

A COMPARATIVE EVALUATION OF THE SURFACE ROUGHNESS AND ANTIFUNGAL PROPERTY OF VORICONAZOLE, ITRACONAZOLE AND TEA TREE OIL COMBINED WITH SOFT LINER IN INHIBITING THE GROWTH OF CANDIDA ALBICANS -AN IN VITRO STUDY

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**ABSTRACT**

**Aim**

This in vitro study aimed to evaluate and compare the antifungal efficacy and surface roughness, heat-cure acrylic resin with soft liners incorporated with voriconazole, itraconazole and tea tree oil.

**Methods and Materials**

The antifungal drugs (voriconazole and itraconazole) were crushed using mortar pestle and added to the polymer in a specified ratio (10% w/w). After completing the process, 30 samples each of Permasoft, Permasoft with Voriconazole, Permasoft with Itraconazole and Permasoft with Tea tree oil were made (Fig 3).

The finished samples were stored in distilled water in separate labelled containers at room temperature for 4 weeks. At intervals of 1st, 3rd, 7th, 14th, 21st and 28th day, samples were checked for surface roughness as well as antifungal efficacy.

**Preparation for Surface Roughness Assessment**

Thin discs of 20 mm diameter \* 4mm thickness of Group D, E and F were prepared by using brass metal mould where the brass metal mould was manufactured in IIT Jammu and these were incubated at 37°C and were stored in distilled water. After 1st, 3rd, 7th, 14th, 21st and 28th day, the specimens were checked under Olympus microscope.

**Results and Conclusion**

Incorporation of Tea tree oil significantly enhanced the antifungal activity against *Candida albicans*, as indicated by a larger zone of inhibition ( $p < 0.05$ ). Surface roughness significantly increased in the Soft Liner without incorporation of any antifungal drug, considerably increasing for itraconazole ( $p < 0.05$ ), whereas tea tree oil caused a significant reduction ( $p < 0.05$ ).

**Keywords :** Anti-Fungal Drugs, Surface Roughness, Soft Liner

## INTRODUCTION

Removable dental prostheses derive their support from the alveolar ridges and oral mucosa, which undergo continuous remodeling with time. In medically compromised patients such as those with diabetes, nutritional deficiencies, and immunosuppression, the supporting tissues are friable and more susceptible to trauma. Ill-fitting dentures and concentrated occlusal forces can therefore result in soreness and denture stomatitis, necessitating either prosthesis replacement or the use of soft denture liners. Soft liners act as a resilient interface between the rigid denture base and oral tissues, improving force distribution, enhancing comfort, and protecting compromised mucosa. They are especially indicated in cases of ridge atrophy, thin mucosa, bony undercuts, bruxism, immediate dentures, and post-surgical healing.

Soft liners are classified as short-term (tissue conditioners) and long-term liners. Tissue conditioners are used temporarily and are composed mainly of polyethyl methacrylate and plasticizers, whereas long-term liners are either plasticized acrylics or silicone elastomers. Acrylic liners tend to harden over time due to plasticizer loss and water sorption, whereas silicone liners demonstrate better long-term resilience. However, methacrylate-based liners exhibit greater surface roughness and porosity, promoting microbial adhesion, particularly of *Candida* species.

*Candida albicans* plays a significant role in denture stomatitis, with studies reporting colonization in up to 65% of denture wearers. Conventional topical antifungal therapies are often ineffective due to salivary dilution, poor patient compliance, and frequent reinfection. To overcome these limitations, incorporation of antifungal agents into tissue conditioners has been proposed as an effective drug-delivery system, offering sustained release, reduced dosage, improved compliance, and simultaneous tissue healing. Various antifungal agents such as nystatin, fluconazole, clotrimazole, chlorhexidine, and natural agents like tea tree oil have shown promising results. Thus, antifungal-modified soft liners represent a promising approach for the effective management of *Candida*-associated denture stomatitis.

## RATIONALE OF THE STUDY

Soft liners itself has no antifungal property as a result when a patient wear relined dentures(having soft liners)for long time , chances of denture stomatitis have been observed. The conditions under the denture base liners promote the growth of microorganisms

Due to widespread use, resistance has come for fluconazole as well and to new synthetic second generation triazole has been developed with the name, voriconazole. It has broad antifungal spectrum unlike fluconazole and is sensitive for *Candida albicans* given orally.<sup>6</sup>Itraconazole is an antifungal agent with more efficacy than fluconazole and tea tree oil also exhibits the antifungal property.

Hence there arises a need to search for different antifungal agents and their effect on surface roughness. Also, this in vitro study is planned to compare the antifungal effect of voriconazole, itraconazole and tea tree oil incorporated into the soft liners for inhibiting the growth of *Candida Albicans*.

## AIM AND OBJECTIVES

### Aim:

To compare and evaluate the surface roughness and antifungal property of Voriconazole, Itraconazole and Tea tree oil combined with soft liner for inhibiting the growth of *Candida Albicans*.

### Objectives:

1. To study if addition of antifungal agents into the denture liners is an effective antifungal therapy against *Candida albicans* and prevent oral candidiasis.
2. To study the time duration up to which the antifungal drug is effective.
3. To study the addition of antifungal agents upto the effective concentration which maintains the properties like surface roughness of the material along with additional benefit of antifungal therapy.

## REVIEW OF LITERATURE

**Jung Eun Choi, Tracey E Ng, Chloe K Y Leong(2018)<sup>7</sup>** conducted a study on Adhesive evaluation of three types of resilient denture liners bonded to heat-polymerized, autopolymerized, or CAD-CAM acrylic resin denture bases. The tensile bond strength of 3 resilient denture liners, namely Ufi Gel SC, Silagum-Comfort, and Vertex Soft, combined with heat-polymerized (Vertex Rapid Simplified), autopolymerized (Vertex Self-Curing), and computer-aided design and computer-aided manufacturing (CAD-CAM) (IvoBase CAD) denture base resins were tested by using a universal testing machine (total N=138). Half of the specimens were thermocycled between 5°C and 55°C for 1500 cycles before testing. After testing, modes of failure and interface surfaces were examined using light microscopy and scanning electron microscopy, respectively. Thermogravimetric analysis was carried out to analyze the differences in content between the 3 different denture base acrylic resins.

**Özdemir H, Özdoğan A.(2018)**<sup>8</sup> investigated on the Bond Strength of Resilient Lining Materials to Denture Base Resin. Bond strength of resilient lining materials with denture base materials is continuously under development. The purpose of this systematic review is to perform a meta-analysis to find out which form of resilient lining bonds better to the denture base, what surface treatments have the highest efficacy to increase the bonding, and what is the most commonly preferred test method to measure bond strength.

