COMPARATIVE EVALUATION OF THE EFFECT OF ANTIFUNGAL AGENTS INCORPORATED INTO DENTURE SOFT LINER ON *CANDIDA SPECIES*- AN *IN VITRO* STUDY

By

Dr. MERLIN RIYA MATHEW

Dissertation submitted to the

Kerala University of Health Sciences, Thrissur

In partial fulfillment of the requirement for the degree of

MASTER OF DENTAL SURGERY

In

Branch 1

PROSTHODONTICS AND CROWN & BRIDGE

Under the guidance of

Prof. Dr. GEORGE FRANCIS

Dept of Prosthodontics and Crown & Bridge

St Gregorios Dental College, Chelad

Kothamangalam

(2017-2020)

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I hereby declare that this dissertation entitled "**Comparative evaluation of the effect of antifungal agents incorporated into denture soft liner on *Candida species* – An *in vitro* study**" is a bonafide and genuine research work carried out by me under the guidance of **Prof. Dr. George Francis**, Department of Prosthodontics and Crown & Bridge, St Gregorios Dental College, Chelad, Kothamangalam.

Date:

Kothamangalam **Dr. Merlin Riya Mathew**

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Signature of the Guide

Date:
Prof. Dr. George Francis

Kothamangalam
(Dept of Prosthodontics and Crown & Bridge)

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ABSTRACT

Background and objectives:

Denture stomatitis is an inflammatory mucosal disorder associated with a microbial pathogenesis. In most cases, *Candida* species are involved, which are normal commensals of the oral microbiota in most people. The fundamental goal is of analyzing the efficacy of fluconazole, clotrimazole, miconazole incorporated into soft liner against *C.albicans, C.tropicalis, and C.krusei.* Denture induced candidiasis could be treated by various treatment modalities directed towards the oral mucosa, or towards the denture base. A soft liner with antifungal activity could be a great advantage for patients with high risk of denture stomatitis.

Methods:

In this study, 60 samples of each species were taken for each of the drugs including the control group. The study was carried out in 2 stages. In stage 1, GC denture soft liner was mixed according to manufacturer's instructions. Miconazole, Fluconazole, Clotrimazole was separately added into the soft liner based on their minimum inhibitory concentration (Miconazole 1µg/ml, Clotrimazole 0.015µg/ml, Fluconazole 16µg/ml). The mixture was then left to gel on a pre-fabricated stainless steel template having disc size of 5mm diameter. The soft liner discs with the antifungal agents incorporated are the test groups and the disc without incorporation of antifungal agents is the control group. Test and control discs were placed onto the agar plates and incubated aerobically at 37^0C for 24 hours. After incubation, the diameters of the zones of inhibition of *Candida species* were measured using calipers. In stage 2, after gelling, the discs were immersed separately in 10 ml of sterile distilled water and stored at 37^0C for 7, 14, 21 days and the zone of inhibition measured.

Results and discussion:

Anova test or analysis of variance was performed to infer any differences that existed between the groups of drugs. As per the tests it was found that the drugs showed different mean values and the tests show that the difference was statistically significant ($P<0.005$). As Anova showed a significant overall difference between the drugs, Post Hoc test was performed to check the level of difference at individual level.

On comparing the efficacy between the drugs it was found that fluconazole showed higher activity than clotrimazole and miconazole and the *Candida albicans* clinical strain was the most sensitive specie.

Conclusion:

Denture soft liners, when mixed with antifungal agents showed satisfactory inhibition of *candida* species suggesting that incorporation of antifungal agents into soft liners can be recommended for clinical use. The dual requirement of reducing trauma to the denture bearing tissue along with sustained release of antifungal agents was met.

Keywords: Denture stomatitis, soft liner, antifungal agents, *Candida* species

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INTRODUCTION

Introduction

Edentulousness is not a disease condition by itself, but rather it is a consequence of pathology. Increasing incidence of edentulousness in the past few years has questioned the adequacy of dental treatment. Yet, the mainstay for the management of the edentulous state still remains to be a complete denture. Treatment of these edentulous individuals not only rehabilitates them functionally but also aesthetically and psychologically. However, prosthetic rehabilitation of the aged has been of great concern to the practitioners. The difficulties that arise are not only attributed to denture construction but also to the problems associated with continuous denture wearing.

The alveolar bone undergoes resorption which might cause a maladaptation of the prosthesis, which therefore leads to patient discomfort. However, this maladaptation can be solved by relining of prosthesis [1]. Relining is defined as the procedures used to resurface the intaglio surface of a removable dental prosthesis with a new base material, thus producing an accurate adaptation to the denture foundation area [2].

Soft resilient denture liners have a pivotal role in modern removable prosthodontics because of their capability of preventing and restoring health of inflamed and distorted mucosa. They act as a cushion for the denture bearing mucosa which leads to absorption and redistribution of forces transmitted to the stress bearing areas of the edentulous ridges. Soft liners are used in cases of alveolar ridge resorption, soreness, and knife edge ridges to provide comfort for patients who cannot tolerate occlusal pressures. Kawano et al evaluated the cushioning effect of soft denture liners, indicating that a soft liner reduced the impact force during function. [3]

Denture-related stomatitis (also termed denture sore mouth, denture stomatitis, chronic atrophic candidiasis, and denture-associated erythematous stomatitis) is a common condition characterized by mild inflammation and redness of the oral mucosa which is usually seen beneath a denture. In about 90% of the cases, *Candida species* are involved, which are the usual commensals of the oral

microbiota. In many people. *C. krusei, C. glabrata, C. tropicalis, C. parapsilosis and C. dubliniensis,* are involved. The prevalence of denture stomatitis among denture wearers varies between 25 and 66.7% with a higher frequency in the elderly [4].

Candida albicans infection and trauma are significant causes of denture stomatitis. Predisposing factors include inadequate denture hygiene, denture wearing habits, xerostomia, medications and nutritional factors. The condition is managed by: (i) Improvement of denture hygiene, (ii) correction of the adaptation of the denture with a tissue conditioner and (iii) topical application of an antifungal agent when the presence of yeasts has been confirmed. Tissue conditioners have been used to improve adaptation of the denture and also to allow recovery of denture bearing tissues.

Resilient denture liners have been used in the field of dentistry for more than a century; the earliest resilient liners were made from natural rubber. One of the first synthetic resins used as a resilient liner was a plasticized polyvinyl resin, which was developed in 1945. Silicone based materials were introduced in 1958 [5]. Soft lining materials can be divided into silicon based groups and acrylic based group and both groups can be further divided into auto-or heat-cured systems. Soft lining materials is defined as soft compliant, viscoelastic materials which may be applied to the fitting surface of a denture for the purpose of reducing and evenly distributing occlusal forces on the underlying mucosal tissues. Soft liners are used when anatomic or physiological defects exist in denture bearing areas of mandible or maxilla.

Autopolymerized resilient liner materials allow the clinician to reline a removable denture directly, intraorally. The optimum thickness has been reported as approximately 2.5 to 3 mm which is needed to provide good shock absorption.[6] Acrylic resin based resilient liner materials generally consist of polymers along with a liquid containing methacrylate monomer and plasticizers (ethyl alcohol and/or phthalate).

Silicone based resilient liner materials are similar in composition to silicone type impression materials, as they are dimethylsiloxane polymers. Polydimethyl siloxane is a viscous liquid that can be cross linked to form an elastic rubber.

Ideal properties of resilient liners mainly includes resiliency, which is desired over a long period of time, and a good bond to the denture base. These properties makes resilient denture lining materials useful for patients with ridge atrophy or resorption, bony undercuts, congenital or acquired oral defects, dentures opposing natural dentition and over implants during healing period. Soft denture liners should be easy to handle, easy to clean, tasteless, odorless, have minimal water absorption, minimal dimensional change, no change in color, acceptable aesthetics, and having a thickness of 2 mm to 3 mm with high bond strength to denture base. As denture liners are in direct contact with oral tissue, they must be nonirritating, nontoxic, and incapable of supporting bacterial and fungal colonization.

There are several problems associated with the use of resilient denture liners, including bond failure between the liner and denture base, colonization by *Candida albicans*, porosity, poor tear strength, and easy hardening.

One of the most serious problems with these materials is loss of resiliency and bond failure between the resilient denture liner and denture base. Bond failure creates a potential area for bacterial growth, plaque and calculus formation [7]. Disruption of this bond leads to the formation of an area that is difficult to clean and this area supports the proliferation of fungi and bacteria. Patients using removable prosthetic restorations lined with an elastic material should carry out regular cleansing procedures to prevent such infection.

Although the use of short-term denture liners to improve the adaptation of the denture in cases of denture stomatitis is part of routine treatment, it has been shown that these liners also promote or support in vivo *Candida* colonization. [8] In vitro studies covering longer time periods show that the use of soft liners might intensify the formation of fungal biofilms. Colonization of soft liners by *Candida albicans* is favored by the presence of saliva and serum pellicles. Fungal adhesion to material surfaces is the first step of colonization. Fungi can then penetrate into the material. [9]

Douglas and Walker (1973) had the idea of combining the therapeutic effects of a tissue conditioner and an antifungal agent. This had the advantages that the action of the drug was prolonged, the cost was low and tissue recovery from trauma was encouraged [10]. It was then speculated that the incorporation of an antifungal agent in a short-term denture liner may be beneficial.

Antifungal agents may be applied directly to the affected mucosa or the fitting surface of the denture. Treatment option of direct application requires good patient compliance which can be difficult to achieve when the patient is hospitalized or lacking independence. Institutionalized patients may find it difficult to follow the prescription for daily application of the antifungal agent onto the denture and consequently rely on nursing or other healthcare professionals. It would therefore be advantageous to provide treatment that is easier to administer and requiring less frequent application.

