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The evaluation of Rosemary Oil as an anti-candidal agent and its effect on the flexural properties of a thermoplastic denture base resin – An In Vitro Study

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Abstract

Background: Chemical denture cleansers play a vital role in maintaining the hygiene and serviceability of the dentures. They are of utmost importance in patients with compromised dexterity, who cannot use the physical methods of denture cleansing. Bacterial resistance to these chemical agents paved way to plant-extracts as novel denture cleansing agents. Several in vitro studies have verified the antifungal activity of oils against Candida albicans, with 8% Rosemary oil (Rosemarinus officinalis) being one such oil tested on acrylic resin. Candida biofilms had a greater growth upon polyamide resin compared to conventional PMMA and a more stringent disinfection protocol is required. However, the effect of rosemary oil as a denture cleanser on the physical properties of the thermoplastic denture base resin has not been evaluated.

Objectives: Evaluate the anti-candidal activity of 8% rosemary oil on thermoplastic denture base material (Valplast) and its effect on flexural strength of the material on immersion and to compare it with 1% sodium hypochlorite solution.

Methodology: In vitro, 18 acrylic resins were exposed to C. albicans and after a process of 30 minutes in immersion in the three groups, the specimens were washed and incubated for 24 hours in 37°C. The growth was determined by colony counting. Flexural strength assays were evaluated in 30 thermoplastic denture resin specimens distributed into three groups (1 test group and 2 control groups - distilled water and hypochlorite 1%), totaling ten specimens per group for each solution. Immersion was done for 30 days changing the cleanser solution every 8hrs simulating an 8 hour/day immersion for 90 days. The analyses were performed at the end of 30 days immersion. For comparisons between the groups in the disinfection test, ANOVA with post hoc Bonferroni were performed by the SPSS program.

Results: 8% Rosemary oil did not show similar anti-candidal activity as compared to 1% sodium hypochlorite on thermoplastic denture base resin – Valplast. At higher concentration of Candida albicans similar growth was seen in distilled water and rosemary oil whereas there was no growth seen when sodium hypochlorite was used. At lower concentrations rosemary oil showed mild anti-candidal activity but it was not comparable to sodium hypochlorite which showed complete inhibition of the yeast at all concentrations tested. Mean flexural strength was higher for sodium hypochlorite group (32.942 ± 2.467) followed by Rosemary oil (32.228 ± 3.548) and Distilled water (28.757 ± 2.403). After immersion in the respective solutions there was an increase in the flexural strength of the samples immersed in sodium hypochlorite and rosemary oil compared to that in distilled water. There was no statistically significant difference in flexural strength between the samples immersed in sodium hypochlorite and rosemary oil.

Conclusion: 8% rosemary oil influenced the flexural strength of thermoplastic denture base resin Valplast in the same manner as 1% sodium hypochlorite although the anti-candidal property of the essential oil was found to be inferior and non-comparable to 1% sodium hypochlorite which was proven to be successful in completely inhibiting the growth of yeast in all concentrations tested. The outcomes of the investigations have implications on the viability of 8% rosemary oil as a disinfectant solution for denture cleansers, more importantly for thermoplastic denture base resins.

Keywords: Denture Cleanser, Rosemary Essential Oil, Candida Albicans, Thermoplastic Denture Base Resin, Anti-Fungal Activity, Flexural Strength.

Introduction

Denture stomatitis also termed as chronic atrophic candidiasis or denture sore mouth is predominant in almost 11% to 67 % of complete denture users. Though the etiology of denture stomatitis is multifactorial, the influencing factors may include deficiency of dental hygiene, fungal infection primarily Candida infection, compromised immune system, dry mouth, continuous usage of denture for long periods, dietary factors and local irritating factors. Out of these, the most frequently encountered occurrence includes a pathogenic response to Candida infection commonly Candida albicans. [1]

The usage of nylon polyamide as a denture base material was introduced in the 1950s. Its use was restricted due to color change over time, high water sorption, increased surface roughness, staining after a few weeks and also processing difficulties. Improvised nylon polyamides exceeded its confines and are finding novel solicitations in the production of removable partial/complete dentures, occlusal splints etc. It is used as an alternative to poly-methyl methacrylate (PMMA) in circumstances where higher flexibility, flexural fatigue and higher impact strength is required. They also have the added advantage of imparting better esthetics, when non-metal clasps are required for retention and in those patients who exhibit allergy to PMMA. [2]

