Future University.
* Corresponding author.
E-mail addresses: mehtab3184@gmail.com (M.H. Deyab), basma.elawady@kasralainy.edu.eg (B.E. Awady), nahed.bakir@dentistry.cu.edu.eg (N.G. Bakir).

https://doi.org/10.1016/j.fdj.2018.05.002
Received 12 February 2018; Received in revised form 18 March 2018; Accepted 13 May 2018
Available online 22 June 2018

Since lack of denture cleanliness is considered as one of the important etiologic factors of denture stomatitis, different methods have been used to ensure optimal hygiene to denture-wearers. These include brushing with abrasives, soaking in disinfecting solutions, ultrasonic treatment or a combination of more than one of these methods [5,6].

Treatment of denture stomatitis has been attempted via using chemicals. Among these is chlorhexidine, which is active against gram-positive and gram-negative organisms as well as yeast. Although effective in the treatment of oral candidiasis, yet using chlorhexidine was accompanied with unpleasant side effects including staining of the tooth surface and its bitter taste [7].The use of topical antifungal agents such as Amphotericin B and Nystatin were also attempted. Both have unpleasant taste and lead to gastrointestinal side effects such as nausea, vomiting and diarrhea [8].

Due to the drawbacks associated with chemical antifungal cleansing solutions, using natural antifungal solutions may be thought off. Mint was used as a medicinal herb e.g. as an astringent, carminative, decongestant, expectorant, digestive, vasoconstrictor, anesthetic, antiseptic, antioxidant and as an antifungal [9]. In the dental field, using mint as an endodontic irrigant has been attempted and proved effective against enterococcus faccalis and candida albicans [10]. On the other hand, the medicinal application of apple vinegar in cleaning the wounds was based on its ability to inhibit the growth of fungi and bacteria [11]. In the dental field, apple cider vinegar showed decreased candida adherence to acrylic denture base, thus representing a possible therapeutic alternative for patients with denture stomatitis [12]. However, using mint and apple vinegar as natural antifungal solutions has not been adequately studied in the field of dentistry. Therefore, the aim of this study was to evaluate whether immersion in mint oil or apple vinegar solution is effective as an antifungal procedure for acrylic soft liner without compromising its resilience.

The null hypothesis stated that there is no significant difference in fungal activity of acrylic soft liner between immersion in mint oil and apple vinegar solutions.

2. Materials and methods

2.1. Extraction of mint oil from mint leaves

Mint oil was extracted from mint leaves by water and steam distillation at the Research Center of Medicinal Aromatic and Poisoned Plants, Faculty of Pharmacy, Cairo University. The extracted mint oil was then stored in capped glass tubes and refrigerated until used [13].

2.2. Determination of the minimal inhibitory concentration (MIC) of apple vinegar and mint oil solutions

MIC for both apple vinegar (National agricultural research center, Egypt) and extracted mint oil were determined by double fold serial dilution following Thosar N. et al., 2013 [14]. Multiple serial double fold dilutions of apple vinegar were obtained; test tubes were labeled from 1 to 7 with tube no.1containing apple vinegar in a concentration of 100%. Using a micropipette, 1 mL of distilled water was withdrawn and dispensed in each test tube from no. 2 to no. 7. Using another micropipette, 1 mL of apple vinegar was withdrawn from tube no.1, dispensed in tube no.2 and mixed thoroughly with the one ml distilled water. One ml of the mixture in tube no.2 was then withdrawn, dispensed in tube no 3 and mixed thoroughly with the 1 ml distilled water. The same steps were repeated for the next tubes. Finally, each tube from no.2 to no.6 contained 1 mL whereas tube no.7 contained 2 mL of apple vinegar solution in serial dilutions. Another tube containing 2 ml of distilled water was prepared and considered as the control.

The same procedure was followed for mint oil. In order to enhance the solubility of mint oil in distilled water, 0.05% (v/v) tween 80 emulsifier (EL Naser Pharmaceutical Chemicals CO. Egypt) was added to the distilled water used to dilute the mint oil following Marcos-Arias C.et al., 2011 [15]. An additional tube containing 2 ml of distilled water and tween 80 of 0.05% (v/v) was prepared and considered as the control.

The (MIC) of the different dilutions of mint oil and apple vinegar solutions was determined by the inhibition zone on Oxoid sabouraud dextrose agar plates (Oxoid, UK) inoculated by candida via well diffusion method.