One possible strategy is to incorporate an antifungal agent into a tissue conditioner which may be used to improve the denture fit and reduce the trauma by cushioning the tissues against applied loads. An antifungal agent incorporated into a soft liner that can provide a slow continuous release results in a sustained therapeutic effect.

A number of effective antifungal agents have been used, either topically or systemically, for management of oral candidiasis. Amphotericin B and nystatin are common topical antifungal agents, whereas azoles such as fluconazole and ketoconazole are available for systemic antifungal treatment. Fluconazole is commonly used to treat denture-induced candidiasis as it has a broad antifungal activity. It is well-tolerated and has few side effects.

Miconazole is a frequently used antifungal agent in dentistry, and it can be applied both topically and systemically. It is a broad spectrum of antifungal drug, which affects the permeability of the candidal membrane by interfering with ergosterol biosynthesis and thus causes oxidative damage. Experiments to examine the effect of incorporating miconazole into denture liners have been studied and it is shown to be having inhibitory action against *C. albicans*[11].

An *in-vitro* study showed the effect of Clotrimazole incorporation into silicone soft liner on fungal colonization when the specimens are stored in distilled water and washed daily with wet cotton. It has been reported that combining anti-fungal agents into soft liners might be used in treatment and prevention of denture stomatitis and concluded that the addition of clotrimazole significantly reduced the *C. albicans* growth on the surface of the silicone soft liner. The samples continued to inhibit the fungal growth when they were washed daily with wet cotton for 2 months. [12]

Incorporation of an antifungal agent into a soft liner will serve the dual purpose of sustained antifungal activity and providing a cushioning effect to the traumatised tissues. This study was conducted for comparative evaluation of the effect of antifungal agents incorporated into denture soft liner on *candida species*

AIM AND OBJECTIVE

Aim and Objectives:

1) To evaluate the effect of antifungal agents Miconazole, Clotrimazole, Fluconazole added to denture soft liner (GC Liner) on :

- *Candida albicans species*
- *Candida tropicalis species*
- *Candida krusei species*
- *Candida albicans* standard strain MTCC 227

2) To evaluate antifungal activity after storage in an aqueous environment for three varying periods of 7, 14 and 21 days.

REVIEW OF LITERATURE

Review of literature

The literature reviewed in this study is on the basis of the various drugs and the Candida species chosen. The literatures reviewed for the study are from the year 1970 – 2018.

- **Davenport J C in 1970** studied 50 patients with denture stomatitis and 50 wearing dentures but without evidence of inflammation. He concluded that denture stomatitis was associated with proliferation of *candida* on the denture and the treatment to reduce the amount of *candida* should be directed at the denture rather than the mucosa. [13]

- **Allison and Douglas in 1973** did a study on micro colonization of the denture fitting surface by *candida albicans*. The study involved the denture fitting surface of two soft lining materials and one acrylic denture from three patients with denture stomatitis that were examined by light and scanning electron microscopy. The lining material showed overgrowth of the fitting surface by *candida albicans* hyphae, the acrylic denture showed a surface plaque in which organisms could be identified. [14]

- **Masella, Dolan and Laney in 1975** studied the prevention of growth of *Candida* on silastic 390 soft liner for dentures. The data of the study indicated that silastic 390 does not provide nutrients for the growth of *C.albicans*. It was further suggested that routine daily immersion of a denture lined with silastic 390 in Pro-Kern denture cleaner , Zephiran , or water at 60^{o} C , after cleansing the denture mechanically by brushing ,is recommended as an effective measure in preventing the growth of candida species on silastic 390. [15]

- **L P Samaranayake et al in 1980** studied the factors involved in the adhesion of *candida albicans* to the acrylic surfaces, and that they may play an important role in the aetiology of chronic atrophic candidiasis. It was shown here that adhesion of *C. albicans* to acrylic surfaces could be influenced by various factors operating

intra-orally, and that if these factors were to exert comparable action in vivo they would regulate yeast colonization on denture surfaces. [16]

- **Epstein JB et al in 1981** studied the effects of topical nystatin (Mycostatin) therapy of oral candidiasis which showed that effects of treatment were limited to the time in which the drug was used. Two weeks of therapy resulted in significant reduction in number of organisms and marked improvement in signs and symptoms of candidiasis. The condition recurred rapidly following cessation of treatment. [17]

- **D. M. Quinn in 1985** carried out an in-vitro study on the effectiveness of miconazole and keteconazole combined with tissue conditioners in inhibiting the growth of *C. albicans*. It was found that unlike the ineffectiveness of amphotericin B, the effectiveness of miconazole and keteconazole as anti-fungal agents is not altered when used in combination with any of the tissue conditioners tested. It was concluded that the duration of the localized antifungal and tissue conditioning therapy is 2 weeks as in the in vitro studies. [11]

- **Wright et al in 1985** studied the prevelance of different species of yeasts in a group of patients wearing denture soft lining materials and the relationship between the presence of yeasts and inflammatory changes in the mandibular denture bearing mucosa, and the soft lining materials. The results indicated that *C. albicans* was clearly and significantly ($p<0.02$) associated with greater density of colonization when compared with the other species isolated in the study. [18]

- **Okita et al in 1991** studied the microbiological properties of four tissue conditioners, one soft liner and one acrylic resin. In in vitro adhesion experiments, more Streptococcus mutans and *Candida albicans* adhered to the tissue conditioners and the soft liner in comparison with conventional acrylic resin. No difference in bacterial adhesion was found among the tissue conditioners. No difference among the materials was found, but a tendency for subject-dependence in plaque formation on the materials was noted. [19]

- **Segal et al in 1992** through a study observed that adherence of microorganisms to the surfaces of the host is believed to be an initial and essential step in the production of infection. Among all strains of the *Candida species, Candida albicans* strains was more adherent to acrylic surfaces. [20]

- **Schneid TR** in his study in **1992** investigated the feasibility of a sustained-release delivery system for the treatment of denture stomatitis using four antifungal agents (chlorhexidine, clotrimazole, fluconazole, and nystatin) incorporated into a tissue conditioner (Lynal) at zero (control), low, medium, and high concentrations. Hardness of all experimental groups increased with both concentration and time, but remained within the range of clinical acceptability (Shore A < or = 49). Mean tensile strength increased in all experimental groups and was significantly stronger than the control for at least one concentration level of each drug, p < or = 0.05. All failures for tensile-strength testing were characterized as cohesive. All drugs demonstrated release from the tissue conditioner matrix and inhibition of growth of *C. albicans* that was either total, dose-related, or related to incubation time prior to inoculation. All controls supported growth. [21]

- **Nikawa et al in 1993** did a study to learn the interactions between denture lining material, protein pellicle and *candida albicans*.It was investigated by monitoring pH changes associated with protein-free and protein-coated lining material and by ultrastructural observations of yeast colonization. The results suggested that denture pellicle derived from saliva and/or serum may potentiate candidal colonization of denture lining materials. [22]

- **Könsberg R et al in 1994** did a study to compare the efficacy of a topically administered miconazole denture lacquer with that of a placebo lacquer in the treatment of Candida-infected denture stomatitis. The results indicate that a single application of a miconazole denture lacquer considerably reduces the number of Candida yeasts for a substantial period of time. Immersion of the discs in water showed an inverse relationship between time of immersion and degree of inhibition. Miconazole added in gel form to Visco-gel had an inhibitory effect on the growth of *C. albicans* in vitro. [23]

- **Paul S. Wright et al (1998)** evaluated the effect of soft lining materials on the growth of yeast and concluded that majority of soft lining materials neither promote nor inhibit the growth of yeast and the increased prevalence of yeast is related to the available nutrients in the mouth and the difficulty in maintaining and cleaning these materials.[24]

- **D. R. Radford et al in 1999** did a review to understand the mechanism and clinical significance of adhesion of *C. albicans* to denture base materials in relation to denture plaque and denture related stomatitis. In conclusion, the review strongly supports the suggestion that the adherence of *C.albicans* to denture based materials in vitro is related to the hydrophobicity of the organism.[25]

- **C K W Chow et al** in **1999** did a study where he incorporated antifungal agents into tissue conditioners to investigate the effectiveness of this method of drug delivery. Combinations of nystatin, fluconazole, itraconazole and Coe Soft were used. Viscogel, Fitt were tested at 1, 3, 5, 7, 9 and 11wt/wt%, with and without sterilized saliva. 6 mm diameter cores were punched in Sabouraud plates pre-grown with standardized C. albicans. Antifungal agents plus tissue conditioner mixtures were injected into each core. Inhibition diameters were measured tor 14 days. Cores with only tissue conditioners acted as negative control and showed no significant inhibition activity (ANOVA, $p>0.05$). Peak activity was between 65 to 89 hours; followed by a plateau. Itraconazole had greater fungicidal activity than fluconazole; while nystatin was found to have the least fungicidal activity. [26]

- **El Hadary et al in 2000** did a study to evaluate and compare the water sorption, solubility, and tensile bond strength of a recently introduced silicone-based soft liner (Luci-sof) and a plasticized acrylic resin soft liner (Permasoft) using 2 processing techniques, laboratory-processed and autopolymerized at chairside. It was found that on the basis of lower water sorption and solubility and higher tensile bond strength, Luci-sof may provide better clinical success. [5]

- **Bulad et al in 2004** studied the colonization and penetration of denture soft lining materials by *Candida albicans*. This study aimed to monitor this interaction by comparing the short-term adhesion of *C. albicans* to six denture lining materials and to monitor any longer term penetration of material by the yeast. Denture lining materials (Molloplast B, Flexor, Permaflex, Luci-soft, Eversoft and Ufi Gel hard C) were processed against glass slides or dental stone. None of the materials produced a zone of inhibition when compared with the nystatin control. There was no significant difference ($p>0.5$) in cell numbers on any of the smooth surfaces. Significantly, ($p<0.001$) higher numbers of cells were observed on roughened surfaces. Both hyphal and yeast forms were observed when penetration was monitored. Penetration was greatest into Ufi Gel hard C (no hyphae observed), but not at the acrylic-liner junction and least into Eversoft. [27]