Amongst various methods, mechanical cleansing with a soft denture brush and water is considered an effective method for denture hygiene that contributes to a healthful mucosa as a denture bearing tissue surface. Insufficient cleaning by the elderly patients who are under long term hospitalization, or those who have poor dexterity allows pathogenic microbiota to multiply on dentures, thus serving as a niche for disseminating infections. In such situations, chemical denture cleansers can be an alternative to maintain denture cleanliness. [3]

An idyllic denture cleanser must be biocompatible, bactericidal and fungicidal, harmless to the edifice of denture, should efficiently remove organic and inorganic deposits and must be easy to use. Chemical denture cleansers are simple to use and efficacious in reducing biofilm formation both in vitro and in vivo. [4]

Denture cleansers can be Oxidising agents (Alkaline perborates), Reducing solutions (Sodium hypochlorite), Effervescent agents (Perborates, Carbonates), Chelating agents (EDTA), Detergents (Sodium polyphosphate), Enzymes (Protease, Amylase), and Disinfectants (Glutaraldehyde). The daily use of the most commonly used effective denture disinfectant solutions can distress the properties of the materials; toxicity (caused by glutaraldehyde), bleaching(sodium hypochlorite) high cost (peracetic acid), staining of teeth, changes in taste, formation of mucosal lesions, alteration in color of acrylic resin (chlorhexidine), alteration of resin dimensional stability (microwave irradiation) and impaired antimicrobial activity in the presence of proteins and other organic substances (acidic electrolyzed water). Therefore, newer mediators for disinfection remain to be tested as currently none can be reflected idyllic as to harmfulness, rate of action, effortlessness of use, variation of properties and cost efficacy. [5]

The extraordinary requirement for new antimicrobial and antifungal agents due to increased defiance shown by pathogenic microorganisms against drugs has strained attention to plant extracts and essential oils as a novel basis of antimicrobial and antifungal agents as they have been used for a wide assortment of resolutions for thousands of years and essential oils are impending sources of novel antimicrobial compounds. Fungal diseases of oral space are challenging to extravagate owing to the defiance to the existing antifungal mediators and to unsolicited responses offered by consumers of prosthetic devices. Owing to limits on the usage of synthetic antifungals, fresh yields are anticipated in an effort to minimalize these difficulties and permit the decontamination of dentures with harvests of an environmental origin. [6]

Rosemary (Rosmarinus officinalis L.), belonging to mint family Lamiaceae, is a communal dense, evergreen, aromatic shrub produced in several segments of the world. Traditionally, rosemary has been used as a therapeutic mediator to manage renal colic and dysmenorrhea. They have been assessed for their prospective uses as different therapies for the management of several contagious diseases. Due to the likely multiple endurances and draw backs of the synthetic antimicrobial, growing consideration has been focused towards environmental antimicrobial agents. [7]

Plant extracts have been expended for various reasons for centuries now and are a well-established source for novel antimicrobial compounds. Studies comparing various plant extracts have shown that rosemary essential oil is an antimicrobial agent possessing action against Candida albicans and other microorganisms and thus can be used for denture cleansing. [8,9,10,11,12]

Heidrich et al evaluated the in vitro pursuit of 8% rosemary versus Candida albicans, also the physical alterations of charecteristics in acrylic resins after dipping. They reported that 8% rosemary oil instigated a color transformation alike water and less colour and roughness changes when associated to 1% sodium hypochlorite. There was no growing of yeast clusters after dipping in rosemary oil which showed similar activity as 1% sodium hypochlorite. They concluded 8% rosemary oil has the prospective to be expended as an acrylic resin decontaminator. [13]

Candida biofilms had a greater growth upon polyamide resin compared to conventional PMMA resin. Sodium hypochlorite was found to be the more effective cleanser. They highlighted the importance of a more stringent disinfection protocol for a polyamide base denture due to the greater growth rate of biofilms upon its surface when compared to PMMA. [14]

Although the antimicrobial properties are established on acrylic resin, there isn't enough research done to evaluate its efficacy on thermoplastic denture base resins and the physical properties when used as a denture cleanser. The present study is to evaluate the anti-candidal activity of 8% rosemary oil on thermoplastic denture base material (Valplast) and its effect on flexural strength of the material on immersion and to compare it with 1% sodium hypochlorite solution.