2.3. Antifungal activity assessment

Vertex-Soft Heat curing soft acrylic resin liner (Vertex- Dental B.V. The Netherlands): pink opaque shade, Lot no. RQ163P01 was utilized for antifungal activity assessment. The composition of the material; as supplied by personal communication with the manufacturer, is citrate ester and methyl methacrylate. The material was supplied as powder and liquid. A total of sixty discs: 9 mm in diameter and 2 mm in length were prepared using the lost wax technique used for denture fabrication [16] [17], following manufacturer's instructions. The specimens were grouped into three groups; twenty discs in each, to be stored in the three different immersion media. These included Mint oil and apple vinegar solutions and distilled water as the control. Each group was divided into four subgroups, five specimens in each, based on the immersion period: one day, one week, three weeks and six weeks. Throughout these immersion periods, a daily immersion protocol was followed. This involved immersion in the immersion solution for 8 h followed by 16 h immersion in artificial saliva; prepared at Faculty of Pharmacy, Cairo University, following **Lata et al 2010** [18].

Candida was the fungal organism chosen in this study. The antifungal suspensions used were adjusted to match a turbidity of 0.5 McFarland in order to standardize the fungal suspensions used throughout the study [12]. Antifungal activity assessment was performed in accordance with **Kandil MMN et al 2009** [19] using Agar disc diffusion method. Each disc was placed in a separate agar plate and was incubated at 37 ± 2 °C. The inhibition zone (cm) was measured twice: after 24 and 48 h of incubation using a caliber.

2.4. Resilience testing

Sixty cylindrical specimens (twenty specimens in each group): 12.5 mm in diameter and 20 mm in length were prepared following **Gronet Pete et al 1997** [20]. The specimens were subjected to the same immersion protocol previously mentioned.

Each cylindrical specimen was compressed at a crosshead speed of 30 mm/min using an Instron universal testing machine (model 3345, England) and the data were recorded using computer software (BlueHill 3 software, version 3.3). A stress-strain curve was obtained for each specimen and the modulus of resilience was calculated as the area under the stress-strain curve until the proportional limit.

2.5. Statistical analysis

All data were reported as means and standard deviations (SD). Descriptive statistics was calculated using statistical package for social science (SPSS) program, version21. Independent T-test was used to test the effect of immersion solution after 24 and 48 h s incubation. One-way Analysis of variance (ANOVA) was used to test the effect of immersion period in each immersion solution. Mann Whitney-U test was used to compare the antifungal activity measured after 24 and 48 h s incubation to ensure robustness of analysis. Two-way ANOVA was used to test the effect of immersion solution and immersion period on modulus of resilience. Bonferroni post hoc test was used to test the effect of immersion period in each immersion solution. P-values ≤ 0.05 was considered significant.

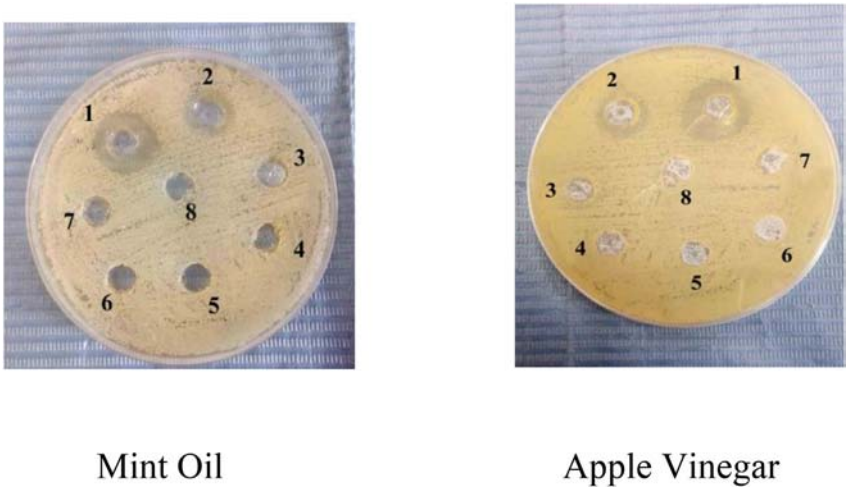

Fig. 1. Minimum and maximum inhibition zones produced by the different dilutions of mint oil and apple vinegar solutions. The numbers from 1:7 indicate double fold serial dilutions.