**Luo Z, Fan S, Gu C, et al.(2019)**<sup>9</sup> examined Metal-Organic Framework (MOF)-based Nanomaterials for Biomedical Applications. Metal-organic frameworks (MOFs), as a new class of porous organic-inorganic crystalline hybrid materials that governed by the self-assembled of metal atoms and organic struts have attracted tremendous attention because of their special properties. Recently, some more documents have reported different types of nanoscale metal-organic frameworks (NMOFs) as biodegradable and physiological pH-responsive systems for photothermal therapy and radiation therapy in the body.

**Wang Y, Yan J, Wen N et al.(2020)**<sup>10</sup> examined Metal-organic frameworks for stimuli-responsive drug delivery. Metal-organic framework (MOF), a novel hybrid porous material which is composited by metal ions and organic linkers, has drawn increasing attention and became a promising material in the biomedical field owing to their unique properties including large pore volume, high surface area, tunable pore size, versatile functionality and high drug loading efficiency. However, the MOF families and members, and the drug release mechanisms in MOF-based stimuli-responsive drug delivery systems (DDSs) are rarely summarized. Here, we systematically classified the families of MOF and introduced some representative members in MOF families. Moreover, the underlying drug release mechanisms were interpreted according to endogenous stimuli (include pH, glutathione (GSH), adenosine-triphosphate (ATP), ion, glucose, enzyme, H<sub>2</sub>S, and etc.) and the exogenous stimuli (include light, temperature, pressure, and etc.)

**Waghule T, Rapalli VK, Gorantla S, et al.( 2020)**<sup>11</sup> examined Nanostructured Lipid Carriers as Potential Drug Delivery Systems for Skin Disorders. Skin diseases affect all the age groups of people and have an impact on patients' physical, mental, and emotional status. Conventional topical preparation is limited with its efficacy due to low permeation, frequent application, and poor adherence to the therapy for prolong time.

**Hosseinpour-Moghadam R, Mehryab F, Torshabi M. et al.(2021)**<sup>12</sup> conducted a study on Applications of Novel and Nanostructured Drug Delivery Systems for the Treatment of Oral Cavity Diseases. Novel drug delivery systems (DDSs) hold great promise for the treatment of oral cavity diseases. The main objective of this article was to provide a detailed overview regarding recent advances in the use of novel and nanostructured DDSs in alleviating and treating unpleasant conditions of the oral cavity. Strategies to maximize the benefits of these systems in the treatment of oral conditions and future directions to overcome these issues are also discussed.

**Karami A, Mohamed O, Ahmed A.et al.(2021)**<sup>13</sup> aimed to study the Recent Advances in Metal-Organic Frameworks as Anticancer Drug Delivery Systems: A Review. Metal-organic frameworks (MOFs), as attractive hybrid crystalline porous materials, are being increasingly investigated in biomedical applications owing to their exceptional properties, including high porosity, ultrahigh surface areas, tailorable composition and structure, and tunability and surface functionality. Of interest, in this review, is the design and development of MOF-based drug delivery systems (DDSs) that have excellent biocompatibility, good stability under physiological conditions, high drug loading capacity, and controlled/targeted drug release.

**Shengjia Ye, Bin Wei, and Li Zeng(2022)**<sup>14</sup> aimed to study the advances on Hydrogels for Oral Science Research. Hydrogels are biocompatible polymer systems, which have become a hotspot in biomedical research. As hydrogels mimic the structure of natural extracellular matrices, they are considered as good scaffold materials in the tissue engineering area for repairing dental pulp and periodontal damages. Combined with different kinds of stem cells and growth factors, various hydrogel complexes have played an optimistic role in endodontic and periodontal tissue engineering studies. Further, hydrogels exhibit biological effects in response to external stimuli, which results in hydrogels having a promising application in local drug delivery. This review summarized the advances of hydrogels in oral science research, in the hopes of providing a reference for future applications.

**Gradinaru I, Ciubotaru BI, Butnaru M, Cojocaru F. et al.(2023)**<sup>15</sup> investigated The Impact of the Addition of Vitamins on a Silicone Lining Material to the Oral Mucosa Tissue-Evaluation of the Biocompatibility, Hydrolytic Stability and Histopathological Effect. One's quality of life depends on overall health, and in particular, oral health, which has been and continues to become a public health issue through frequent manifestations in various forms, from simple oral stomatitis (inflammations of the oral cavity) to the complicated oral health pathologies requiring medical interventions and treatments (caries, pulp necrosis and periodontitis). The aim of this study focused on the preparation and evaluation of vitamins (vitamin A, B1 and B6) incorporated into several silicone-based lining materials as a new alternative to therapeutically loaded materials designed as oral cavity lining materials in prosthodontics.

**Amal Qasim Ahmed et. al (2023)**<sup>16</sup> assessed the Antifungal Activity of a Soft Denture Liner Loaded with Titanium Oxide Nanoparticles (TiO<sub>2</sub> NPs) and the results seen were the SEM images showed fairly homogeneous dispersion, with patches of TiO<sub>2</sub> NPs agglomeration within the PEMA matrix and an increasing concentration of NPs with higher

NP content. The particle map and EDX analysis confirmed the evidence of the TiO<sub>2</sub> NPs. The mean viable count results for the control (0.0 wt.%) and 1.0 wt.%, 1.5 wt.%, and 2 wt.% TiO<sub>2</sub> groups were 139.80, 12.00, 6.20, and 1.00, respectively, with a significant difference from the control group ( $p < 0.05$ ). The antifungal activity also increased with the increase in the concentration of TiO<sub>2</sub> NPs.

## MATERIALS AND METHOD

### Armamentarium and Materials:

The following armamentarium and materials will be used in the study:

- 0.01g Voriconazole tablet
- 0.01g Itraconazole tablet
- 15% Tea tree oil
- Heat cure acrylic soft liner
- Candida albicans specimen
- Sabourad's Dextrose Agar medium
- Petridishes
- Incubator
- Digital microscope (Olympus DS\*510)
- Brass metal die
- Test tubes

The study was conducted in Department of Prosthodontics, IGGDC Jammu. All the samples used were fabricated by the same operator to prevent any inter operator bias. The Brass metal rectangular sheet of dimensions 18cm x 10cm x 4cm which was cut in the form of 15 circular dies of dimensions (20\*4 mm) were fabricated from IIT. Thin discs of 20mm diameter and 4mm thickness of **Group I(A)** and **Group II( A), (B)** and **(C)** were prepared by using brass metal mould. The Soft liner PermaSoft(Heat cure acrylic) was mixed according to manufacturer's recommendation measures (0.99g) in a mixing jar. The antifungal drugs (voriconazole and itraconazole) were crushed using mortar pestle and added to the polymer in a specified ratio (10% w/w). After completing the process, 30 samples each of Permasoft, Permasoft with Voriconazole, Permasoft with Itraconazole and Permasoft with Tea tree oil were made (**Fig 3**).