Materials and Methods

Fabrication of the specimen

Dental stone was mixed as per the manufacturer's instructions for investing the injection molding Valplast flask. A powder:liquid ratio of 100mg of stone to 30ml of water was used to create the mold . Wax samples of apposite measurements was invested within the stone to craft a mold space for introduction of liquefied flexible denture base resin material. Wax sprues were ascribed to the samples and upper portion of flask was precisely placed and packed with stone under vibration to avoid air bubbles incorporation. After setting of the gypsum mold, wax elimination was performed below steaming water and the flask was unlocked. The stone mold was heated as per the company's directives. Resin was inserted in the mold space, by means of a manual injection unit. The flasks were bench cooled beforehand de-flasking and sprues cut using carborundum disk. Final finishing and polishing was done with silicon carbide discs and a polishing lathe.

Microbiological study

18 Valplast samples were subjected to Ethylene oxide sterilization prior to be used for the microbiological study. The specimens of Valplast were fabricated with dimensions of 10 mm diameter and 2 mm thickness. (fig 1)

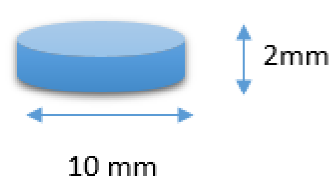
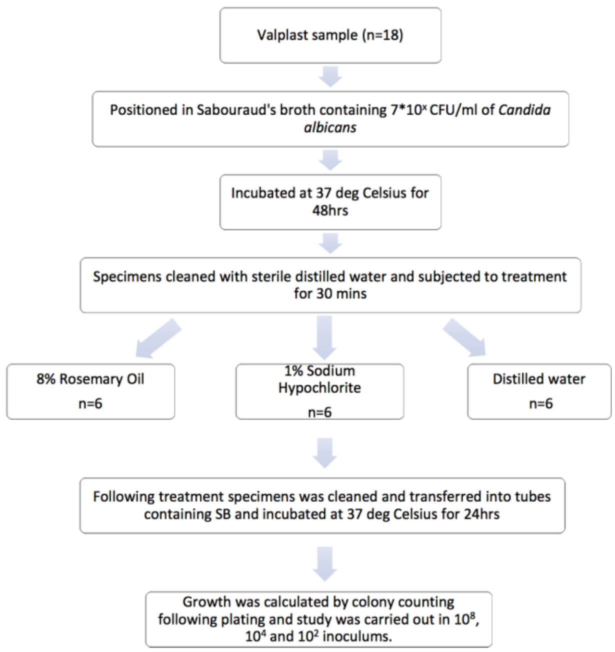


Figure 1

The study was conducted in the following steps:



Study for flexural strength

Wax samples constructed with the specifications of 64 × 10 × 2.5 mm were fabricated, according to ISO specification 1567. (fig 2)

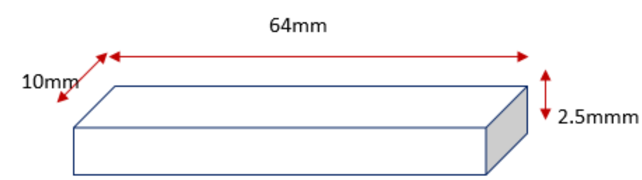
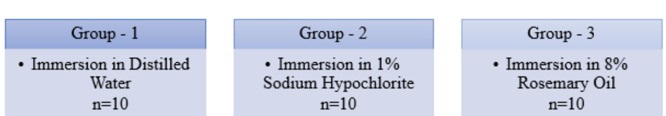


Figure 2

The 30 samples were divided into three groups with 10 samples each and were immersed in their respective solutions. Group 1 (control group): distilled water. Group 2 (Sodium hypochlorite):1% sodium hypochlorite Group 3 (Rosemary oil): 8% of rosemary essential oil solution.



Immersion was done for 30 days changing the cleanser solution every 8hrs simulating an 8 hour/day immersion for 90 days.

Flexural strength evaluation

The dimensions of each sample were confirmed before exposing them to testing. The samples were placed in a Universal Testing Machine (INSTRON 3366, Norwood USA), which imperiled the samples to a 3-point bending test.