3. Results

3.1. MIC of mint oil and apple vinegar solutions

Photographs showing the inhibition zones of the different dilutions of mint oil and apple vinegar solutions as determined by well agar diffusion method; are presented in Fig. 1.

As shown in Fig. 1, the minimum inhibition zone produced by both mint oil and apple vinegar solutions corresponded to no. 2 (50% dilution).

3.2. Antifungal activity

All control subgroups showed no inhibition zone and therefore were not included in the tables of results antifungal activity.

3.2.1. Effect of immersion solution and immersion period on the antifungal activity measured after 24 h incubation

The antifungal activity, as measured by the inhibition zone, after 24 h incubation following immersion in mint oil solution was significantly higher than that revealed after immersion in apple vinegar solution at all immersion periods (Table 1).

As shown in Table 1, the antifungal activity following immersion in mint oil solution was the highest after three and six weeks immersion followed by that after one week immersion whereas the one day immersion showed the lowest antifungal activity. However, following immersion in apple vinegar, the highest antifungal activity resulted after three weeks immersion followed by one week and one day immersion periods whereas the lowest antifungal activity was revealed after six weeks immersion.

3.2.2. Effect of immersion solution and immersion period on antifungal activity measured after 48 h incubation

The results obtained for mint oil solution after 48 h incubation (Table 2) revealed significantly higher antifungal activity compared to apple vinegar solution following all immersion periods.

As shown in Table 2, following immersion in mint oil solution, the inhibition zones measured after 48 h incubation were significantly different between three and six weeks immersion. Both periods revealed significantly higher antifungal activity compared to one day and one week immersion periods. On the other hand, following immersion in apple vinegar solution, no significant difference in the antifungal activity was noted between all immersion periods.

Table 1
Means and standard deviations (cm) of the inhibition zones measured after 24 h s incubation following immersion in mint oil and apple vinegar solutions for different periods.

Immersion period	Immersion solution				
	Mint oil		Apple Vinegar		P_1^*-value
	Mean	SD	Mean	SD	
1 day	2.7^c	0.4	1.7^b	0.3	0.002*
1 week	3.4^b	0.2	1.5^b	0.3	< 0.001*
3 weeks	4.3^a	0.2	1.9^a	0.3	< 0.001*
6 weeks	4.3^a	0.2	1.4^c	0.1	< 0.001*
P_2^{**}-value	< 0.001**		0.022**		

Independent T-test results between immersion solutions, $P_1^* \leq 0.05$ is statistically significant.
One-way ANOVA test results between immersion periods, $P_2^{**} \leq 0.05$ is statistically significant.
Similar letters indicate non-significant difference within the same column.

Table 2
Means and standard deviations (cm) of the inhibition zones measured after 48 h incubation following immersion in mint oil and apple vinegar solutions for different periods.

> Immersion period	Immersion solution				
	Mint oil		Apple Vinegar		P_1^*-value
	Mean	SD	Mean	SD	
1 day	2.4^b	0.4	1.6^a	0.3	0.009*
1 week	2.2^b	0.2	1.3^b	0.1	< 0.001*
3 weeks	3.4^a	0.2	1.4^b	0.2	< 0.001*
6 weeks	3.8^a	0.3	1.3^b	0.2	< 0.001*
P_2^{**}-value	< 0.001**		0.072		

Independent T-test results between immersion solutions, $P_1^* \leq 0.05$ is statistically significant.
One-way ANOVA test results between immersion periods, $P_2^{**} \leq 0.05$ is statistically significant.
Similar letters indicate non-significant difference within the same column.