- **Yanikoglu et al in 2004** studied two acrylic based materials and three silicone rubber soft lining materials were investigated to determine the percentage of absorption and solubility in artificial saliva, distilled water, and denture cleanser. In addition, the effect of denture cleanser on surface properties of soft lining materials was also evaluated. For sorption and solubility testing, 75 discs (50 mm x 0.5 mm) were prepared and divided into 5 groups with 15 samples in each group. It was found that the acrylic resin soft lining materials had higher solubility (3.432% Visco-gel in artificial saliva) and absorption (3.349% Visco-gel in distilled water) than Molloplast-B after 16 weeks of aging. The greatest hardness and color change were shown in the acrylic soft lining materials. [28]

- **H. Lamfon et al in 2005** did a study to investigate the composition of denture plaque biofilms and the susceptibility of *candida sp.* Within these biofilms, it was found that exposure to single agents like Miconazole, fluconazole or chlorhexidine did not inhibit the growth of *candida spp*. However, combinations of miconazole and chlorhexidine, pulsed into the system to mimic patient use, did reduce the bacterial and candidal growth for several days. Hence, the use of dual therapy appeared to be useful in reducing viable oraganisms in denture plaque biofilm. [29]

- **Dorocka et al in 2007** did a study to determine the susceptibility of *Candida* isolates obtained from patients with Candida-associated denture stomatitis to 4 antimycotics. A total of 120 *Candida* strains were identified: *C albicans* (59.2%), *C glabrata* (20%), *C tropicalis* (12.5%), and *C parapsilosis* (8.3%). Amphotericin B, 5-fluorocytosine, fluconazole, and itraconazole were effective against 100%, 98.6%, 88.7%, and 87.3% of *C albicans* and 79.6%, 77.6%, 71.4%, and 79.6% of the other *Candida* strains, respectively. [30]

- **Bilge T.Bal in 2008** studied and compared the adhesion of oral microorganisms to different types of soft liner and acrylic resin surfaces at 1,7 and 14 day time period. They concluded that the total number of oral microorganisms adhering to the soft liner material was the greatest after each of the time period tested. [31]

- **Marta Radnai et al in 2009** aimed to study and examine the effectiveness of antifungal gels incorporated into the tissue conditioner which inhibit the growth of *candida albicans* in vitro. To investigate antifungal property over time, visco-gel discs containing chlorhexidine digluconate and 20% v/v miconazole were prepared inoculated with *C. albicans* and placed on SDA immersed in water for different time periods. And it was concluded that chlorhexidine digluconate gel added has no inhibitory effect on growth of *C.albicans*. Incorporation of miconazole has dose related inhibitory effect on candidal growth. [4]

- **Redding et al in 2009** did a study to determine the ability of several thin film polymer formulations, with and without antifungal incorporated, to inhibit *candida albicans* biofilm growth on denture material. It was found that thin-film polymer PMMA coatings alone, without an antifungal agent, produced a small significant reduction in *C. albicans* biofilm formation compared with control PMMA. However, incorporation of antifungal medications into the thin-film polymer reduced biofilm formation between 70% and 80% with nystatin, and between 50% and 60% with amphotericin B. Biofilm reduction with chlorhexidine (up to 98%) was significantly greater than all other formulations tested ($P < .025$). Hence, it was concluded that thin film coating of antifungals can be a potential preventive therapy for denture stomatitis. [32]

- **Eino Makila et al in 2009** studied mycotic flora from the dentures and denture bearing mucosae of 39 persons who wore soft-lined (Molloplast B) mandibular dentures and heat-cured acrylic resin maxillary dentures. The specimens revealed 7 different yeasts and 2 moulds. The most common fungi identified were *Candida albicans*, (86 %,) *C .glabrata*, (31%), and *C. tropicalis*, (14%). The uncured Molloplast material caused a definite inhibition of *candida* growth in vitro, while the cured material indicated no growth inhibition. [33]

- **Isham N and Ghannoum in 2009** did a study to determine the antifungal activity of miconazole (MICON) and comparators against recent clinical isolates of *candida spp.* Using standard clinical and laboratory standards institute methodology. One hundred and fifty isolates, consisting of 25 strains each of *Candida albicans, C. krusei, C. glabrata, C. tropicalis, C. parapsilosis* and *C. dubliniensis*, were tested. MICON demonstrated potent inhibitory activity against all of the strains tested. This indicated that recent clinical isolates remain susceptible to this antifungal agent and that MICON could be used as first-line treatment for candidiasis. [34]

- **Vojdani M et al in 2009** did an in-vitro study to determine whether incorporating clotrimazole (C) into the silicone soft liner (S), would inhibit the growth of *C. albicans* when the specimens are stored in distilled water and washed daily with wet cotton. It was found that in comparison to those of the control disks, clotrimazole in treated disks was effective in inhibiting *C.albicans* growth significantly following storage in water for 2 months. Conclusively it was deduced that the addition of clotrimazole significantly reduced *C.albicans* growth to the surface of the silicone soft liner. The samples continued to inhibit the fungal growth when they were washed daily with wet cotton for 2 months. [12]

- **Mutluay et al in 2010** did a study to investigate in vitro adhesion and in vivo biofilm formation of *Candida species* on polysiloxane liner surfaces.The materials used in this study were Molloplast B, GC Reline soft, Mollosil Plus, Silagum Comfort and Palapress Vario. There was found to be a significant decrease in the

number of cells attached in vitro to saliva-coated surfaces compared with non-treated surfaces. An oral Candida carriage of 78% was found. *Candida albicans, C. glabrata, C. intermedia* and *C. tropicalis* were identified. In vivo biofilm formation on the liners appeared as massive colonisation by microorganisms. The results of the in vitro experiments suggest that salivary film influences early colonisation of different *C. albicans* strains. [35]

- **Falah-Tafti A in 2010** did a comparative study on the efficacy of Nystatin and Fluconazole incorporated into tissue conditioner, on the in vitro attachment and colonization of *Candida Albicans*. Nystatin showed a potentially higher effect in inhibition of candida attachment and colonization (P = 0.0001) compared to that of fluconazole and a statistically significant difference was seen between 5% and 1% fluconazole (P = 0.0001).Tissue conditioner with 1% to 10% nystatin or 10% fluconazole can completely inhibit the adhesion and colonization of *Candida albicans.* [36]

- **Jose Julian et al in 2010** did a study to evaluate the better drug delivery system. Three different drug delivery systems for the treatment of denture stomatitis were observed. The study proved that the tissue conditioner (Viscogel) can be used as a delivery system for the antifungal drugs like Nystatin, Clotrimazol and Ketoconazole. Topical application of drugs can be used but the purpose is defeated by the copious flow of saliva. Systemic administration of drugs may not be that effective against candidal infection because the organism usually limits its activity to the oral mucosa. So in the treatment of denture stomatitis, it is always desirable to have the drug release at the site of infection constantly at therapeutic level. [37]

- **Zomordian et al in 2011** aimed to investigate the risk factors associated with progression to *Candida* related denture stomatitis in patients using complete dentures, and then genetically identify the *candida* isolates associated with disease and colonization. Morphologic analysis was used to identify potential yeast-positive cultures, which were then characterized further by RFLP analysis. *C*

albicans was the most frequently recovered species (61; 41.5%), followed by *C.glabrata* (27; 18.4%) and *C.tropicalis* (19; 12.9%). [38]

- **Himanshu Gupta et al in 2011** did an in vitro study aimed to test the efficacy of ketoconazole and itraconazole, combined with two tissue conditioners (Viscogel [Dentsply] and GC Soft [GC India]), in inhibiting the growth of *Candida albicans*. Four control groups tested were Viscogel (VGC), GC Soft (GCC), ketoconazole (KTZ) and itraconazole (ITZ). Four combination groups tested were ketoconazole with GC Soft (KGC), ketoconazole with Viscogel (KV), itraconazole with Viscogel (IV) and itraconazole with GC Soft (IG). There was absolutely no inhibition of *C albicans* observed in VGC and GCC. For KTZ and ITZ, all the inhibition diameter values were within the standard prescribed limits. There was absolutely no inhibition in IG and IV. For ketoconazole combinations, KGC was found to be significantly more inhibiting than KV, (p value< 0.001). The inhibition of drug effect by embedding the drug into tissue conditioners was significant in all the groups (p value< .001). VGC and GCC do not have any antifungal property of their own. IG and IV were found to be completely ineffective. KGC was found to be significantly more inhibiting than KV. [39]

- **Chopde N et al in 2012** did a study to determine and compare antifungal activity of two tissue conditioners combined with nystatin, miconazole and fluconazole against *Candida albicans*.The results showed maximum inhibition in the fluconazole groups followed by miconazole and the least inhibition was seen in case of nystatin group. It was hence concluded thattissue conditioners when mixed with antifungal agents showed satisfactory inhibition of *Candida albicans.* [40]

- **Altarawneh et al in 2012** attempted to explore the association between *candida*, denture, and mucosal tissue using exfoliative cytology, also evaluating the *candida* levels in saliva, on mucosal surfaces and denture surfaces along with the salivary flow rate and xerostomic conditions. It was found that the prominent etiological factor for denture stomatitis in this population is the presence of *candida* in denture and saliva. Therefore, it was conclusively determined that the

treatment of denture stomatitis in healthy patients should first focus on the sanitization of an existing denture and / or fabrication of a new denture. [41]

