Original Article

Effect of plant extract denture cleansing on heat-cured acrylic denture base resin: An *in vitro* study

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Abstract

Aim: Various chemical solutions have been recommended for denture disinfection; however, the immersion effect on the properties of denture base resin may also have effect on strength of the resin material. The purpose of this *in vitro* study was to compare and evaluate the flexural strength of heat-cure acrylic denture base resin by combination method of denture cleansing and plant extract immersion.

Materials and Methods: In this study, ninety specimens of heat-cure acrylic denture base resin material were fabricated with dimensions of 65 mm × 10 mm × 3.3 mm. The specimens were divided into three groups, namely, Group I–III of thirty specimens each and were immersed in distilled water, denture cleanser, and thyme essential oil, respectively. The flexural strength of specimen was evaluated.

Results: Natural plant extract showed better flexural strength than immersed in denture cleanser and control group but statistically was not significant.

Conclusion: The study concluded that plant extract, namely, thyme essential oil, showed better flexural strength than combination method and can be used as denture cleanser.

Keywords: Fittydent®, flexural strength, thyme essential oil

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INTRODUCTION

Adequate mechanical properties, sufficient esthetics, hygiene, and easy handling are basic requirements for denture base polymers. In long-term use, denture base material can get colonized and infected by microorganisms.[1] Efficient and regular hygiene is important for long-term upkeep of complete denture and is indispensible for the general health of edentulous people. Denture can be cleaned mechanically, chemically, or by combination of these methods.[2] Different types of chemical solutions have been recommended for denture disinfection. An ideal denture cleanser should be biocompatible, bactericidal, fungicidal, harmless, and nontoxic to the structure of denture; should be effectively remove deposits; and should be easy to use.[3]

The hypothesis of the study lies in that plant essential oils and extracts that have been used in food preservation, pharmaceuticals, alternative medicine, and natural therapies for many years.[4] Natural products can be an alternative to synthetic chemical substances and the interest in medicinal plants as a source of antimicrobial agents has

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grown dramatically. Natural products and essential oils are promising therapeutic tools for oral infection. These oils are complex mixtures of volatile compounds obtained from plant with antioxidant and antimicrobial properties against wide range of pathogens, including *Candida albicans* and dermatophytes.

Mechanical properties of denture resin and its strength can be affected when dentures are immersed in certain cleansing and disinfecting solution. The assessment of transverse strength of acrylic resins reported as a reliable method to estimate resin behavior under different experimental condition.[5]

The aims and objectives of the study are as follows:

1. To evaluate the influence of combination method of denture cleaning on flexural strength of heat-cured acrylic denture base resin
2. To evaluate the influence of immersion in plant extract on flexural strength of heat-cured acrylic denture base resin
3. To compare the influence of combination methods of dental cleansing and plant extract immersion on flexural strength of heat-cured acrylic denture base resin.

MATERIALS AND METHODS

Preparation of specimens

In this study, 90 specimens of heat-cure acrylic denture base resin were fabricated with dimensions of 65 mm × 10 mm × 3.3 mm (according to ISO/FDIS 1567), for which wax patterns were made from the addition silicon impressions obtained from metal die. The wax patterns were flasked according to conventional technique ensuring complete closure between the counter parts of the flask. Dewaxing was carried out. The molds formed were immersed in hot water to remove any traces of petroleum or wax and also facilitate the application of separating medium. The mold cavities thus obtained were used for the fabrication of the specimens. Heat-cured acrylic resin was packed and trial done before final closure. After bench curing, polymerization was accomplished by immersion in water bath for 9 h at 74°C. To avoid distortions, the flask was cooled to room temperature and then deflasked. The acrylic samples were then retrieved, and finishing and polishing were done [Figure 1].

Minimal inhibitory concentration assay

The minimal inhibitory concentration (MIC) is defined as the lowest concentration of a substance that visibly inhibits the growth of an organism on dilution tubes or agar plates.

Figure 1: Retrieved acrylic samples

Dilution method is used to determine the minimal fungicidal concentration (MFC) value of thyme essential oil. This method involves the testing of microorganisms for their ability to produce visible growth in the media and measured for turbidity units using turbidimeter (photometrically using 530 nm). The method described is intended for testing clinically significant "fungus."