The finished samples were stored in distilled water in separate labelled containers at room temperature for 4 weeks. At intervals of 1st, 3<sup>rd</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day, samples were checked for surface roughness as well as antifungal efficacy. To measure antifungal efficacy, zone of inhibition was measured: 1 L of pure, deionized water was utilised to dissolve 61.36 grams of Sabourad's Dextrose Agar. It underwent autoclave sterilization. Approximately 25 mL of SDA were added to a petri dish after the mixture had cooled to 40°C.

A culture of *C. albicans* was attained from Microbial Type Cell Culture(MTCC), Chandigarh. Sabourads Dextrose Agar (SDA) Petri plates received an inoculation with that *Candida* inoculum following solidification. The incubation process lasted for 24 hours at 37°C. After the intended growth obtained on petri plate, few colonies were picked up using sterile inoculation loop (**fig.7**)

These colonies were suspended in 5ml of sterile saline (0.9%) to obtain suspension. The resulting suspension was centrifuged and turbidity was set. This suspension (**fig.8**) obtained and then uniform spreading of candida was done by swabbing the suspension all over agar media in petri plate (**Fig 9**). One sample from each group was placed in the center of the petri dish and the petridish was incubated at 37°C for 24 hrs. The visible zone of inhibition created by the drug was measured in (cm) on the petri dish with a metallic ruler. The same procedure was repeated at time interval of 1,3,7,14,21,28 days and results were noted down. This procedure conducted was examined in the Department of Microbiology, Gmc Jammu.

### Preparation for surface roughness assessment:

Thin discs of 20 mm diameter \* 4mm thickness of Group D, E and F were prepared by using brass metal mould where the brass metal mould was manufactured in IIT Jammu and these were incubated at 37°C and were stored in distilled water. After 1<sup>st</sup>, 3<sup>rd</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day, the specimens were removed from container and blotted to remove excess water. The Surface roughness measurement was carried out by using Digital microscope (Olympus DS\*510) by simply placing the disc sample Under the microscope(Fig 36) and the availability of this equipment was in the Deptt of mechanics, IIT Jammu.

## ILLUSTRATIONS



**Fig. No. 1(A): Heat cure acrylic**



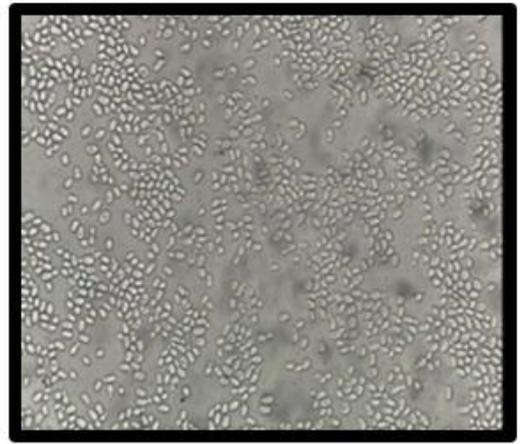
**Fig. No. 2: Brass metal dies**



**Fig. No. 3: Prepared specimens**



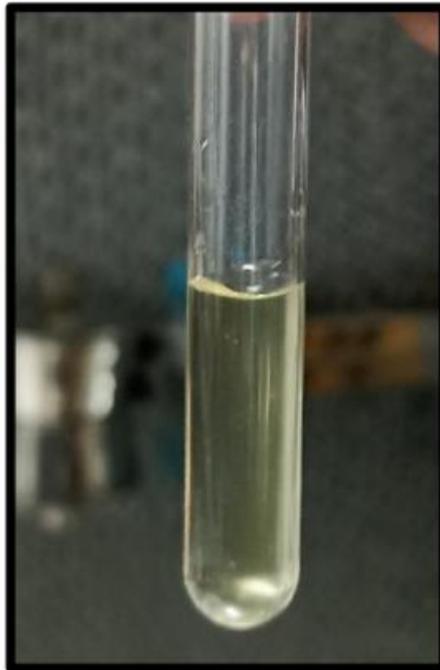
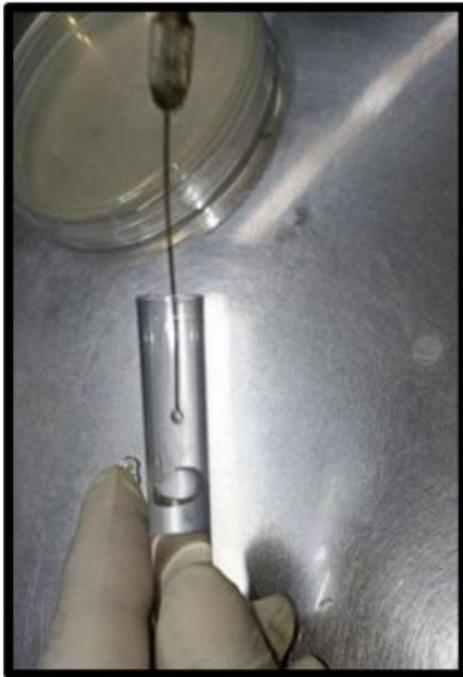
**Fig. No. 4: Lyophilized Form (Candida albicans)**