- **Salim N et al** in **2012** did a study to investigate the efficacy of a polymeric delivery system impregnated with chlorhexidine or fluconazole against *Candida species*. Self-cure poly-ethyl methacrylate and tetrahydro-furfuryl methacrylate (PEM/THFM) discs impregnated with pure fluconazole substance (FLUp), fluconazole powder from capsules (FLUc) or chlorhexidine powder (CHX) were incubated in water for up to 28 days at 37 °C. The water was replaced at 24h and 3, 7, 14, 21, 28 days. The amount of released drugs and antifungal activity of the leachates was measured by bioassay. The minimal inhibitory concentration (MIC) of each drug for 46 *Candida* isolates was determined and compared to the released concentrations. Both chlorhexidine and fluconazole become readily leached from PEM/THFM polymer up to four weeks and that the polymerization of the acrylic does not affect the antimicrobial activity of the agents. [42]

- **Vijeta Jadhav et al** in **2013** aimed to evaluate and compare the hardness of two different tissue conditioners before and after the addition of three types of antifungal agents. Two types of tissue conditioners: viscogel and coe-soft were manipulated with three types of antifungal agents (fluconazole, clotrimazole, and neem) and placed in the metal mold as per the manufacturer's instructions. A total of 240 samples were fabricated and divided into two groups of 120 samples each and were grouped as, T1 – Viscogel, T2 – Coe-soft. These were subjected to hardness test on 1st, 7th 14th days with the Shore-A-Durometer equipment. Tissue conditioners showed an increase in hardness values with the use of antifungal agents in all the groups. The study can be concluded that hardness of Viscogel was statistically significant when mixed with fluconazole and compared with coe-soft as on 1st day, 7th day and 14th day. [43]

- **Salim N et al** in **2013** did a study to investigate the efficacy and rate of killing of a fluconazole- or chlorhexidine-impregnated polymeric delivery system against fluconazole-susceptible and -resistant *Candida albicans* and fluconazole-resistant,*Candida glabrata*. Poly (ethyl methacrylate)/tetrahydrofurfuryl

methacrylate (PEM/THFM) discs impregnated with chlorhexidine, pure fluconazole (FLCp) or fluconazole from capsules (FLCc) were prepared by substituting a portion of PEM powder with an equivalent amount of each drug. Discs were incubated in sterile water for 1, 3, 7, 14, 21 and 28 days. The amounts of drugs in the leachates were measured spectrophotometrically and their antifungal activity against fluconazole-susceptible (n=1) and fluconazole-resistant (n=2) candidal isolates was determined using a time-kill method and by comparing the released concentrations with the corresponding MICs. Fluconazole and chlorhexidine leached from PEM/THFM polymer for up to 28 days and the released concentrations were fungicidal against all three *Candida* isolates for at least the first 7 days. Chlorhexidine leachates killed all *Candida* isolates more rapidly than the two fluconazole formulation leachates throughout the study period. [44]

- **Gebremedhin S et al in 2014** studied the miconazole activity against *candida* biofilms developed on acrylic films.The minimum inhibitory concentrations (MICs) of miconazole against Candida species were determined by the microdilution method. The MICs for miconazole for the investigated strains ranged from 0.016-32 μg/ml. Treatment with miconazole resulted in a significant reduction of biofilm metabolic activity for all strains. The highest inhibition was observed at 96 μg/ml miconazole. The study provides support for the use of miconazole as an effective agent for the treatment of candida associated denture stomatitis. [45]

- **Koteswara Rao Pachava et al in 2014** did a study to determine and compare antifungal activity of two soft liners combined with Clotrimazole against *candida albicans*. Two soft liners Coe soft (Acrylic) and GC Reline soft (Silicone) combined with Clotrimazole pure powder form and microsphere form at different concentrations (0.5, 1 and 1.5% w/v) were tested against *candida albicans* by agar disc diffusion method. Maximum inhibition was seen in the GC Reline soft with pure Clotrimazole followed by GC microsphere form followed by coe soft pure and microsphere forms at 1.5 and 1% W/V concentration. In conclusion, it was found that denture soft liners, when mixed with antifungal

agents showed satisfactory inhibition of candida albicans suggesting that incorporation of antifungal agents into soft liners can be recommended for clinical use. [46]

- **Ginjupalli, Kishore et al in 2014** aimed to determine and compare antifungal activity of two soft liners combined with Clotrimazole against candida albicans. Results showed maximum inhibition in the GC Reline soft with pure Clotrimazole followed by GC microsphere form followed by coe soft pure and microsphere forms at 1.5 and 1% W/V concentrations.It was deduced that denture soft liners, when mixed with antifungal agents showed satisfactory inhibition of candida albicans suggesting that incorporation of antifungal agents into soft liners can be recommended for clinical use. [47]

- **Maria Helena Figueiral et al in 2015** observed the effect of denture related stomatitis fluconazole treatment on oral *Candida albicans* susceptibility profile and genotypic variability and concluded that fluconazole presents a good short-term but not a good long term denture stomatitis treatment. [48]

- **Ellepolla et al in 2015** did a study to evaluate the PAFE (post antifungal effect) and hemolysin production of oral *C. albicans* isolates following brief exposure to sublethal concentrations of the foregoing antifungals. Nystatin, amphotericin B, caspofungin and ketoconazole induced mean PAFE (hours) of 2.2, 2.18, 2.2 and 0.62, respectively. Fluconazole failed to produce a PAFE. Conclusively it were summarized as brief exposure to sublethal concentrations of antifungal drugs appears to exert an antifungal effect by interfering with the growth as well as hemolysin production of *C. albicans.* [49]

- **Bharathiprakash et al in 2015** did a study to understand the prevalence of *Candida species* among healthy denture wearers and nondenture wearers with respect to their age and hygiene status. Swabs were collected from 50 complete dentures and 50 non-denture wearers and processed on Sabouraud's dextrose agar. Data obtained was correlated with age & oral hygiene and statistical analysis was performed. Prevalence of different *Candida* species was significantly higher in

denture wearers and found predominated by *C. albicans*, *C. tropicalis*, *C. dubliensis* and *C. glabrata*. Among nondenture wearers, *C. albicans* and *C. tropicalis* were isolated. Prevalence of Candida increased with increasing age among denture wearers.[50]

- **Iqbal Z et al in 2016** did a study to investigate the current state of knowledge on the incorporation of antifungal agents into the tissue conditioners for the treatment of denture induced stomatitis. The review of literature reported that incorporation of antifungal agents into tissue conditioners is effective with minimal or no effects on physical and mechanical properties of tissue conditioners. [51]

- **Aditi and Garg in 2016** studied the water sorption and solubility of commercially available acrylic based self-cure soft denture lining material (GC RELINE™ Tissue Conditioner) after immersion in three different storage media (distilled water, Shellis artificial saliva, 5.25% sodium hypochlorite disinfectant solution) at time interval of 4, 7, 11, and 15 days. It was concluded that water sorption of the GC RELINE™ soft denture liner material was highest in distilled water followed by 5.25% sodium hypochlorite and least in Shellis artificial saliva at 4, 7, and 11 day interval. However, on the 15th day, the results showed maximum water sorption in 5.25% sodium hypochlorite followed by distilled water and least in artificial saliva. The results on solubility showed highest solubility of GC RELINE soft denture liner in artificial saliva followed by distilled water and least in 5.25% sodium hypochlorite at 4, 7, 11, and 15 day interval. [52]

- **Lima et al in 2016** studied the porosity of a tissue conditioner (Softone) and a temporary resilient liner (Trusoft) modified by minimum inhibitory concentrations (MICs) of antifungal agents for *Candida albicans* biofilm. The addition of antifungals at MICs resulted in no harmful effects for the porosity of both soft liners in different periods of water immersion, except for Chlorhexidine and Nystatin in Softone and Chlorhexidine in Trusoft at 14 days. [53]

- **Nowakowska et al in 2016** did a study investigate the effect of artificial saliva storage on the color stability of soft silicone liners. Four silicone-based liners

(Elite Soft Relining, GC Reline Soft, Megabase, and Mucopren Soft) (n=10) were tested after 7, 30, and 90 days of storage in artificial saliva at 37°C in darkness. The color of each specimen was measured with a spectrophotometer using the CIELab color scale. Significant differences were found in the color changes of silicone-based denture liners after storage in artificial saliva. With regard to color stability, GC Reline Soft may be recommended for use in dental practices as a silicone soft relining material for long-term applications. [54]

- **Sushma Krishnamurthy et al in 2016** evaluated retention, colonization and penetration of the four denture lining materials namely Molloplast B, Permaflex, GC Soft Liner and Ufi Gel Hard C by *Candida albicans*. It was concluded that Smoother surfaces retain fewer cells than rough surfaces. Denture lining materials permit infiltration of *Candida* through their structure and have insignificant anti-fungal properties. [55]

- **Iqbal Z et al** in **2016** did a review to investigate the current state of knowledge on the incorporation of antifungal agents into the tissue conditioners for the treatment of denture induced stomatitis Various studies reported the efficacy and effectiveness of adding conventional organic antifungal medicines (nystatin, azole group derivatives and chlorhexidine, antimicrobials /antifungals other than organic (silver zeolite, silver nano-particles, photo-catalysts and metallic oxides) and natural and herbal antimicrobials (tea tree oil, lemongrass essential oil and origanum oil) into various tissue conditioners. The review literature reported that incorporation of antifungal agents into tissue conditioners is effective with minimal or no effects on physical and mechanical properties of tissue conditioners. Incorporation of different antifungal medicaments to commercially available tissue conditioners can be recommended for the management of denture induced stomatitis. [56]