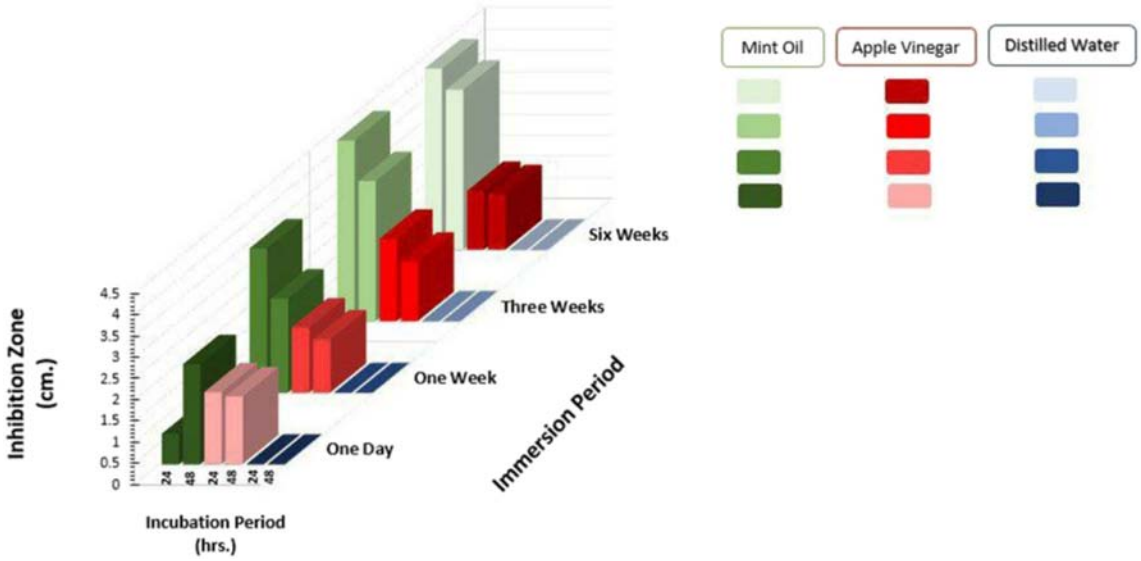

Fig. 2. 3-D Bar chart of the mean inhibition zones (cm) measured after 24 and 48 h s incubation following immersion in mint oil and apple vinegar solutions for different periods.

3.2.3. Effect of incubation period (24 vs. 48 hrs) on antifungal activity

The means and standard deviations of inhibition zones (cm) measured after 24 vs. 48 hrs incubation following the different immersion periods in each solution together with the results of Mann Whitney-U test are presented in Table 3.

The results of Mann Whitney-U test revealed that for both immersion solutions after all immersion periods, except after six weeks immersion in apple vinegar, the inhibition zones measured after 48 h incubation were significantly lower than those measured after 24 h incubation for the same group.

The 3-D bar chart (Fig. 2) summarizes the effects of immersion solution, immersion period and incubation time on antifungal activity.

Table 3
Means and standard deviations of antifungal activity measured after 24 and 48 h incubation following immersion in mint oil and apple vinegar solutions for different periods.

Incubation Period	Mint Oil		Apple Vinegar	
	Mean	SD	Mean	SD
One day immersion				
24 h	2.7	0.4	1.7	0.3
48 h	2.4	0.4	1.6	0.3
P- value	0.001*		0.034*	
One week immersion				
24 h	3.4	0.2	1.5	0.3
48 h	2.2	0.2	1.3	0.1
P- value	0.001*		0.031*	
Three weeks immersion				
24 h	4.3	0.2	1.9	0.3
48 h	3.4	0.2	1.4	0.2
P- value	< 0.001*		0.004*	
Six weeks immersion				
24 h	4.3	0.2	1.4	0.1
48 h	3.8	0.3	1.3	0.2
P- value	0.007*		0.099	

Mann Whitney *U* test results, *P ≤ 0.05 is statistically significant.

3.3. Resilience

3.3.1. Effect of immersion solution and immersion period on modulus of resilience

The results of modulus of resilience in MPa of the acrylic soft liner immersed in the three immersion solutions (Mint oil, Apple vinegar and distilled water) for different immersion periods are presented in Table 4 and Fig. 3.

Immersion solution was a significant variable for the modulus of resilience. Lowest modulus of resilience/immersion period curve (Fig. 3) was obtained after immersion in mint oil solution. Following one day immersion, no significant difference in modulus of resilience of acrylic soft liner existed between the apple vinegar and the control groups. After both one and three week-immersion periods, the apple vinegar group had the highest modulus of resilience followed by control group. However, after six weeks immersion, the control group showed the highest modulus of resilience followed by the apple vinegar group.

Modulus of resilience of the acrylic soft liner was adversely affected by immersion in mint oil solution for more than one day and in apple vinegar solution for more than one week.