**Fig. No. 6: Candida culture in microscopic view**



**Fig. No. 7: Colonies pickup via sterilized loop**



**Fig. No. 8: Suspension Formation**



**Fig. No. 9: Uniform spreading of candida on agar plate( Swabbing)**



**Fig. No. 10: Weighing the specimen soft liner**



**Fig. No. 12. Tablet Voriconazole**



**Fig. No. 13: Tablet itraconazole**



Fig. No. 15: Zone of inhibition -Day1



Fig. No. 16: Zone of inhibition -Day3



Fig. No. 17: Zone of inhibition -Day7



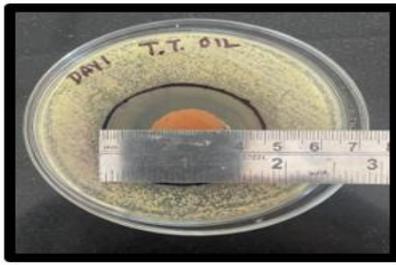
Fig. No. 18: Zone of inhibition -Day19



Fig. No. 19: Zone of inhibition -Day21



Fig. No. 20: Zone of inhibition -Day28



**Fig. No. 21: Zone of inhibition-Day1(T.T. OIL)**



**Fig. No. 22: Zone of inhibition- Day 3**



**Fig. No. 23: Zone of inhibition- Day 7**



**Fig no.24. Zone of inhibition-Day 14**



**Fig. No. 25: Zone of inhibition- Day 21**



**Fig. No. 26: Zone of inhibition-Day 28**



**Fig. No. 27: Zone of inhibition- Day 1**



**Fig. No. 28: Zone of inhibition- Day 3**



**Fig. No. 29: Zone of inhibition- Day 7**



**Fig. No. 30: Zone of inhibition- Day 14**



**Fig. No. 31: Zone of inhibition- Day 21**



**Fig. No. 32: Zone of inhibition- Day 28**



**Fig. No.33: Zone of inhibition Day 1 (Soft liner without antifungal drug)**



**Fig. No. 34: Zone of inhibition- Day 3**



**Fig. No. 35: Zone of inhibition- Day 7**



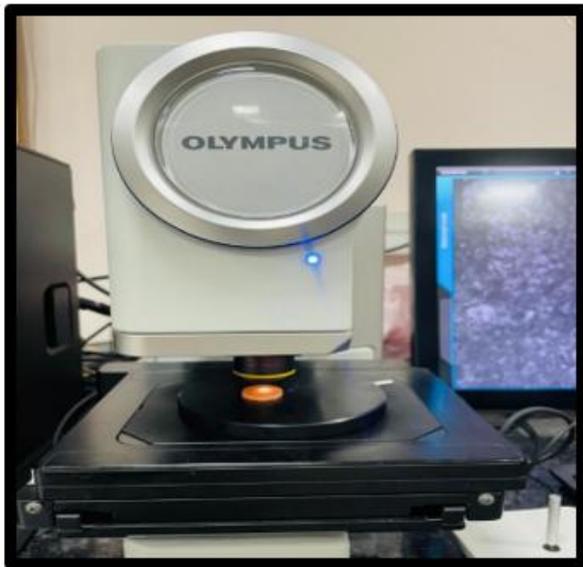
**Fig. No. 36: Zone of inhibition- Day 14**



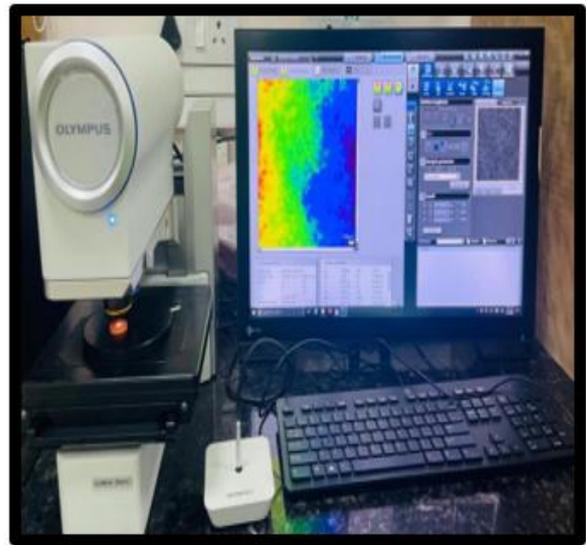
**Fig. No. 37: Zone of Inhibition Day 21**



**Fig. No. 38: Zone of inhibition Day 28**



**Fig. No. 39: Digital Microscope**



**Fig. No. 40: Digital Microscope with computer reading**



**Fig no. 1(B): Heat cure acrylic soft liner**



**Fig no.1(C):Heat cure acrylic soft liner**

**Result**

Table: Distribution of samples of different groups studied at different points of time in respect of zone of inhibition.

Groups	Measurement time	N	Minimu m	Maximu m	Mean	Std. Deviation
GROUP - A	Day 1 (cm)	30	2.8	3.2	3.483	0.0937
	Day 3(cm)	30	3.6	3.9	4.417	0.0740
	Day7 (cm)	30	3.8	4.2	4.443	0.0999
	Day 14(cm)	30	3.8	4.2	4.457	0.0952
	Day 21(cm)	30	4.3	4.7	4.460	0.0855
	Day 28(cm)	30	4.3	4.6	4.470	0.0679
GROUP - B	Day 1(cm)	30	3.2	3.7	3.013	0.1147
	Day 3(cm)	30	4.2	4.6	3.793	0.0950
	Day 7(cm)	30	4.3	4.6	4.037	0.0817
	Day 14(cm)	30	4.4	4.6	4.030	0.0626
	Day 21(cm)	30	4.4	4.5	4.036	0.0466
	Day 28(cm)	30	4.4	4.5	4.040	0.0498
GROUP - C	Day 1 (cm)	30	4.3	4.7	4.517	0.0874

	Day 3(cm)	30	4.6	4.9	4.803	0.0718
	Day 7(cm)	30	4.7	4.9	4.820	0.0610
	Day14(cm)	30	4.7	4.9	4.810	0.0662
	Day21(cm)	30	5.1	5.3	5.197	0.0669
	Day28(cm)	30	5.8	6.2	5.973	0.0980

**Table: Comparison of different groups at different points of time in respect of zone of inhibition**

Measurement time	Groups	N	Mean	Std. Deviation	F-value	P-value
Day 1 (cm)	A	30	3.483	.0937	1799.16	< 0.0001
	B	30	3.013	.1147		
	C	30	4.517	.0874		
	Total	90	3.671	.6390		
Day 3(cm)	A	30	4.417	.0740	1189.12	< 0.0001
	B	30	3.793	.0950		
	C	30	4.803	.0718		
	Total	90	4.338	.4260		
Day7 (cm)	A	30	4.443	.0999	677.413	< 0.0001
	B	30	4.037	.0817		
	C	30	4.820	.0610		
	Total	90	4.433	.3318		
Day 14(cm)	A	30	4.457	.0952	790.504	< 0.0001
	B	30	4.030	.0626		
	C	30	4.810	.0662		
	Total	90	4.432	.3294		
Day 21(cm)	A	30	4.470	.0855	1151.09	< 0.0001
	B	30	4.460	.0466		
	C	30	5.197	.0669		
	Total	90	4.709	.3534		
Day 28(cm)	A	30	4.460	.0679	4122.89	< 0.0001
	B	30	4.457	.0498		
	C	30	5.973	.0980		
	Total	90	4.963	.7220		