- **Neppelenbroeke et al in 2017** investigated the ultimate tensile strength of temporary soft denture liners modified by minimum inhibitory concentrations (MICs) of antifungal agents for *Candida albicans* biofilm. The results showed that the addition of nystatin, chlorhexidine, and ketoconazole at MICs for *C. albicans*

biofilm resulted in no harmful effects on the tensile strength and elongation percentage of the temporary soft denture liner materials up to 14 days. [57]

- **Beuno et al in 2017**studied the addition of minimum inhibitory concentrations (MICs) of antifungals for *Candida albicans* biofilm on the hardness and roughness of temporary denture soft liners. The addition of all antifungals, except for the miconazole in Softone, resulted in no deleterious effects on the materials' hardness over the evaluation time. The MICs of nystatin in both temporary soft lining materials, ketoconazole in Softone and chlorhexidine in Tru soft resulted in no deleterious effects for roughness up to 14 days.[58]

- **Pragati Rawat et al in 2017** did a study that compares the anti-fungal and viscoelastic properties of tissue conditioner containing different antifungal agents. The antifungal property and viscoelasticity of Viscogel containing the antifungal agents were assessed after 24 hours, three days and seven days. Results showed that the highest antifungal activity was shown by Viscogel containing fluconazole. Although Viscogel alone and in combination of fluconazole showed deterioration in viscoelasticity, Viscogel in combination of natural agents showed no significant changes over the period of seven days. Conclusively it was seen that incorporation of the natural agents in the tissue conditioner can be used as an effective alternative to systemic or topical synthetic antifungal agents. [59]

- **Khadka S et al in 2017** aimed to study *Candida species* and to determine antifungal susceptibility pattern of *Candida species* to antifungal agents. Out of 100 *Candida* isolates, *Candida albicans* (56%) was the most common species. Among the non-albicans Candida species, *Candida tropicalis* (20%) was the predominant isolate followed by *Candida glabrata* (14%). Regarding antifungal susceptibility pattern, *Candida species* were more susceptible to clotrimazole (82%) followed by fluconazole (64%) and miconazole (44%). It was concluded that *Candida albicans* was the predominant species responsible for various Candidal infections. Among commonly used antifungal drugs clotrimazole, miconazole and fluconazole were most effective. [60]

- **Gauch et al in 2018** did a study to isolate and identify *Candida species* from the oral cavity of denture wearers with denture-related stomatitis. *Candida spp.* were isolated from 89% of the cases and included five different *Candida species*. *C. albicans* was the most frequently recovered species (78% of the cases), followed by *C. famata* and *C. tropicalis.* [61]

- **Ali Abdul Hussein S. AL-Janab et al in 2018** studied the ability of antifungal agents to reduce or eliminate biofilm formation by *Candida albicans* after incorporating into heat-cured acrylic denture base materials .Different concentrations of Amphotericin B (AmB) and Clotrimazole (CT) were incorporated into polymethylmethacrylate (PMMA) specimens (10 mm × 10 mm × 2 mm). It was found that the incorporation of AmB and CT into denture materials has a significant inhibitory effect on the biofilm produced by *C. albicans*, especially at low concentrations. Decrease in porosity level is another advantage evidenced by incorporating low concentrations of AmB and CT within denture. [62]

- **Swapnil Chincholikar et al in 2019** conducted a study to evaluate the leaching of fluconazole and herbal neem extract incorporated into auto polymerising acrylic resin, heat polymerising acrylic resin and permanent silicone soft liner over a period of 21 days and also evaluated the effect of the leached antifungal agents on the growth of *Candida albicans*. Results showed that Fluconazole exhibited significantly better elution profile and antifungal activity against *Candida albicans* as compared to herbal neem extract. Amongst the materials tested, permanent silicone soft liner exhibited significantly higher elution and better antifungal activity in terms of colony inhibition of *Candida albicans* followed by auto polymerising acrylic resin and heat polymerising acrylic resin.It was concluded that Fluconazole was more potent than herbal neem extract against *Candida albicans*. Permanent silicone soft liner was established to be the most effective polymeric system for sustained release of antifungal agents up to 21 days. [63].

RELEVANCE

Relevance

Denture stomatitis or denture sore mouth is an inflammatory process of the oral mucosal areas that underlies a removable denture and may affect 15% to more than 70% of denture wearers. The cause of denture stomatitis is believed to be multifactorial, and the severity of the denture stomatitis has been correlated with the presence of yeast colonizing the denture surface. Dentures can produce a number of etiological changes that facilitate the accumulation of bacteria and yeast. *Candida species* particularly *Candida albicans* have been identified in most patients. Knowledge of a prosthetic material or technique which can minimize this association would be useful for a better and long term outcome for a patient. One possible strategy is to incorporate an antifungal agent into a tissue conditioner whose prime role is to improve the denture fit and reduce trauma by cushioning the tissues against applied loads.

This study aims to comparatively evaluate the effect of antifungal agents (fluconazole, clotrimazole, miconazole) when used with soft liner (GC soft liner) as well as the status of their efficacy when immersed in water for varying periods. Incorporation of an antifungal agent into a soft liner will serve the dual purpose of sustained antifungal activity and providing a cushioning effect to the traumatized tissues. The presence or absence of leaching of the antifungal agents from the soft liner can be observed for 7, 14 and 21 days to determine the time period for which the antifungal agents would remain active. Immersion in water would simulate conditions of the oral cavity. Since water is the storage media of dentures for all patients, the efficacy of antifungal incorporated liner after storage in water can be evaluated.

METHODOLOGY

Methodology

Materials used:

- Soft liner : GC soft liner (GC Corporation, Japan).
- Antifungal powders : Fluconazole (HiMedia Laboratories Private Limited, Mumbai).
  : Miconazole (HiMedia Laboratories Private Limited, Mumbai).
  : Clotrimazole (Glenmark)
- *Candida albicans* standard strain (MTCC 227).
- *Candida albicans* clinical strain.
- *Candida tropicalis* clinical strain.
- *Candida krusei* clinical strain.
- Microbiological culture media used: Mueller Hinton Agar (Himedia).
  : CHROMagar (BD).
  : Sabourauds dextrose agar (Himedia).
  : BHI (brain heart infused broth – Himedia).

Sampling

Sample Size:

Sample size required for the study is calculated by using the formula:

$$n \geq \left[\frac{Z^2 \times P(1-P)}{ð^2} \right]$$

where P stands for expected prevalence and ð stands for precision

The sample size worked out from the above formula is 60.

Sampling Procedure:

The Candida species chosen were *Candida albicans* standard strain MTCC 227, *Candida albicans* clinical strain, *Candida tropicalis* clinical strain and *Candida krusei* clinical strain. The drugs chosen were fluconazole, miconazole and clotrimazole as they have proven to be efficient against the *Candida* species.

A total of 60 samples comprising of each of the *candida* species (i.e 15 samples for *Candida albicans* standard strain, 15 samples for *Candida albicans* clinical strain, 15 samples for *Candida krusei*, 15 samples for *Candida tropicalis*) were taken for each group of antifungal incorporated soft liner (fluconazole, clotrimazole, miconazole) including control group.

The soft liner used in this study is commercially available GC Soft liner (GC Corporation, japan). Incorporation of the above mentioned antifungal agents into GC soft liner was done to study the sustained antifungal activity over determined time intervals which was at day 1,7 14 and 21.

The groups were divided into the following:

GROUPS	DESCRIPTION		SAMPLE NUMBER
Group I	Test disc inoculated with *Candida albicans* standard strain		60
	Sub group A	Miconazole incorporated	
	Sub group B	Fluconazole incorporated	
	Sub group C	Clotrimazole incorporated	
	Sub group D	Control	
Group II	Test disc inoculated with *Candida albicans* clinical strain		60
	Sub group A	Miconazole incorporated	
	Sub group B	Fluconazole incorporated	
	Sub group C	Clotrimazole incorporated	
	Sub group D	Control	
Group III	Test disc inoculated with *Candida tropicalis*		60
	Sub group A	Miconazole incorporated	
	Sub group B	Fluconazole incorporated	
	Sub group C	Clotrimazole incorporated	
	Sub group D	Control	
Group IV	Test disc inoculated with *Candida krusei*		60
	Sub group A	Miconazole incorporated	
	Sub group B	Fluconazole incorporated	
	Sub group C	Clotrimazole incorporated	
	Sub group D	Control	

Method:

Clinical strains of *Candida species* collected from cases of denture stomatitis was used for the study. Standard *Candida albicans* strain MTCC 227 was used (procured from MTCC Chandigarh)

The experiments were carried out in two stages:

- Stage I - effect of antifungal incorporated soft liner on *Candida species*
- Stage II - effect of antifungal incorporated soft liner on *Candida species* after immersion in water for varying periods of 7,14 and 21 days

This study was conducted in the Department of Microbiology, Mar Baselios Dental College, Kothamangalam, Kerala.

Candida albicans inoculation preparation

Clinical isolates of *Candida* species obtained from the Department of Microbiology in Mar Baselios Dental college was inoculated on to CHROM agar and used as test organisms for the current experimental study.

Candida species was cultured onto sabourauds dextrose agar plate and incubated at 37^0C for 24 hours. Each species gave rise to a variety of colony colours ranging from pink to green to blue of different colony characteristics. Therefore the chromogenic agar was found to be useful in the study for identifying clinical *Candida* isolates

Preparation of the tissue conditioner

The standard powder liquid ratio is 2.2gms/1.8gms as given by manufacturer. The appropriate antifungal taken in its MIC was added to the powder weighed according to the manufacturer's instruction and then mixed with the liquid using an L- spreader

Preparation of control group without antifungal agents (Group A)

Soft liner was mixed in the powder liquid ratio as given by manufacturer. The discs were prepared without addition of antifungal agents

Preparation of fluconazole incorporated soft liner discs (Group B)

Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the test molecule that causes inhibition of visible growth of *Candida* cells as compared with the controls. MIC was determined in vitro in liquid medium by several dilution methods. 16µg/ml of fluconazole which was obtained after calculating MIC was then added to the powder of soft liner and then mixed with the liquid to a uniform consistency.