4. Discusion

Denture stomatitis; due to fungal infection, is the major problem associated with dentures lined with soft liners. Therefore, several chemicals and therapeutic agents were recommended to disinfect the denture liners. Unfortunately, they had multiple drawbacks and limitations [21]. Nowadays, surface sealers can be used to reduce microorganism growth and biofilm formation. However, such sealers may contain hazardous components e.g. methyl ethyl ketone [22]. Therefore, it was desirable to search for safer natural antifungal products that have fewer side effects, as well as reasonable cost. The aim of this study was to assess the validity of immersion in mint oil and apple vinegar solutions as antifungal approach for acrylic soft liner.

In this study, extraction of mint oil from the plant material was carried out by steam distillation, one of the oldest and easiest methods

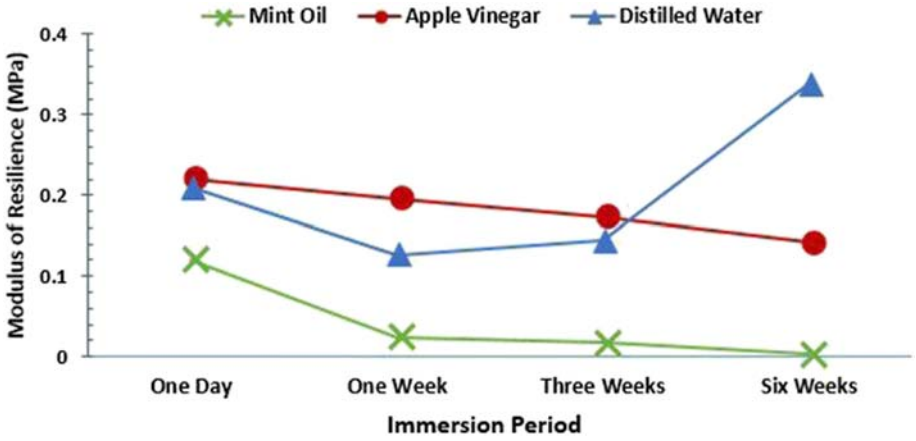

Fig. 3. Mean modulus of resilience of acrylic soft liner after immersion in the different solutions for different periods.

Table 4
Means and standard deviations of the modulus of resilience (MPa) of the acrylic soft liner following immersion in the three solutions (Mint oil, apple vinegar and distilled water) for different periods.

Immersion period	Immersion solution						
	Apple Vinegar		Mint oil		Distilled water		P_1*-value
	Mean	SD	Mean	SD	Mean	SD	
One Day	$0.220^{a,1}$	0.023	$0.119^{a,2}$	0.027	$0.210^{b,1}$	0.009	0.009
One Week	$0.196^{a\text{-}b,1}$	0.026	$0.025^{b,3}$	0.007	$0.127^{c,2}$	0.007	0.002
3 Weeks	$0.174^{b\text{-}c,1}$	0.012	$0.017^{b\text{-}c,3}$	0.003	$0.144^{c,2}$	0.013	0.003
6 Weeks	$0.142^{c,2}$	0.011	$0.003^{c,3}$	0.001	$0.338^{a,1}$	0.020	0.002
P_2**-value	<0.001		<0.001		<0.001		

One-way ANOVA test between immersion solutions, $P_1^* \leq 0.05$ is statistically significant.
Similar numbers indicate non-significant difference within same raw.
Bonferroni post hoc test between immersion periods, $P_2^{**} \leq 0.05$ is statistically significant.
Similar letters indicate non-significant difference within the same column.

to ensure purity of the extracted mint [23]. Mint oil produced by such extraction method produced the widest inhibition zone in a pilot study. The same was applicable with the utilized commercial apple vinegar (data not reported).

Immersion in the storage medium was carried out for 8 h followed by 16 h immersion in artificial saliva. The aim of this protocol was to mimic the common practice of immersing a denture for 8 h at night then wearing it for the rest of the day.

Since candida is the main cause of denture stomatitis and persistent infections, it was the fungal organism targeted in this study. Antifungal activity assessment was performed using disc diffusion method. Disc diffusion is the official method used in many clinical microbiology laboratories for routine testing. This may be attributed to its simplicity and ease to interpret the results. Disc diffusion is commonly used for the antimicrobial screening of plant extracts, essential oils and other drugs standards as documented by the Clinical and Laboratory Standards Institute (CLSI) [24]. The inhibition zone was measured after two incubation time intervals 24 and 48 h in order to determine whether the incubation period might have affected the antifungal activity after immersion in either mint oil or apple vinegar solutions [25].