Each sample was positioned in a 50mm – long support for 3-point flexural testing. A vertical load was then pertained at the mid-point of the specimen at a crosshead speed of 5mm/min on a load testing machine. Testing was carried out for each sample up to 5mm deflection, since the material in question is flexible in nature. Standards of flexural strength for each sample were documented in MPa.

Flexural testing was computed using the formula:

Flexural strength S= 3FL/2BH2 F= Maximum load (N), L= Span distance (20mm), B= Width (mm) of the specimen, H = Thickness (mm) of specimen.

Statistical Analysis

The statistical evaluation was accomplished using SPSS (Statistical package for social science, SPSS Inc.) version 20. Data was subjected to normalcy test (Shapiro-Wilk test) to check for distribution. One-way analysis of variance (ANOVA) test was done to compare the flexural strength.

Results

This in-vitro study evaluated the activity of 8% rosemary oil, 1% sodium hypochlorite and distilled water versus Candida albicans as well as the alterations in the mechanical property – Flexural strength of thermoplastic denture base material (Valplast) after immersion in these three solutions for 30 days, changing the solution every 8 hours thus simulating 8 hour/day immersion for 90 days.

The use of colony counting and INSTRON machine served to provide the evaluations of the samples. All the samples were measured for their thickness, width and length before being tested.

All the readings obtained were tabulated and subjected to statistical analysis for the comparison of flexural properties of denture base resin after treatment with three different disinfectant solutions.

Microbiological study results

Numerous in vitro studies have substantiated the antifungal action of oils against Candida albicans, with 8% Rosemary oil (Rosemarinus officinalis) being one such oil tested on acrylic resin.

This study was done to assess the in-vitro action of Distilled water, 1% sodium hypochlorite and 8% rosemary oil against Candida albicans tested on thermoplastic denture base resin - Valplast.

Growth was established by colony counting following plating and experimentation was completed in $7*10^8$, $7*10^4$ and $7*10^2$ CFU/ml of Candida albicans.

In $7*10^8$ CFU/ml of Candida albicans there was no significant difference in growth of Candida albicans in Distilled water and 8% Rosemary oil group. They showed similar growth >105 CFU/ml. There was no growth seen in 1% Sodium hypochlorite group.

In $7*10^4$ CFU/ml of Candida albicans there was significant difference in growth in all the three groups. Distilled water showed highest growth - >105 CFU/ml followed by 8% Rosemary oil-75000 CFU/ml whereas there was no growth seen in 1% Sodium hypochlorite.

And in $7*10^2$ CFU/ml of Candida albicans there was significant difference in growth in all the three groups. Distilled water showed highest growth – 75000 CFU/ml followed by 8% Rosemary oil – 1000 CFU/ml and there was no growth seen in 1% Sodium hypochlorite.

Dilution	Growth of *Candida albicans* (CFU/ ml)		
	Distilled water	8% Rosemary oil	1% Sodium hypochlorite
$7*10^8$	$> 10^5$	$> 10^5$	NO GROWTH
$7*10^4$	$> 10^5$	75,000	NO GROWTH
$7*10^2$	75,000	1000	NO GROWTH

Table 1

Results for study of flexural strength

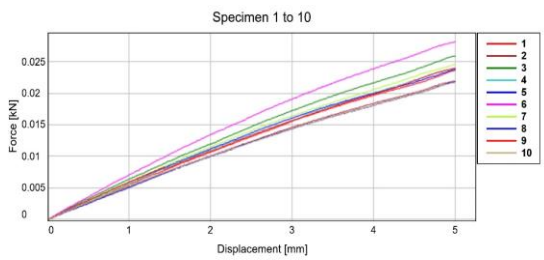
Specimens of denture base material were constructed accordance to the stated guidelines in relation to measurements. 30 specimens were divided in three groups of 10 specimens each for immersion in the respective solutions.

Mean and standard deviation of Maximum flexural stress (MPa), Flexural load at maximum flexural stress (N) and Young's modulus(MPa) for thermoplastic resin samples immersed in Distilled water, 1% Sodium hypochlorite and 8% Rosemary oil are given in table.