Preparation of miconazole incorporated soft liner discs (Group C)

Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the test molecule that causes inhibition of visible growth of *Candida* cells as compared with the controls. MIC was determined in vitro in liquid medium by several dilution methods. 1µg/ml of miconazole which was obtained after calculating MIC was then added to the powder of soft liner and then mixed with the liquid to a uniform consistency.

Preparation of clotrimazole incorporated soft liner discs (Group D)

Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the test molecule that causes inhibition of visible growth of *Candida* cells as compared with the controls. MIC was determined in vitro in liquid medium by several dilution methods. 0.015µg/ml of clotrimazole which was obtained after calculating MIC was then added to the powder of soft liner and then mixed with the liquid to a uniform consistency.

Stage 1- effect of antifungal incorporated soft liner on *Candida species*

GC denture soft liner was mixed according to manufacturer's instructions.

Miconazole, Clotrimazole, Fluconazole, was separately added into the soft liner based on their minimum inhibitory concentration (Miconazole 1µg/ml, Clotrimazole 0.015µg/ml, Fluconazole 16µg/ml) as per CLSI M44-A2 [64, 65, 66]. It was mixed well and spread on a sterile petridish using an L- spreader. The mixture was then left to gel on a pre-fabricated stainless steel template having disc size of 5mm diameter. The soft liner discs with the antifungal agents incorporated are the test groups and the disc without incorporation of antifungal agents is the control group. Routine antifungal

susceptibility was done on all isolates by CLSIM44-A2 [67]. Cells were harvested by centrifugation at 3000 rpm for 10 min, washed by brief vortexing for 1 minute and spread onto Meuller Hinton with methylene blue agar plates uniformly by pipetting 0.5 ml *Candida species* suspension [4].Test and control discs was placed onto plates and incubated aerobically at 37^0C for 24 hours. After incubation, the diameters of the zones of inhibition of *Candida species* were measured using calipers.

Stage 2- effect of antifungal incorporated soft liner on *Candida species* after immersion in water for varying periods of 7, 14 and 21 days

Test and control discs as well as plates inoculated with *Candida species* culture was prepared as described earlier for Stage I. After gelling, discs was immersed separately in 10 ml of sterile distilled water and stored at 37^0C for 7, 14, 21 days [1]. After removal from the water, the discs were washed by dipping into sterile distilled water, blotted on sterile blotting paper to remove excess fluid and placed on the MHA plates. The plates were then incubated for 24 hours at 37^0C.The zone of inhibition was measured after 24 hours and calculations were made using caliper.

Once the measurements were made, the results were calculated and the statistical analysis was done using ANOVA and Post Hoc Tukey test.

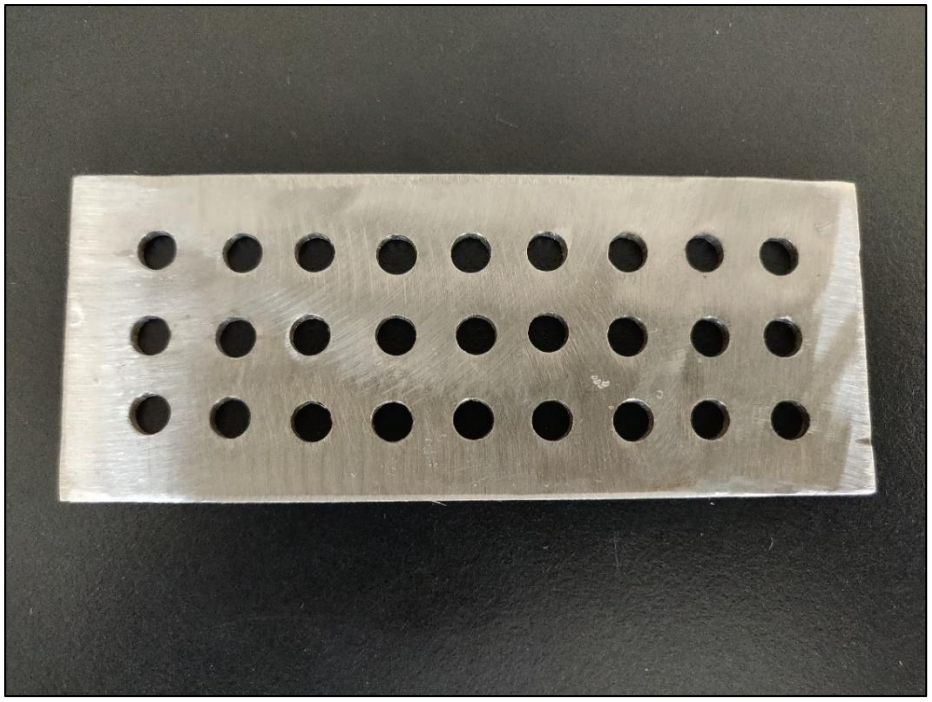


Figure 1: Pre-fabricated stainless steel template

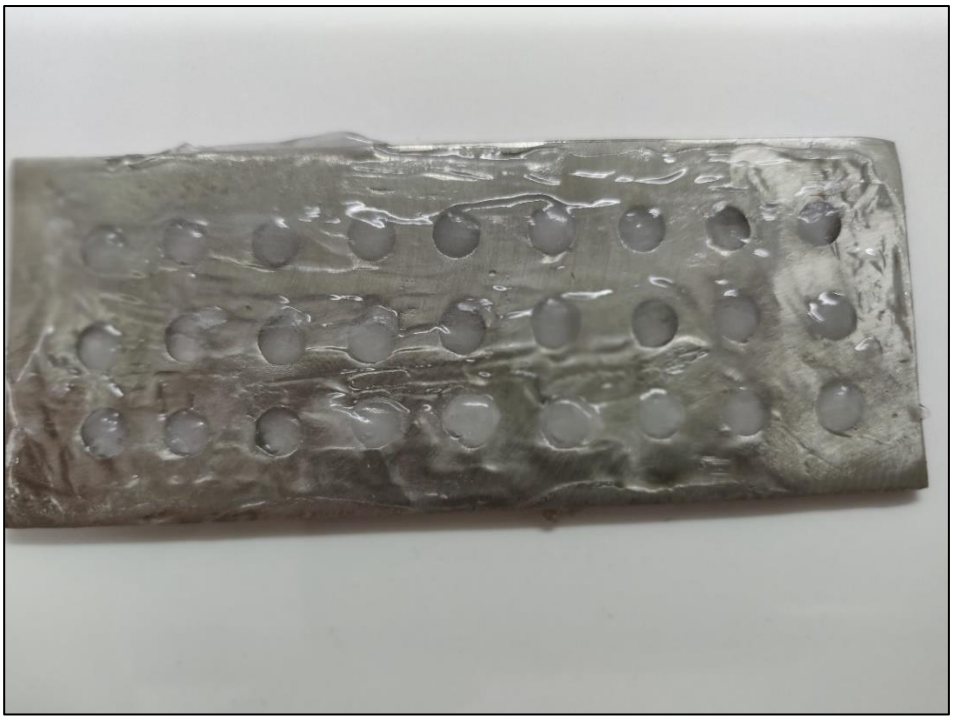


Figure 2 : Pre fabricated stainless steel template filled with the soft liner to which the antifungal agent is added

Figure 3: Colonies of *Candida krusei*

Figure 4: Colonies of *candida tropicalis*

Figure 5 : Colonies of *candida albicans*

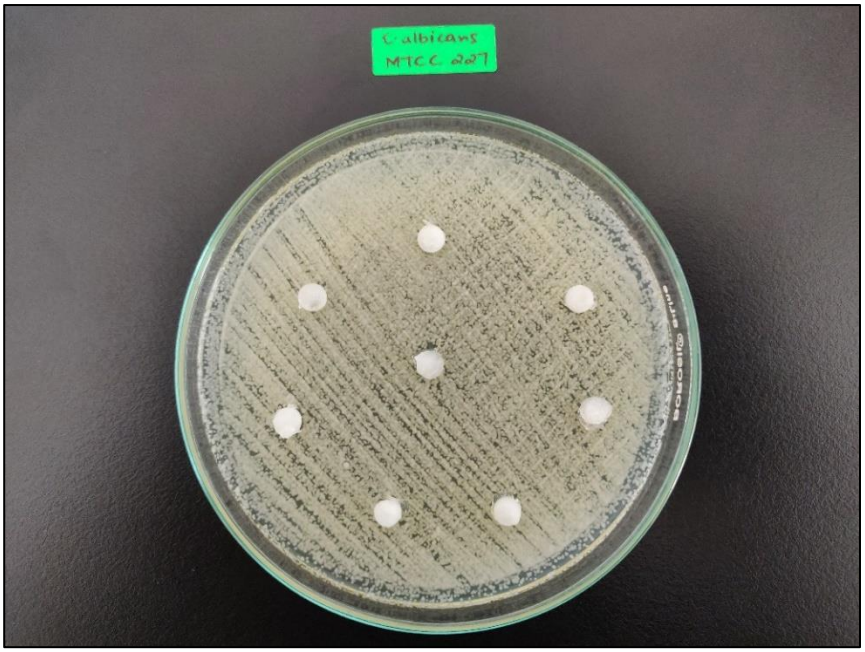


Figure 6: Agar plate inoculated with *candida albicans* standard strain having soft liner discs