Although many studies have suggested that natural antifungal compounds act by disrupting cytoplasmic membranes of candida, the specific mechanisms involved in their mode of action remain not fully understood [26] [27]. Ergosterol, which is specific to fungi, is the major sterol component of the fungal cell membrane that is responsible for maintaining cell function and integrity. The effect of mint and its main constituents (Menthol, and Menthone) on the structural and functional aspects of membrane integrity may arise from reducing ergosterol levels that ultimately cause cell death rendering mint fungicidal, **Neha Samber et al 2015** [28]. On the other hand, the mechanism of action of acetic acid, which is the main component of apple vinegar, is probably related to the reduced hydrogen potential (pH). This facilitates diffusion of the acid across the plasma membrane of candida cells acidifying the cell interior, and thus inhibiting nutrient transport [28]. Reduction in fungal adherence associated with apple vinegar and its probable fungistatic action may support their medicinal use. The significantly higher antifungal activity of mint compared to apple vinegar may be explained by the fungicidal effect of mint as opposed to the fungistatic action of apple vinegar [12] [27], [29].

The results of this study revealed that immersion in mint oil solution resulted in a significantly higher antifungal activity after 24 compared to 48 h incubation for all immersion periods. This may be due to the chemical nature of mint as a crystalline cyclic alcohol and to its volatile nature. However, this did not agree with the findings of **El-Masry Lalia et al 2010** [10]. who reported that the antifungal effect of mint increased as the incubation period was increased. This contradiction may be due to the fact that they used a different method for assessing the antifungal activity, the counting test. Similarly, the antifungal activity results following immersion in apple vinegar solution for the different immersion periods were significantly higher after 24 h compared to those after 48 h incubation (Table 3), which may have resulted from volatility of acetic acid.

The immersion period was a significant variable for modulus of resilience. Modulus of resilience of the acrylic soft liner was adversely affected by immersion in mint for more than one day and in apple vinegar for more than one week. On the contrary, an abrupt increase in

the modulus of resilience of the control group was evident after six weeks of immersion. The results of modulus of resilience are probably a combination of main features as material swelling, retention of water and loss of substances. The osmotic pressure of the external solution may affect water uptake of the material. Higher rate of loss of plasticizers as opposed to water sorption may be evident throughout the first three intervals. However, after three weeks of immersion, water uptake may be predominant. Water may have acted as a plasticizer that may have increased the modulus of resilience. Therefore, further investigations of water sorption and solubility of the acrylic soft liner following different periods of immersion in the tested solutions is recommended. Testing of other properties of acrylic soft liners e.g. tear strength, surface roughness as well as bond strength to denture base following immersion should be considered as well.

5. Conclusions

1) Mint oil and apple vinegar solutions represent possible natural disinfectants for acrylic soft liner.
2) Care should be taken not to repeat the immersion protocol of the tested acrylic liner for more than one day in mint oil solution and one week in apple vinegar solution otherwise modulus of resilience will be compromised.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or, not-for-profit sectors.