**Table: Post Hoc (Bonferroni correction) pair-wise comparison of different groups at different points of time in respect of zone of inhibition**

Dependent Variable at time	(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Day 1 (cm)	A	B	-.4700*	.0256	.000	-.533	-.407
		C	-1.5033*	.0256	.000	-1.566	-1.441
	B	C	-1.0333*	.0256	.000	-1.096	-.971
Day 3(cm)	A	B	-.6233*	.0209	.000	-.674	-.572
		C	-1.0100*	.0209	.000	-1.061	-.959
	B	C	-.3867*	.0209	.000	-.438	-.336
Day7 (cm)	A	B	-.4067*	.0213	.000	-.459	-.355
		C	-.7833*	.0213	.000	-.835	-.731
	B	C	-.3767*	.0213	.000	-.429	-.325
Day 14(cm)	A	B	-.4267*	.0196	.000	-.475	-.379
		C	-.7800*	.0196	.000	-.828	-.732
	B	C	-.3533*	.0196	.000	-.401	-.305
Day 21(cm)	A	B	-.0100	.0176	1.000	-.053	.033
		C	-.7367*	.0176	.000	-.780	-.694
	B	C	-.7267*	.0176	.000	-.770	-.684
Day 28(cm)	A	B	-.0033	.0193	1.000	-.050	.044
		C	-1.5167*	.0193	.000	-1.564	-1.470
	B	C	-1.5133*	.0193	.000	-1.560	-1.466

**Table: Distribution of samples of different groups studied at different points of time in respect of surface roughness**

Groups	Measurement time	N	Minimum	Maximum	Mean	Std. Deviation
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Group - B1	Day 1(μm)	30	6.10	6.14	6.1213	0.00973
	Day3(μm)	30	6.11	6.14	6.1223	0.00817
	Day7(μm)	30	6.10	6.13	6.1187	0.00937
	Day 14(μm)	30	6.11	6.14	6.1243	0.00858
	Day 21(μm)	30	10.16	10.19	10.1723	0.00774
	Day 28(μm)	30	10.16	10.18	10.1753	0.00629
Group - D	Day1(μm)	30	3.093	3.099	3.09780	0.00161
	Day 3(μm)	30	3.53	3.57	3.5467	0.01373
	Day7(μm)	30	3.54	3.57	3.5557	0.00898
	Day14(μm)	30	3.76	3.81	3.7853	0.00900
	Day 21(μm)	30	6.88	6.92	6.8947	0.01106
	Day 28(μm)	30	6.87	6.91	6.8900	0.01114
Group - E	Day 1(μm)	30	3.53	3.57	3.5487	0.00937
	Day3(μm)	30	3.54	3.57	3.5543	0.00817
	Day 7(μm)	30	3.77	3.79	3.7810	0.00607
	Day14(μm)	30	3.77	3.79	3.7803	0.00669
	Day 21 (μm)	30	8.47	8.50	8.4877	0.00679
	Day 28(μm)	30	8.48	8.51	8.4870	0.00837
Group – F	Day1 (μm)	30	3.77	3.79	3.7810	0.00662
	Day 3(μm)	30	3.08	3.12	3.1017	0.01117
	Day 7(μm)	30	3.09	3.12	3.1043	0.00971
	Day14 (μm)	30	3.09	3.12	3.1007	0.01202
	Day 21(μm)	30	.91	.95	.9280	0.00847
	Day28(μm)	30	.75	.79	.7693	0.00980

**Table: Comparison of different groups at different points of time in respect of surface roughness**

Measurement time	Groups	N	Mean	Std. Deviation	F-value	P-value
Day 1(μm)	B1	30	6.12133	.009732	959433.779	< 0.0001
	D	30	3.09780	.001606		
	E	30	3.54867	.009371		
	F	30	3.78100	.006618		
	Total	120	4.13720	1.176515		
Day3(μm)	B1	30	6.1223	.00817	509333.874	< 0.0001
	D	30	3.5467	.01373		
	E	30	3.5543	.00817		
	F	30	3.1017	.01117		
	Total	120	4.0813	1.19763		
Day7(μm)	B1	30	6.1187	.00937	728577.931	< 0.0001
	D	30	3.5557	.00898		
	E	30	3.7810	.00607		
	F	30	3.1043	.00971		
	Total	120	4.1399	1.17305		
Day 14(μm)	B1	30	6.1243	.00858	612158.692	< 0.0001
	D	30	3.7853	.00900		
	E	30	3.7803	.00669		
	F	30	3.1007	.01202		
	Total	120	4.1977	1.15154		
Day 21(μm)	B1	30	10.1723	.00774	6477540.652	< 0.0001
	D	30	6.8947	.01106		
	E	30	8.4877	.00679		
	F	30	.9280	.00847		
	Total	120	6.6207	3.49965		
Day 28(μm)	B1	30	10.1753	.00629	6116141.507	< 0.0001
	D	30	6.8900	.01114		
	E	30	8.4870	.00837		
	F	30	.7693	.00980		
	Total	120	6.5804	3.56536		

**Table: Post Hoc (Bonferroni correction) pair-wise comparison of different groups at different points of time in respect of surface roughness**