Dilution of the sample (thyme essential oil) to be tested is done by dilution method (10 ml of diluted solution is made by adding 1 ml of thyme essential oil, 1 ml of ethanol, 1 ml of 0.5% tween [it increases the solubility], and 7 ml of distilled water). This diluted solution was used in different concentrations in different test tubes of inoculated Nutrient Broth Media [Figure 2] to know the MFC value of thyme essential oil using turbidimeter. After 72 h, turbidity of test tube was checked and MFC of thyme essential oil was found to be 0.5 µl/ml. To make 1000 ml of diluted solution for immersion procedures, 5 ml of thyme essential oil, 5 ml of 0.5% tween, 80 ml ethanol, and 910 ml distilled water is added. This solution was used for immersion of Group III specimen.

Immersion of specimens

- Group I (control group): Specimens were immersed in distilled water for 15 min, 3 times a day for 10 days (simulating a 15 min, daily soaking for 30 days) changing distilled water for every immersion
- Group II (combination method): Specimens were brushed on all faces with a soft bristle toothbrush for 20 s [Figure 3], then were immersed in a container with 250 ml of distilled water, and one effervescent tablet of alkaline peroxide was dissolved in it for 15 min, 3 times a day for 10 days (simulating a 15 min, daily soaking for 30 days)
- Group III: Specimens were immersed in thyme

essential oil with MFC value of 0.5 µl/ml for 15 min, 3 times a day for 10 days.(simulating a 15 min, daily soaking for 30 days) changing solution for every immersion [Figure 4].

After immersion is completed, the specimens were subjected to three-point bending test

Measuring flexural strength–three-point loading

The flexural strength was measured using a three-point bending test in a Instron 3369 universal testing machine [Figure 5]. The specimens were subjected to flection until fracture. Three-point bending tests were carried out with a distance of 50 mm between the supporting points [Figure 6].

Flexural strength was calculated using the formula:

$$S = 3PL/2bd^2$$

Where, S is flexural strength, P is peak load applied, L is span length (50 mm), b is the specimen width (10 mm), and d is the specimen thickness (3.3 mm). Mean flexural strength was calculated in MPa.

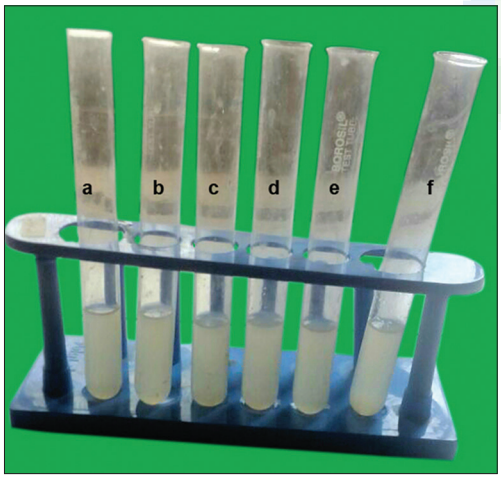

Figure 2: Minimal fungicidal concentration assay

Figure 4: Specimens immersed

RESULTS

Flexural strength results were analyzed statistically by analysis of variance (ANOVA) and unpaired t-test. For all tests, $P \leq 0.05$ was considered statistically significant. Mean ± standard deviation value of flexural strength of control was 86.45 ± 6.53, by combination method was 88.037 ± 14.3, and thyme essential oil was 90.27 ± 9.41 [Tables 1 and 2] (Group III > Group II > Group I).

ANOVA test for flexural strength (maximum stress) (P = 0.3759) [Table 3] and unpaired t-test for between groups showed insignificant ($P > 0.05$) [Table 4].

Table 1: Manufacturer details for all materials

Material	Manufacturer detail
Thyme essential oil	Deve Herbes, India
Fittydent®	Dr. Reddy's Pharmaceutical, India
Heat-cured acrylic denture base resin	DPI-India
Modeling wax	Hindusthan Products, India
Ethanol	Triveni Chemicals, Bombay
Tween 0.5%	SD Fine Chemical Ltd., Bombay

Figure 3: Brushing all the surfaces in combination method

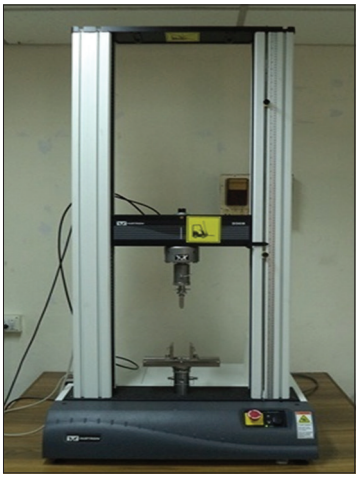

Figure 5: Universal testing machine

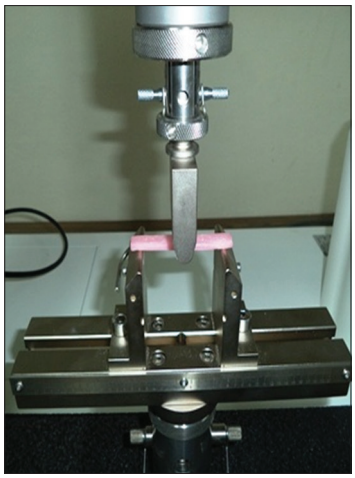

Figure 6: Three-point bending test

Table 2: Mean±standard deviation of all groups (Group III > Group II > Group I)