References

[1] Liao W. The chemical degradation of denture soft lining materials: a study of the interactions between denture soft lining materials and food simulating liquids Wen-Chien Liao thesis submitted to the university of London for the degree of doctor of philosophy Ce. 2006.
[2] Hashem MI. Advances in soft denture liners: an update. J Contemp Dent Pract 2015;16(4):314–8.
[3] Chladek G, Mertas A, Barszczewska-Rybarek I, Nalewajek T, Zmudzki J, Król W, et al. Antifungal activity of denture soft lining material modified by silver nanoparticles-a pilot study. Int J Mol Sci 2011;12:4735–44.
[4] Khan MA. Commercial and plant extract denture cleansers in prevention of Candida albicans growth on soft denture reliner: in vitro study. J Clin Diagn Res 2016;10(2):42–5.
[5] Nikawa H, Jin C, Makihira S, Egusa H, Hamada T, Kumagai H. Biofilm formation of Candida albicans on the surfaces of deteriorated soft denture lining materials caused by denture cleansers in vitro. J Oral Rehabil 2003;30(3):243–50.
[6] Nishi Y, Seto K, Kamashita Y, Kaji A, Kurono A, Nagaoka E. Survival of microorganisms on complete dentures following ultrasonic cleaning combined with immersion in peroxide-based cleanser solution. Gerodontology 2014;31(3):202–9.
[7] Epstein JB. Oral and pharyngeal candidiasis. Topical agents for management and prevention. Postgrad Med 1989;85(5):257–8. 263–5, 268–9.
[8] Webb BC, Thomas CJ, Willcox MDP. Candida-associated denture stomatitis. Aetiology and management: a review. Part 3. Treatment of oral candidosis 1998;43(4):3–8.
[9] Souto WM, Mourão JS, Barboza RR, Mendonça LE, Lucena RF, Confessor MV, et al. Medicinal animals used in ethnoveterinary practices of the “Cariri Paraibano”, NE Brazil. J Ethnobiol Ethnomed 2011;7(1):30.
[10] Elmansy LH. Evaluation of antimicrobial effect of different medicinal herbs against single species of enterococcus faccalis and candida albicans: an in-vitro study Master Thesis Endodontic Dep Univeristy; 2010 p. 87. CU Theses.
[11] Fukaya M, Park YS, Toda K. Improvement of acetic acid fermentation by molecular breeding and process development. J Appl Bacteriol 1992;73(6):447–54.
[12] Mota ACLG, de Castro RD. de Araújo Oliveira J, de Oliveira Lima E. Antifungal Activity of Apple Cider Vinegar on Candida Species Involved in denture stomatitis. J Prosthodont 2015;24(4):296–302.
[13] Organic Chemistry Laboratory. Steam distillation of an essential oil. 2013. p. 1–11.
[14] Thosar N, Basak S, Bahadure RN, Rajurkar M. Antimicrobial efficacy of five essential oils against oral pathogens: an in vitro study. Eur J Dermatol 2013;7(5):71–7.
[15] Marcos-Arias C, Eraso E, Madariaga L, Quindós G. In vitro activities of natural products against oral Candida isolates from denture wearers. BMC Compl Alternative Med 2011 Jan;11(1):119.
[16] de Castro DT, Holtz RD, Alves OL, Watanabe E, Valente ML, da Silva CH, et al. Development of a novel resin with antimicrobial properties for dental application. J Appl Oral Sci 2014;22(5):442–9.
[17] Radnai M, Whiley R, Friel T, Wright PS. Effect of antifungal gels incorporated into a tissue conditioning material on the growth of Candida albicans. Gerodontology 2010;27(4):292–6.
[18] Lata S, Varghese NO, Varughese JM. Remineralization potential of fluoride and amorphous calcium phosphate-casein phospho peptide on enamel lesions: an in vitro comparative evaluation. J Conserv Dent 2010;13(1):42–6.
[19] Monia K, Jaffer N, Shehab E. The effect of three coating materials on the candidal growth, on the surface and Color of A heat. Cure Acrylic Resin Denture Base 2009;9:279–88.
[20] Gronet PM, Driscoll CF, Hondrum SO. Resiliency of surface-sealed temporary soft denture liners. J Prosthet Dent 1997;77(4):370–4.
[21] 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;110(5):356–62.
[22] Report IC. DENTSPLY international inc. Business 2014;2:1–10.
[23] Mondello F, De Bernardis F, Girolamo A, Salvatore G, Cassone A. In vitro and in vivo activity of tea tree oil against azole-susceptible and -resistant human pathogenic yeasts. J Antimicrob Chemother 2003;51(3):1223–9.
[24] Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: a review. J Pharm Anal 2016;6(2):71–9.
[25] Kumar R, Shrivastava SK, Chakraborti A. Comparison of broth dilution and disc diffusion method for the antifungal susceptibility testing of Aspergillus flavus. Am J Biomed Sci. 2010;2(3):202–8.
[26] Palmeira-de-Oliveira A, Salgueiro L, Palmeira-de-Oliveira R, Martinez-de-Oliveira J, Pina-Vaz C, Queiroz JA, et al. Anti-Candida activity of essential oils. Mini Rev Med Chem 2009;9(11):1292–305.
[27] Ahmad A, Khan A, Akhtar F, Yousuf S, Xess I, Khan LA, et al. 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.
[28] Samber N, Khan A, Varma A, Manzoor N. Synergistic anti-candidal activity and mode of action of Mentha piperita essential oil and its major components. Pharm Biol 2015;53(10):1496–504.
[29] de Castro RD, Mota ACLG, de Oliveira Lima E, Batista AUD, de Araújo Oliveira J, Cavalcanti AL. Use of alcohol vinegar in the inhibition of Candida spp. and its effect on the physical properties of acrylic resins. BMC Oral Health 2015;15(1):52.