Dependent Variable at time	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	p-value	95% Confidence Interval	
						Lower Bound	Upper Bound
Day 1( $\mu\text{m}$ )	B1	D	3.023533*	.001953	< 0.001	3.01829	3.02878
		E	2.572667*	.001953	< 0.001	2.56742	2.57791
		F	2.340333*	.001953	< 0.001	2.33509	2.34558
	D	E	-.450867*	.001953	< 0.001	-0.4561	-0.44562
		F	-.683200*	.001953	< 0.001	-0.6884	-0.67796
		F	-.232333*	.001953	< 0.001	-0.2375	-0.22709
Day3( $\mu\text{m}$ )	B1	D	2.57567*	.00273	< 0.001	2.5683	2.5830
		E	2.56800*	.00273	< 0.001	2.5607	2.5753
		F	3.02067*	.00273	< 0.001	3.0133	3.0280
	D	E	-.00767*	.00273	.035	-0.0150	-0.0003
		F	.44500*	.00273	< 0.001	0.4377	0.4523
		F	.45267*	.00273	< 0.001	0.4453	0.4600
Day7( $\mu\text{m}$ )	B1	D	2.56300*	.00223	< 0.001	2.5570	2.5690
		E	2.33767*	.00223	< 0.001	2.3317	2.3437
		F	3.01433*	.00223	< 0.001	3.0083	3.0203
	D	E	-.22533*	.00223	< 0.001	-0.2313	-0.2193
		F	.45133*	.00223	< 0.001	0.4453	0.4573
		F	.67667*	.00223	< 0.001	0.6707	0.6827
Day 14( $\mu\text{m}$ )	B1	D	2.33900*	.00239	< 0.001	2.3326	2.3454
		E	2.34400*	.00239	< 0.001	2.3376	2.3504
		F	3.02367*	.00239	< 0.001	3.0172	3.0301
	D	E	.00500	.00239	.233	-0.0014	0.0114
		F	.68467*	.00239	< 0.001	0.6782	0.6911
		F	.67967*	.00239	< 0.001	0.6732	0.6861
Day 21( $\mu\text{m}$ )	B1	2	3.27767*	.00224	< 0.001	3.2717	3.2837
		3	1.68467*	.00224	< 0.001	1.6787	1.6907
		4	9.24433*	.00224	< 0.001	9.2383	9.2503
	D	3	-1.59300*	.00224	< 0.001	-1.5990	-1.5870
		4	5.96667*	.00224	< 0.001	5.9607	5.9727
		4	7.55967*	.00224	< 0.001	7.5537	7.5657
Day 28( $\mu\text{m}$ )	B1	D	3.28533*	.00234	< 0.001	3.2790	3.2916
		E	1.68833*	.00234	< 0.001	1.6820	1.6946
		F	9.40600*	.00234	< 0.001	9.3997	9.4123
	D	E	-1.59700*	.00234	< 0.001	-1.6033	-1.5907
		F	6.12067*	.00234	< 0.001	6.1144	6.1270
		F	7.71767*	.00234	< 0.001	7.7114	7.7240

## DISCUSSION

A single or multiple prosthetic teeth replacements are in high demand in today's society where socializing is valued and older patients wish to appear younger and have a beautiful smile. However, there appears to be loosening of the denture bases because bone naturally remodels in response to stress and pressure in the form of occlusal loads and denture bases. In addition to maintaining aesthetics, a suitable fit for the prosthesis is essential for effective speech and mastication. Also, the adherence and colonization of candida on denture soft liners is the most important contributing factor in development of denture stomatitis. Denture stomatitis, more commonly known as 'denture sore mouth', is a term used to describe certain pathologic changes (bright erythema) found in the oral mucosa of denture-bearing tissues under complete or partial dentures in both jaws, but more frequently in the maxilla of, usually, elderly denture wearers. Prevalence has been reported at 11–67% in complete denture wearers. The frequent association of *Candida* species with denture stomatitis has been reported in the literature. As of this, a substantial proportion of people wear Removable complete dentures and encounter from Denture stomatitis. This was in accordance with BT Bal. et al where a study was done to evaluate the adhesion of oral microorganisms to soft liners. The greatest strategy for preserving denture hygiene is to stop the biofilm from growing on dentures. Although Denture stomatitis is common amongst denture-users and is linked to poor denture cleanliness, it appears that only a small % of denture-users actually clean their prostheses thoroughly. Also, in accordance with **Reecha et.al** where in one study they investigated that only a few number of subjects knew how well to clean their dentures and to maintain the prosthesis hygiene.<sup>42</sup>

According to **Von Fraunhofer and Loewy's** review, future strategies for lowering the formation of biofilms may include coverings that can inhibit bacterial attachment or altering denture-constituents to offer a comparatively anionic surface.<sup>33</sup> In the present study, we checked the growth of only *Candida albicans* because, amongst the fungi seen in the oral cavity, about 80% of the microorganisms that are recoverable from the oral mucosa of denture wearers consist of *Candida albicans*.

Soft Liner is a great tool for achieving the shortcomings of a denture fit. Commercially available dental materials include silicone-based long-term soft denture linings (SLTSDLs), acrylic-based long-term soft denture liners (ALTSDLs) and, sporadically, materials based on other polymers. Long-term soft denture lining (LTSDL) materials constitute a group of polymer materials that can remain in the oral cavity for at least four weeks; in practice, however, their use can extend to several months or even years. Keeping in this point of view, LTSDLs have been used in the study that can help to evenly distribute the biting loads transferred onto the soft tissues during chewing and to relieve the mucosa from high mechanical stress. Note that LTSDLs cannot, as has sometimes been suggested, reduce the forces transmitted to the denture-bearing area, as clearly explained by **Brenciaglia MI et al.**<sup>10</sup> Patients are typically given complete dentures with balanced occlusion, which minimizes the amount of damage to the underlying tissues. In this study, heat cure acrylic soft denture liner (HCASDL) manufactured by Perma Soft corporation, is used, called Perma Soft-Liner. It is intended to cushion and comfort denture users by absorbing pressure and shock when speaking and chewing. The special zinc free soft liner has good flexibility and durability, improving denture fit and retention.<sup>12</sup> A salient characteristic of Perma Soft-Liner is its enduring softness, which contributes to the long-term maintenance of patient comfort. Its lifespan and durability are further enhanced by its resistance to both tearing and stains. Moreover, dental practitioners can use Perma Soft-Liner conveniently chairside due to its ease of use and only it requires the placement in warm water for few minutes for its manipulation.

As the Pemasoft Liner optimize the formulation and dosage of antifungal drugs for long-term efficacy in dental prostheses, it becomes of utmost importance. Given its impact on microbe adherence, surface roughness is a crucial polymer attribute. The initial step in the colonization and development of diseases such as denture stomatitis is the adherence of bacteria on denture base materials. In order to minimize biofilm formation and the ensuing inflammation of the oral mucosa, these materials should ideally have smooth surfaces. This will also improve oral hygiene.<sup>32</sup>

**Gruber RG et al.** investigated that the threat of DS can be significantly elevated by several key factors, including inadequate denture fit, inadequate denture care, and *C. albicans* proliferation of the oral-tissue and denture surface, particularly the tissue in contact with denture-seating surfaces.<sup>50</sup> It is evident that unhygienic conditions is associated with a higher risk of *Candida* infection. The threat of DS might be increased by denture materials themselves because parts of Surface roughness (Ra) and hydrophobic nature of denture surfaces may promote the adhesion of bacteria. These shortcomings have stimulated the development of other methods of drug elution, such as the incorporation of antifungal or antimicrobial agents with denture acrylic resin or with soft liners.<sup>34</sup>

A method of treatment by combining soft liner and antifungal agents was suggested initially. After that several attempts have been made to incorporate different antifungal agents such as propolis, zeolite, chlorhexidine, Fluconazole, punica granatum, Nystatin, Miconazole, Ketoconazole, Clotrimazole in the resilient liners with varying degree of success.