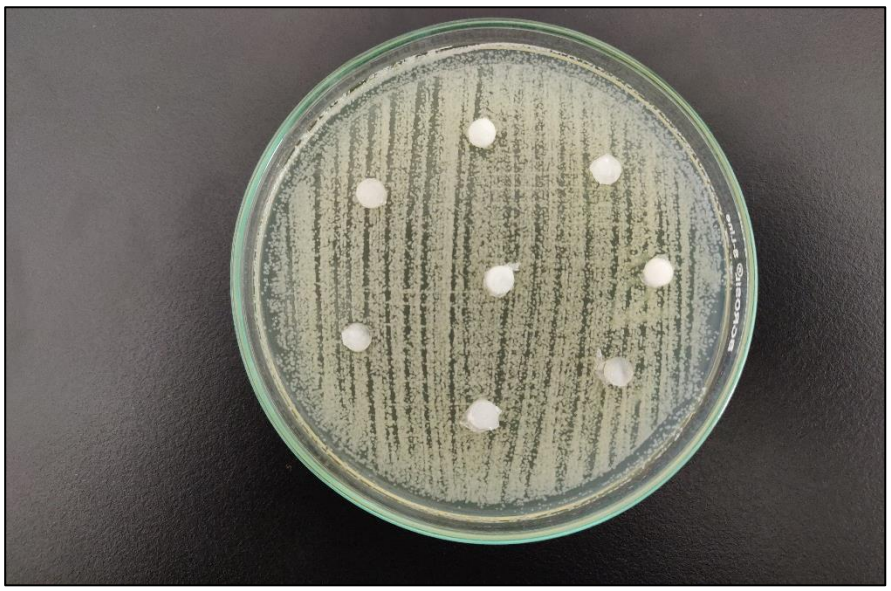


Figure 7: Agar plate inoculated with *candida albicans* clinical strain having soft liner discs

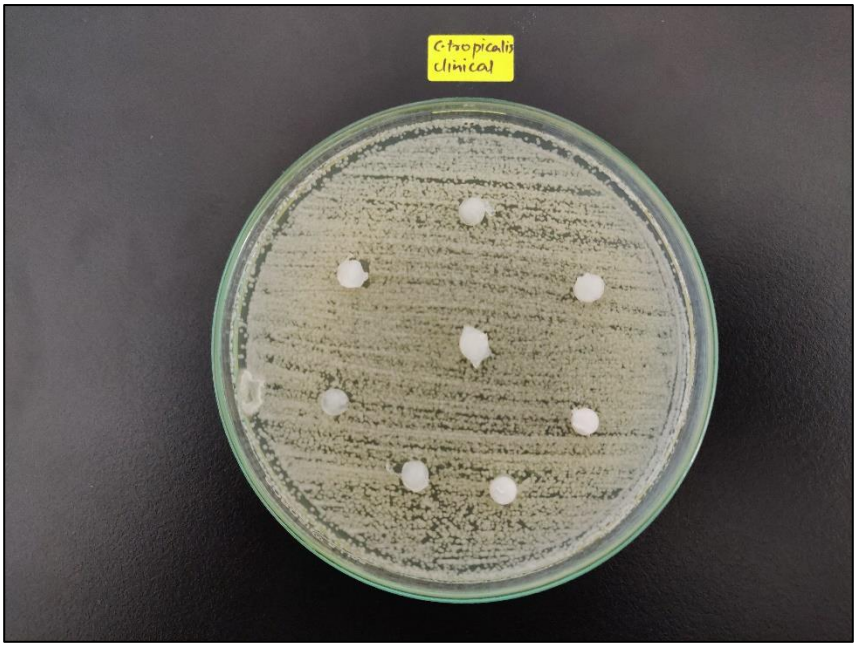


Figure 8: Agar plate inoculated with *candida tropicalis* having soft liner discs

Figure 9: Agar plate inoculated with *candida krusei having* soft liner discs

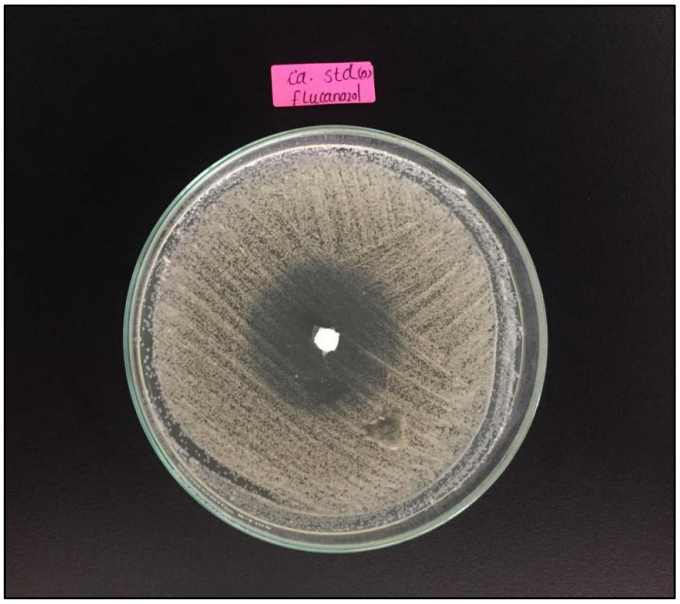


Figure 10: Soft liner disc incorporated with fluconazole showing its activity against *candida albicans* standard strain

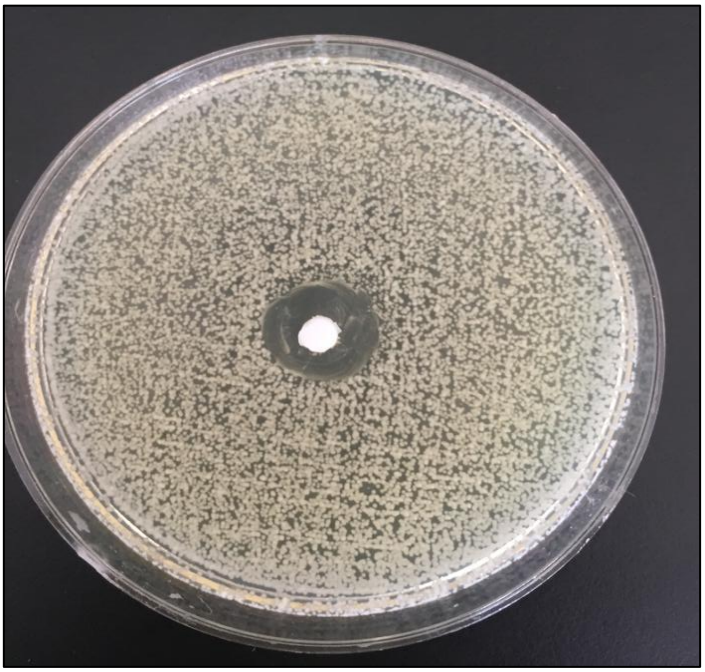


Figure 11: Soft liner disc incorporated with miconazole showing its activity against *candida albicans* standard strain

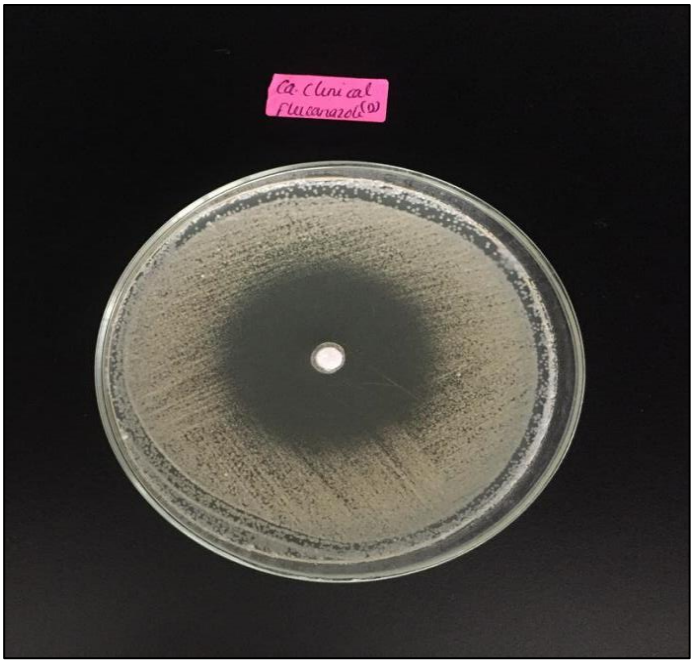


Figure 12: Soft liner disc incorporated with fluconazole showing its activity against *candida albicans clinical strain*

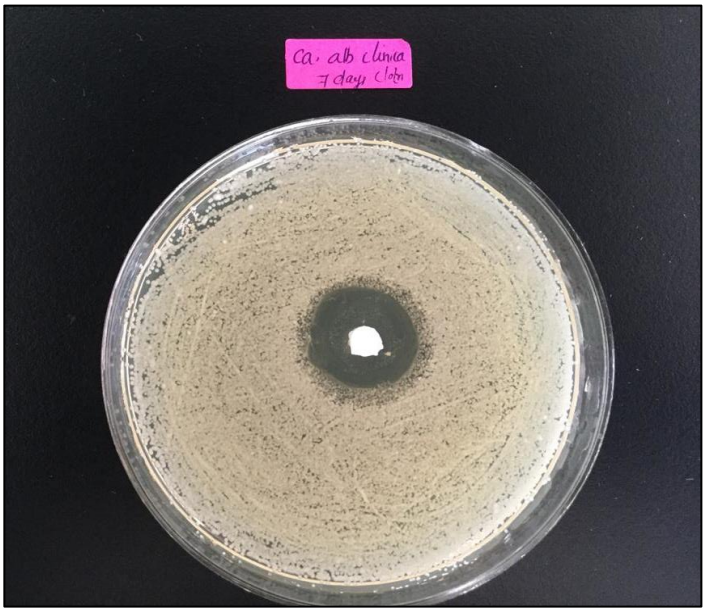


Figure 13: Soft liner disc incorporated with miconazole showing its activity against *candida albicans* clinical strain