Distilled Water Group:

Specimen	Maximum Flexure stress [MPa]	Maximum Force [N]	Young's Modulus [MPa]
1	28.38	23.65	1098.57
2	26.31	21.93	1051.85
3	31.12	25.94	1281.65
4	28.79	23.99	1132.78
5	28.52	23.76	1182.32
6	33.86	28.22	1415.86
7	29.47	24.56	1171.87
8	26.24	21.87	1022.50
9	28.83	24.02	1199.84
10	26.05	21.71	1084.49
Mean	28.76	23.96	1164.17
Standard deviation	2.40	2.00	116.97

Table 2

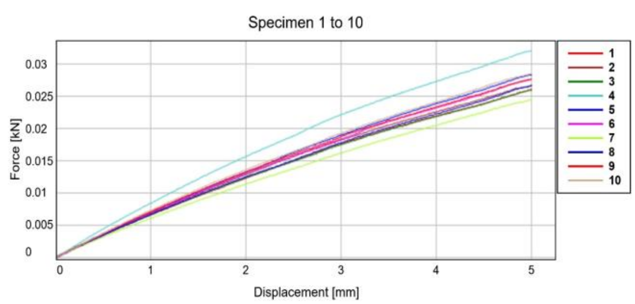


Graph 1

Sodium Hypochlorite Group

Specimen	Maximum Flexure stress [MPa]	Maximum Force [N]	Young's Modulus [MPa]
1	31.35	26.13	1347.46
2	32.01	26.68	1390.08
3	31.21	26.01	1309.62
4	38.57	32.14	1748.77
5	34.07	28.39	1428.48
6	33.26	27.72	1383.96
7	29.39	24.49	1231.94
8	32.04	26.70	1376.42
9	33.24	27.70	1467.64
10	34.28	28.57	1509.98
Mean	32.94	27.45	1419.43
Standard deviation	2.46	2.05	139.61

Table 3

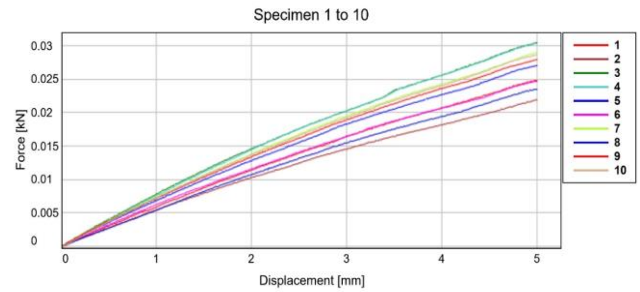


Graph 2

8% Rosemary Oil Group

Specimen	Maximum Flexure stress [MPa]	Maximum Force [N]	Young's Modulus [MPa]
1	29.88	24.90	1206.55
2	26.34	21.95	1092.36
3	36.59	30.49	1571.80
4	36.50	30.42	1524.25
5	32.46	27.05	1391.92
6	29.63	24.69	1272.52
7	34.78	28.98	1486.07
8	28.20	23.50	1126.81
9	33.51	27.92	1461.90
10	34.39	28.66	1504.16
Mean	32.23	26.86	1363.83
Standard deviation	3.55	2.96	175.43

Table 4



Graph 3

Test of normality

Data was subjected to normality test - Shapiro-wilk test. Data showed normal distribution, hence parametric tests ANOVA with post-hoc Bonferroni were applied.

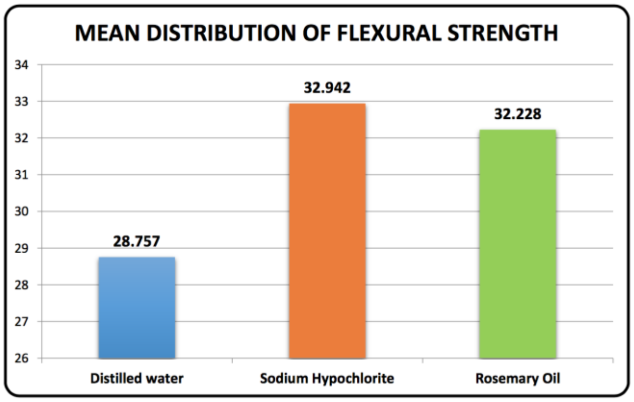
Test of Normality							
Groups		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
1	Flexural strength	.188	10	.200*	.897	10	.204
2	Flexural strength	.194	10	.200*	.911	10	.285
3	Flexural strength	.146	10	.200*	.938	10	.533
*. This is a lower bound of true significance.							
a. Lilliefors Significance Correction							

Table 5

Mean flexural strength was higher for sodium hypochlorite group (32.942 ± 2.467) followed by Rosemary oil (32.228 ± 3.548) and Distilled water (28.757 ± 2.403).