Because of its multiple causes, treating denture stomatitis linked with *Candida* can be challenging. Options for treatment include applying orally applied antifungal medications as well as thorough denture cleaning.<sup>44</sup> Because they are diluted by saliva, swallowing, and tongue motions, topical antifungals frequently don't perform properly. Patients must adhere to the treatment plan since it is necessary for them to apply them several times. Despite their effectiveness, systemic treatments can cause adverse reactions, drug interactions, and the establishment of resistant strains.

Rather than applying topically or systemically, antifungals can be incorporated in to the substance as a method of drug delivery to achieve greater results because they release slowly and have longlasting effects. In the present investigation, Voriconazole, itraconazole and Tea tree oil were added to Perma soft liner and samples were created to offer a local delivery method. This finding is also in accordance with **Pingo et al's** study which demonstrated that clotrimazole at 1.0% concentration by weight when incorporated into silicon samples was effective in inhibiting in-vitro growth of the fungus for 5 months at room temperature, but the effect of long-term water storage was not tested.<sup>45</sup>

Newer medications have been introduced to treat *Candida* infections more effectively in order to battle resistance to classic antifungal treatments such as nystatin and miconazole. In this study, Tea tree oil, Voriconazole and itraconazole were used as substitutes. These medications show promising results in treating problems linked to *Candida* and might be more effective against resistant strains.

In light of the study's encouraging findings, natural products can be employed as less side-effect-prone replacements for chemical medications to treat *C. albicans*. These findings urge additional research into both the numerous synergistic interactions between plant oils or plant oils and antifungal medications as as the effectiveness of various other plant oils against *C. albicans*.

### **Zone of Inhibition**

In the results of this study, Perma Soft liner showed absolutely no inhibition of *C. albicans* and voriconazole was more effective antifungal medication than itraconazole. By comparing the mean zone of inhibition, the antifungal activity of the drugs added to the soft liner disc samples was assessed. It was found that voriconazole, Itraconazole and Tea Tree oil combined with soft liner produced statistically significant differences in the mean zone of inhibition. During the course of four weeks, the mean zone of inhibition of tea tree oil was markedly elevated than the mean zone of inhibition for voriconazole, and the mean zone of voriconazole was considerably elevated than-that of itraconazole, indicating that Tea tree oil likely exhibited superior anticandidal activity.<sup>43</sup> This study was in agreement to an invitro study by **Merta et al.**, who suggested that the clinical candida strains that are resistant to Fluconazole when exposed to sublethal concentrations of TTO and fluconazole, explored a change in the activity of Fluconazole.<sup>2</sup> TTO enhanced the activity of

Fluconazole against resistant strains and it was concluded that TTO can be used as a single therapy or in combination with other conventional drugs like Fluconazole that can be used to treat difficult yeast infections.

TTO can block the development of germ tubes and mycelia conversion, slowing the growth of *C. albicans*. TTO contains terpenine-4-ol, which increases yeast cell permeability by rupturing the barrier of microbial membrane structure, increases membrane fluidity, and prevents the medium from becoming too acidic, which prevents the fungi from breathing. This study was supported by **Pachava KR in 2015**, where the mean of colony forming units in tea tree oil treated acrylic discs were checked after one day, 30 days, and 60 days and the colonization was lower in tea tree oil treated discs with a p-value of 0.001.<sup>44</sup> There were mathematical substantial disparities between the groups when comparing intragroup comparisons across time periods; Day 1 showed the lowest zone of inhibition, gradually increased on Day 3 and Day 7, considerable increase on Day 14 while Day 21 and 28 showed the highest. This indicates a gradual increase in antifungal activity over time in all groups, most likely as an outcome of the antifungal drugs' delayed release from the soft liner, which inhibits fungal development for a longer period of time.

In the previous studies, **Bhatia Tania et al** investigated itraconazole and voriconazole incorporation in soft liner and the results showed voriconazole to be better antifungal drug than itraconazole.<sup>25</sup> But contrary to this study, there was no remarkable difference observed as seen the former between the two drugs and addition of another group (Tea tree oil + soft liner) proved to be the most efficient. Overall, the results indicate that Tea tree oil, in particular, can be added to soft liner to effectively increase their antifungal effectiveness against *Candida* species.

The study's findings with respect to zone of inhibition, throughout the duration of 28 days were observed. As on Day 1, The change in the values and change in statistical significance between the groups suggests that the addition of tea tree oil to the long term soft liners makes them a suitable carrier for the drug releasing property. The comparison of three groups with respect to differences in zone of inhibition at different time interval was done using Tukey post hoc test. These results are in co-ordination with the study done by **Pigno MA et. al** who showed that methacrylate soft denture liners would support the growth of *C. albicans*, so accordingly the Group I A showed no zone, and out of all the three groups Group B with mean value of zone measured in cm is 3.013 which showed that its efficiency is least when compared to other groups with mean values of 3.483 in Group A and Group C with 4.517.<sup>47</sup> Comparison of zone of inhibition in between groups and within groups by One way ANOVA. ( $p < 0.001$ ) statistically significant indicates that zone of inhibition constantly increases over time. If the antifungal drug stays in contact with the mucosa for a longer period of time there is a possibility of development of inactivity within the strains.<sup>45</sup> However, in this study the antifungal activity of the drug was seen increasing for all the three groups upto the period of 28 days and thus the possibility of development of resistant strains is not seen upto 28 days, as evidently observed in the results.