Variables	Mean±SD
Group I	
Maximum stress (flexural strength) (Mpa)	86.45±6.53
Group II	
Maximum stress (Mpa)	88.037±14.3
Group III	
Maximum stress (Mpa)	90.27±9.41

SD: Standard deviation

Table 3: Analysis of variance test

ANOVA	*F*	*P*	Result
Maximum stress (Mpa)	0.9895	0.3759	NS

ANOVA: Analysis of variance, NS: Not significant

Table 4: Unpaired *t*-test

Unpaired *t*-test	*P*	Result	CI	
			Lower boundary	Upper boundary
Group I versus Group II	0.582	NS	−4.155	7.334
Group I versus Group III	0.073	NS	−0.362	8.0097
Group II versus Group III	0.478	NS	−4.042	8.489

CI: Confidence interval, NS: Not significant

DISCUSSION

Complete denture wearers are associated with several problems which may be transient and may be essentially disregarded by the patient or they may be serious enough that the patient feels discomfort and unable to bear the denture and some patients fail to maintain denture sanitation. Nevertheless, several studies indicate that denture cleanliness and oral hygiene of denture wearers are generally poor, thereby facilitating the formation and accumulation of an oral biofilm, resulting to risk of denture stomatitis.[6] Denture cleanliness is, thus, of paramount importance to prevent the various oral diseases and also for treatment of denture stomatitis among edentulous patients. The combination of mechanical and chemical methods is routinely recommended for denture cleansing.

Higher and aromatics plants have been used and showed inhibition against bacteria, fungi, and yeasts. Plant-derived products as disease control agents have low mammalian toxicity, less environmental effects, and wide public acceptance.[7] Liu *et al*. had stated that thyme essential oil is the best among plant extracts.[8] Gutiérrez *et al*. had proved that thymol showed complete inhibition of microorganisms such as *C. albicans*, *Aspergillus flavus,*[9] and Goncalve had concluded that thyme oil was efficient against *Streptococcus mutans*.[10] Studies have been done on the thyme essential oil and its properties, but few studies have been done on the effect of thyme essential oil on denture base resin. Therefore, plant extract thyme essential oil was used in this study, and influence of it on flexural strength of heat-cured acrylic denture base resin was evaluated.

The denture is in constant contact with either water or oral fluids. They come in contact with water when the patient uses water as storing medium, but the contact with denture cleanser is limited to 15–20 min used for denture cleansing. In the present study, results obtained in combination method (88.037 ± 14.3) were in agreement with Sato et al.[11] where chemical method was used for denture cleansing 15 min/day for 30 days and concluded that there was no significant change in flexural strength which was found to be 86.75 ± 7.625 MPa and also was in agreement with Raj and D'Souza[1] where there is no change in the flexural strength when denture was continuously immersed in cleanser for 4 months and also with Paranhos Hde et al.[12] who concluded that overnight immersion in a denture cleansing solution simulating a year half of use did not alter the flexural strength which was 97.61 ± 11.09 MPa, since flexural strength depends on bulk of material, and insignificant change in flexural strength indicates that the bulk of the material remained intact from the influence of cleansers. ion method is not in agreement with Peracini *et al*.[13] and with Shah *et al*.[14] as there is decrease in flexural strength of the acrylic resin when immersed in peroxide solution according to their study also flexural strength obtained in plant extract immersion has slight higher values compared to combination method (90.27 ± 9.41).

CONCLUSION

Within the limitation of this *in vitro* study, it can be concluded:

1. Thyme essential oil being a natural plant extract may have absolutely no or very minimal side effect and can be used for denture cleansing without any effect of it

on flexural strength
2. A very low concentration of thyme essential oil (0.5 μl/ml) is sufficient for efficient denture cleansing which is viable in terms of cost and sensory characteristics
3. Finally, it can be concluded that thyme essential oil due to its antibacterial, antifungal, antiviral, and antioxidant property can fulfill some requirements of ideal denture cleanser.

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Conflicts of interest
There are no conflicts of interest.

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