Groups		Minimum	Maximum	Mean	Std. Deviation
1	Distilled water	26.05	33.86	28.757	2.403
2	1% Sodium Hypochlorite	29.39	38.57	32.942	2.467
3	8% Rosemary Oil	26.34	36.59	32.228	3.548

Table 6



Graph 4

Comparison of flexural strength among the groups using ANOVA

ANOVA test was applied to compare the flexural strength among the three groups of distilled water, 1% sodium hypochlorite and 8% rosemary oil. ANOVA test showed statistical significant difference between the groups (p=0.006).

	F value	P value
Flexural Strength	6.14	0.006*

Table 7

Post Hoc Bonferroni Test:

Groups	Groups	Mean Difference	p value
Distilled water	Sodium Hypochlorite	-4.18	.009*
	Rosemary Oil	-3.47	.034*
Sodium Hypochlorite	Rosemary Oil	.714	1.000

Table 8

Post hoc Bonferroni test was applied to associate the flexural strength between the three groups.

Statistical significant difference was seen between distilled water and Sodium hypochlorite(p=0.009) & also between Distilled water and Rosemary oil (p=0.034) There was no statistical significant difference seen between Sodium Hypochlorite and Rosemary oil (p=1.00) with respect to flexural strength

The results displayed that after immersion in the respective solutions there was increase in the flexural strength of the samples immersed in sodium hypochlorite and rosemary oil compared to that in distilled water.

There was no statistically significant difference in the flexural strength between the samples immersed in sodium hypochlorite and rosemary oil.

Discussion

Oral cavity ports plethora of microorganisms, of which most are harmless commensal, with the potential of turning into pathogenic in response to alterations in the oral environment. Fungal diseases are closely associated with the health of individuals. The progress in age and decline in immunity capitulate the individuals wearing removable prosthesis to a condition known as Denture Stomatitis. It is a common occurrence in denture wearers and is linked with a multitude of factors, but usually found associated with Candida species, especially Candida albicans. [15] In the oral cavity maintaining an effective concentration of anti-fungals is very challenging owing to the fact that saliva continuously flows and wipes the oral cavity. Therefore, immersion disinfection is important to maintain the hygiene of the prosthesis. The treatment modalities have now shifted to natural alternatives which are cheap, diverse and easily available with less adverse effects. [16]

Chemical denture cleansing methods are often used in addition to physical cleaning methods to maintain the denture cleanliness and hygiene. Geriatric patients with severe debilitating conditions, impaired manual dexterity and neuromuscular dysfunction tend to depend entirely on the chemical methods for denture maintenance. Traditional chemical denture cleansers although being effective anti- microbials are being replaced with natural plant extracts due to the developing resistance shown by microbes against them. [3]

Duration of immersion in the denture cleanser was chosen to be mimicking the overnight immersion (8 h) which signifies a common state, recommended by dentists for patients to remove dentures during the resting period and immerse in solutions for relieving the underlying tissues and clean the dentures.

The main necessity of denture base material is acceptable physical and mechanical properties. High flexural and impact strength produce a prosthesis that is long lasting, sturdy and more defiant to destruction which is of more importance when prostheses are used in high-stress conditions.

The intrinsic flexibility of nylon dentures gives such materials advantages over conventional PMMA prostheses. A major advantage is the ability to flex and engage unfavourable undercuts where a orthodox denture base may be dreadful to insert. They may be caused by bony protuberances, severe angulation of teeth, cancer resection or cleft palate. An important property in this respect is elastic memory, permitting the prosthesis to go back into its initial form after flexing over an undercut. Flexible dentures also use this property in the retentive 'fingers' which behave similarly to clasps in the retention of the prosthesis. Also, translucency of these 'clasps' make them more aesthetic than conventional metal clasps. [2]

Agreeing to Lambert et al the usage of natural oils might display alterations in their structure, chiefly due to their environmental origin, obtained from the plant, being open to genetics, weather, soil and type of nourishment also the method of oil abstraction, which validate the variances discovered in the studies. [17]