The drug releasing or diffusing properties amongst the three groups can be compared as follows from maximum to minimum. Corresponding to Day 1, other days including all the three groups showed constant increase on Day 3 in Group A with mean zone value of 4.417cm, Group B mean zone value of - 3.793cm and Group C with mean zone value of 4.803cm while Day 7 showed considerable increase in Group A with mean zone value of 4.443cm, Group B with mean zone value -4.037cm and Group C with mean zone value - 4.820cm. The Day 14 showed that Group A with mean zone value -4.443, Group B with mean zone value -4.037 and Group C- with mean zone value 4.820. Day 21: Group A- with mean zone value 4.457, Group B- with mean zone value of 4.030, Group C- with mean zone value 4.810 and lastly on Day 28 with Group A- with mean zone value 4.460, Group B- with mean zone value 4.457 and Group C- with mean zone value 5.973 suggesting that TTO treated disks showed significant antifungal efficacy against *C. albicans* compared to treated disks with voriconazole and itraconazole upto 28 days, and this was in agreement with **Al-Mashadane et al.**, showed that 15% TTO had significant antifungal effect against *C. albicans* on the surface of heat cure acrylic denture base material.<sup>2</sup> This study also supports the results of **Hammer et al.**,<sup>17</sup> suggested that the treatment of *C. albicans* with TTO exert antifungal action by altering membrane properties of fungal cells, which may alter their permeability and affect the membranes ability to osmoregulate the cells adequately or to exclude toxic materials. Our study results are also in agreement with **Emira et al.**, suggested that plants essential oils significantly prevent the formation of biofilm at low concentrations and the potential bio active compounds in TTO has distinct influence on candida cell growth, function and biofilm formation by interfering any of the steps involved in bio film development and has a potential anti-adhesive effect of candida strains on PMMA.<sup>22</sup> Therefore, in this study incorporation of antifungal drugs in the soft liner proved to be a boon in inhibiting the growth of candida and among them Tea tree oil proved to be the most effective antifungal drug.

## Surface Roughness

As the study went on, the soft lining materials are susceptible to roughness changes. An invitro study done by **Verran Jet. al** investigated the retention of *Candida albicans* on acrylic resin and silicone of different surface topography. However, the present study investigated only the surface topography of the acrylic permanent soft liners.<sup>48</sup> The study's findings show that, throughout the duration of 28 days, there were notable variations in the surface roughness of the four groups and were measured in ( $\mu\text{m}$ ). Group I(B) had marked surface roughness of-6.1213, Group II(F) (Perma Soft Liner + Tea tree oil) had the mean surface roughness on Day 1 (3.7810), followed by Group II(D) PermaSoft Liner + Voriconazole) (3.097), followed by Group II(E) PermaSoft Liner + Itraconazole (3.54). This implies initially voriconazole showed the lowest mean surface roughness This infers that refined-surfaces were obtained in the Soft Liner group with the addition of antifungal drugs as opposed the control group. The more rougher the material, the more ability of candida biofilm to bind with soft liners. Maintaining a satisfactory roughness is one of the most complicated factors for acrylic liners because they are not stable in an aqueous medium, The values of surface roughness

were analysed and plotted on a graph. Thus from start to end of the study, due to the leaching out of plasticizers and ethanol content of the acrylic soft liner, the roughness value increased gradually. This does not go in accordance with the studies done by **Mirian et al.**<sup>22</sup>The probable reason why the surface roughness of the Permasoft with itraconazole and voriconazole (Group 2) increased can be due to the low molecular weight of the same as these two drugs will allow greater diffusion into the medium in which it is immersed, leading to more roughness of the material itself, while 15 % tea tree oil showed that this conc was potent enough and the molecular size of the essential oil was larger than plasticizers and ethanol content of the acrylic soft liner that it did not cause the leaching out of these products. Also, **Adelaida SANCHEZ-ALIAGA et. Al** investigated surface morphology and in vitro leachability of soft liners incorporated with antifungal drugs and their findings were itraconazole presented higher surface roughness values initially upto 7 days followed by a reduction to values lower than the initial ones at 14 days while the present study results show that the mean surface roughness of (itraconazole+ soft liner) showed constant rise in surface roughness from Day 1<sup>st</sup> to Day 28<sup>th</sup>.<sup>46</sup>On Day 3, Group IB's mean surface roughness (6.12) stayed largely constant, Group II'S(D) had increase in surface roughness and had a value of 3.54 and group(E) mean surface roughness (3.55) nearly stayed constant, and Group II(F)'s mean surface roughness (3.10) reduced slightly.

Next on Day 7 Group I(B) had a mean surface roughness of (6.12), Group II(D) had a mean roughness of 3.55, and Group II(E) had a mean surface roughness of 3.78 and Group II(F) had a mean roughness of 3.10 which remained constant. The Day 14 showed Group IB- 6.12 and only the rise in surface roughness was seen in Group IID -3.78 and Group E- 3.78 having the same significant values while Group F showing the least mean surface roughness value of 3.10.

On Day 21 Group I B showed marked rise with the mean surface roughness value of (10.17). Group D with mean surface roughness value of 6.89, Group E with the mean surface roughness value of 8.48 and Group F showing the least surface roughness with the mean surface roughness value of 0.92.

Lastly on Day 28, Group IB-had the same mean surface roughness value as on 21<sup>st</sup> day for all the groups except for Group F with mean surface roughness value of 0.76 signifying that the TTO showing the most refined area with minimum roughness value, initially less affecting the soft liner roughness but lately improving the smoothness by the 21<sup>st</sup> and 28<sup>th</sup> day in such a way as essential oils present in TTO significantly prevent the formation of biofilm at low concentrations and the potential bio active compounds in TTO has distinct influence on candida cell growth.

Hence, surface topography of these samples are evident enough in proving that the the antifungal drugs incorporated in soft liner help in improving the surface smoothness of the soft liner as a result of which candida biofilm binding is seen to less extent.

## Limitations

- 1.The study had the following limitations: it only tested *Candida albicans* as the microorganism causing denture stomatitis.
- 2.The effect of other microflora on denture stomatitis was not evaluated.
- 3.Further in vivo studies on a large scale should be conducted to confirm the findings of the efficacy of disinfectants.

## SUMMARY AND CONCLUSION

Within the limitations of the laboratory testing conditions and materials the following conclusions can be drawn

1. Significant changes were seen in the antifungal property of voriconazole, itraconazole and Tea tree oil added to the soft liner. Addition of tea tree oil to denture soft liner significantly reduced growth of *C.albicans* suggesting a new form of intra oral effective antifungal management for denture stomatitis.
2. The mean zone of inhibition in case of Tea tree oil added to soft liner is higher than the mean zone of inhibition of voriconazole added to soft liner followed by the mean zone of inhibition of voriconazole added to soft liner which was significantly higher than itraconazole in soft liner, thus showing that voriconazole had exhibited significantly better antifungal property in-vitro contrary to *C. albicans* inhibition with itraconazole.
3. Significant changes were seen in surface roughness of a Soft Liner when combined with three antifungal drugs, Tea tree oil, voriconazole and itraconazole and among them Tea tree oil added soft liner proved to exhibit the least surface roughness followed by voriconazole added to soft liner and lastly followed by itraconazole added to soft liner thereby Tea tree oil being the most effective in depleting the binding of candida to the soft liner surface.

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