There are various reports on the relationship of Rosmarinus officinalis (rosemary) against various microorganisms, demonstrating its antibacterial and antifungal potential. Numerous in vitro reports have verified the anti-fungal action against C. albicans of rosemary oil (Rosmarinus officinalis). [8,10,11,12,13]

According to Heidrich et al, [13] there was not at all development of yeast clusters in PMMA following dipping in 8% rosemary oil and its anti-candidal effect was comparable to 1% sodium hypochlorite which is considered as a gold standard material. 8% rosemary oil might be used to sanitize dental prostheses, as 30 mins of dipping in these liquids were adequate to attain complete reticence of C. albicans. They concluded that 8% rosemary oil has the prospective to be used as an acrylic resin disinfectant establishing the protocol of disinfection. The 8% rosemary oil displayed a statistically alike color alteration to water and a smaller roughness variation than 1% sodium hypochlorite on the acrylic resin, which is a significant outcome, since it produces fewer modification in acrylic resin when associated to 1% hypochlorite sodium, one of the utmost expended disinfection mediators in dental exercise. [18]

Freitas Fernandes FS et al in 2011 [14] revealed that Candida biofilms had a greater growth upon polyamide resin compared to conventional PMMA resin. Sodium hypochlorite was found to be the more effective cleanser. They highlighted the importance of a more stringent disinfection protocol for a polyamide base denture due to the greater growth rate of biofilms upon its surface when compared to PMMA.

So the purposefulness of this in-vitro study was to assess the activity of 8% rosemary oil, 1% sodium hypochlorite and distilled water against Candida albicans also the changes in the mechanical property – Flexural strength of thermoplastic denture base material (Valplast) after immersion in these three solutions for 30 days, changing the solution every 8 hours thus simulating 8 hour/day immersion for 90 days.

In our study 8% Rosemary oil did not show similar anti-candidal activity as compared to 1% sodium hypochlorite on thermoplastic denture base resin – Valplast when growth was determined by colony counting following plating and test was completed in 7*108, 7*104 and 7*102 CFU/ml of Candida albicans.

At higher concentration (7*108 CFU/ml) of Candida albicans similar growth (>105 CFU/ml) was seen in distilled water and rosemary oil whereas there was no growth seen when 1% sodium hypochlorite was used. At lower concentrations (7*104 and 7*102 CFU/ml) of Candida albicans rosemary oil showed mild anti-candidal activity but it was not comparable to 1% sodium hypochlorite which showed 100% inhibition of the yeast at all concentrations tested.

According to our study 8% rosemary oil did not perform similarly to 1% sodium hypochlorite as an anti-candidal agent on thermoplastic denture base resin and cannot be used as an immersion disinfectant on thermoplastic denture resins. The results obtained are in accordance with a study by Fernandes FS et al which stated that Candida biofilms had a greater growth upon polyamide resin compared to conventional PMMA resin and the importance of a more stringent disinfection protocol for a polyamide base denture. Also Sodium hypochlorite was found to be the more effective cleanser which was similar to the results in our study. [14]

In this study, to compare and estimate the Flexural strength of Valplast- flexible denture base material, after immersion in distilled water, 8% rosemary oil and 1% sodium hypochlorite for 30 days changing the solution every 8 hours using a Universal Testing Machine. (Instron 3366) it was seen that the Mean flexural strength was highest for sodium hypochlorite group (32.942 ± 2.467) followed by Rosemary oil (32.228 ± 3.548) and Distilled water (28.757 ± 2.403) showed the lowest flexural strength.

ANOVA test was applied to compare the flexural strength among the three groups and it showed statistical significant difference between the groups (p=0.006). Post hoc Bonferroni test was applied to compare the flexural strength between the groups and statistical significant difference was seen between Group 1 and 2 (distilled water and Sodium hypochlorite) (p=0.009) & also between Group 1 and 3 (Distilled water and Rosemary oil) (p=0.034) which shows that there was a significant rise in flexural strength of Valplast when immersed in both rosemary oil and sodium hypochlorite as compared to distilled water. The increase in flexural strength after immersion in rosemary oil was lesser than that in sodium hypochlorite but it was not statistically significant. (p=1.00)

According to our study, 8% rosemary oil influenced the flexural strength of thermoplastic denture base resin Valplast in the same manner as 1% sodium hypochlorite although the anti-candidal property of the essential oil was found to be inferior and non-comparable to 1% sodium hypochlorite which was proven to be successful in completely inhibiting the growth of yeast in all concentrations tested.

Conclusion

Within the limitations of this study, the following conclusions can be drawn:

1. 8% Rosemary oil did not show equivalent anti-fungal activity as compared to 1% sodium hypochlorite when tested as an immersion disinfectant solution on thermoplastic denture base resin – Valplast.
2. There was no statistically significant alteration in the flexural strength of the thermoplastic resin samples immersed in rosemary oil and sodium hypochlorite solutions. Both the solutions equally effected the flexural strength but both were significantly different from those immersed in distilled water which least effected the flexural strength.

The outcomes of the investigations have implications on the viability of 8% rosemary oil as a disinfectant solution for denture cleansers, more importantly for thermoplastic denture base resins.

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Legend Figure

Figure 1: Valplast cartridges

Figure 2: Solution materials

Figure 3: Rosemary oil & Polysorbate 20

Figure 4: Distilled water & Sodium hypochlorite

Figure 5: Ethylene oxide(EtO) sterilizer

Figure 6: Universal testing machine (Instron 3366)

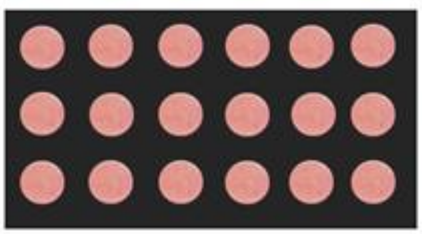

Figure 7: Valplast samples for microbiological study

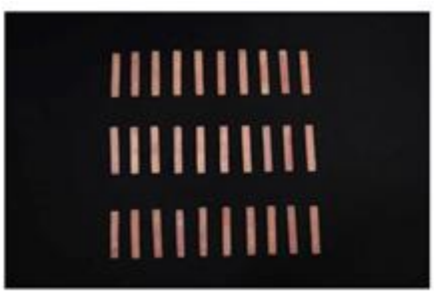

Figure 8: Valplast samples for study of flexural strength

Figure 9: Specimens in Sabouraud's broth

Figure 10: Specimen under treatment

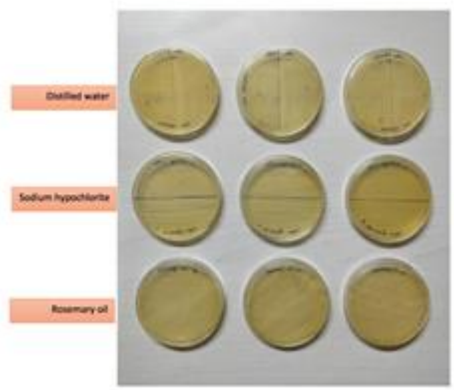

Figure 11: Colony counting in $7*10^4$ CFU/ml of Candida albicans

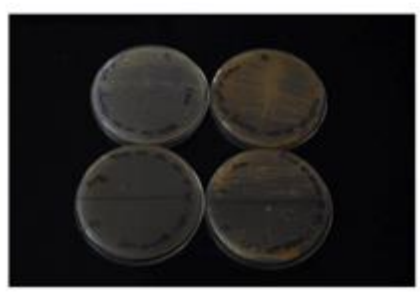

Figure 12: Colony counting in $7*10^4$ CFU/ml of Candida albicans

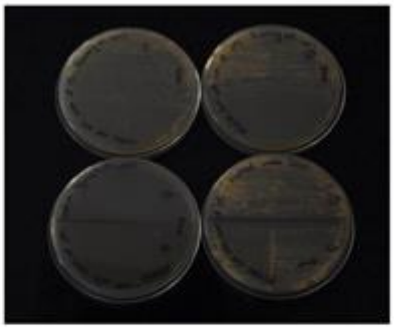

Figure 13: Colony counting in $7*10^2$ CFU/ml of Candida albicans

Figure 14: Specimen immersion in distilled water, 8% Rosemary oil and 1% Sodium hypochlorite solutions

Figure 15: Flexural strength test in Universal testing machine

Figure 16: Data obtained for specimens from all three solutions