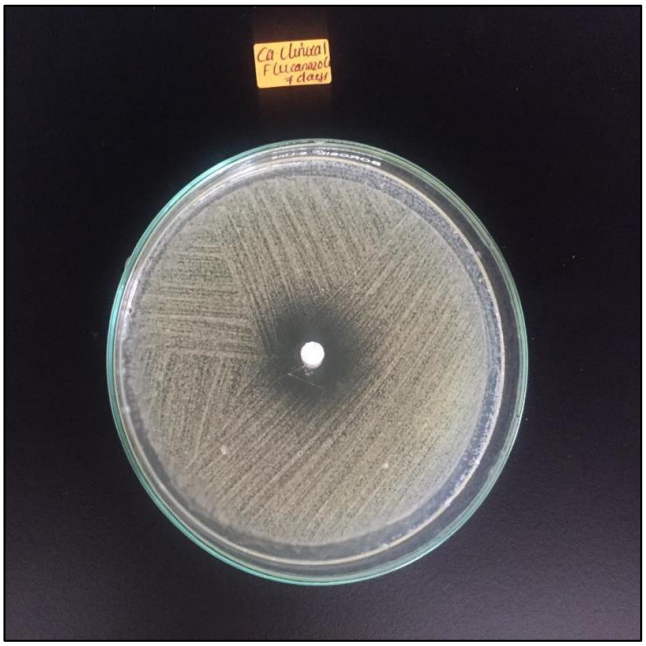


Figure 14: Soft liner disc incorporated with clotrimazole showing its activity against *candida albicans* clinical strain

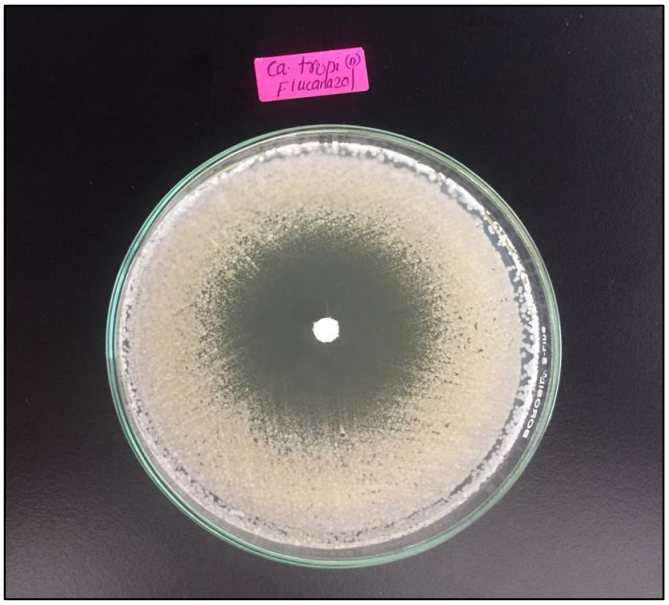


Figure 15: Soft liner disc incorporated with fluconazole showing its activity against *candida tropicalis* clinical strain

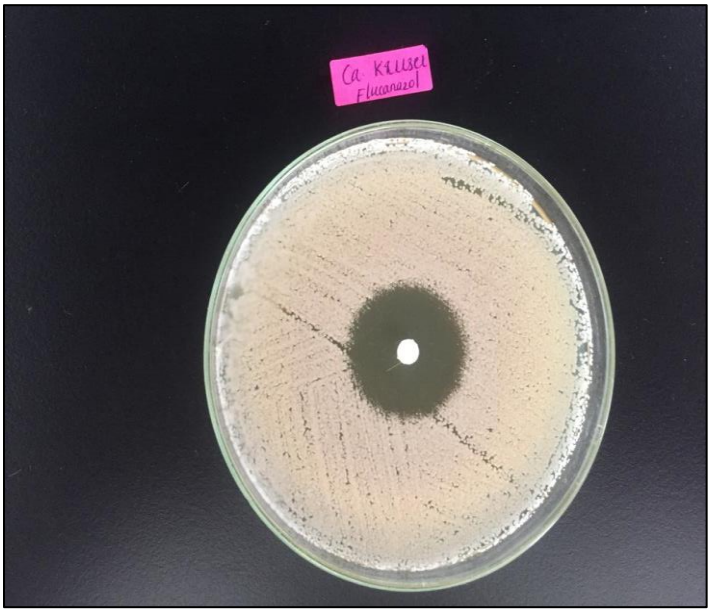


Figure 16: Soft liner disc incorporated with fluconazole showing its activity against *candida krusei* clinical strain

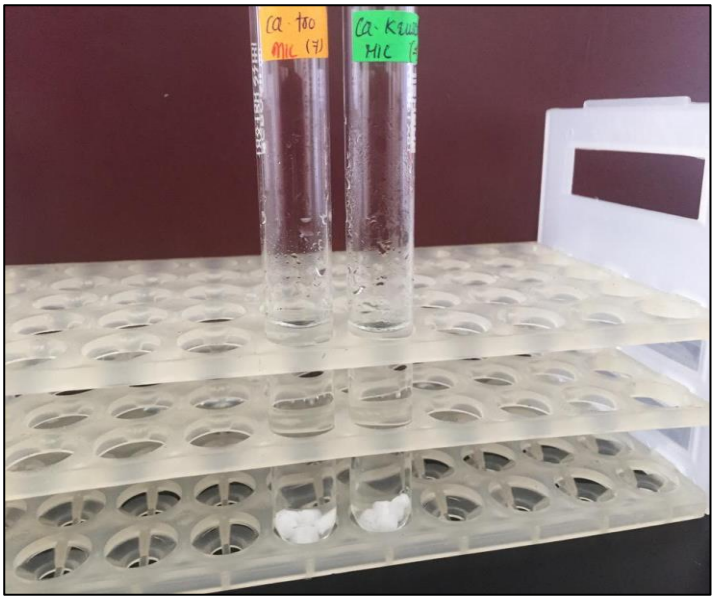


Figure 17: Soft liner discs incorporated with antifungal agents stored in distilled water for varying time periods.

RESULTS

Results

In this chapter the results of the data analysis are presented. The data were collected and then processed in response to the problems posed in the objectives and aims of this dissertation. The fundamental goal of analyzing the efficacy of fluconazole, clotrimazole, miconazole incorporated into soft liner against *C.albicans, C.tropicalis, and C.krusei* drove the collection of the data and the subsequent data analysis objectives were accomplished. The findings presented in this, demonstrate the potential for blending theory and practice.

Hypothesis testing:

To verify the data derived from the experiments, statistical tests for hypothesis testing – ANOVA test and POST-Hoc test are followed. The zone of inhibition were calculated on the day of inoculation, 7 days after immersion in water, 14 days after immersion in water, 21 days after immersion in water, 15 times each for each strain.

Null hypothesis

There is no difference in the sensitivity of *Candida species* to different antifungal agents incorporated into denture soft liner.

Alternate hypothesis

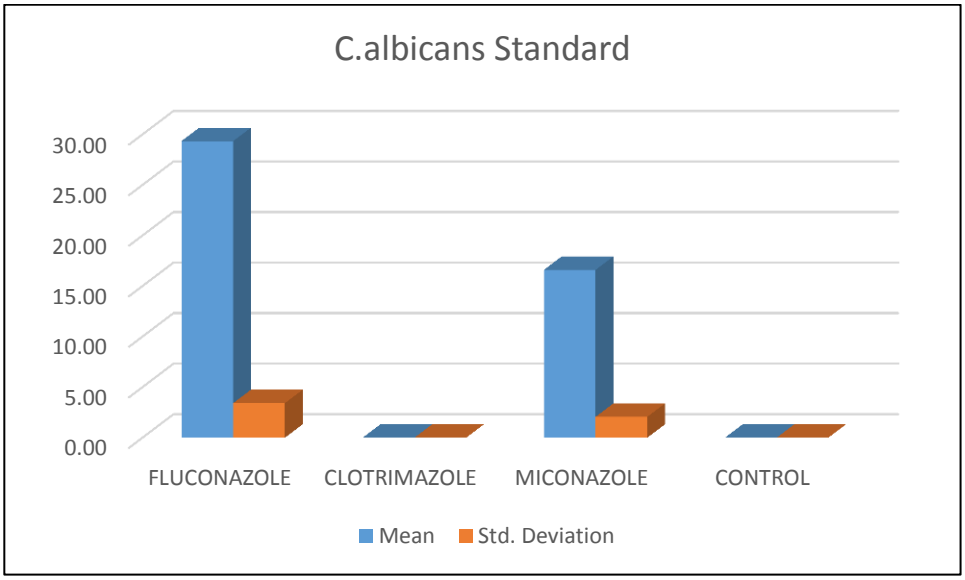
There exists a difference in the sensitivity of *Candida species* to different antifungal agents incorporated into denture soft liner

Level of significance, α=0.05

CANDIDA ALBICANS STANDARD

Descriptive Analysis				
C. albicans (Std)				
	N	Mean	Std. Deviation	Std. Error
Fluconazole	15.00	29.33	3.42	0.88
Clotrimazole	15.00	0.00	0.00	0.00
Miconazole	15.00	16.60	2.06	0.53
Control	15.00	0.00	0.00	0.00
Total	60.00	11.48	12.59	1.63

Table 1: Descriptive analysis table for *Candida albicans* standard strain on day 1 of inoculation.

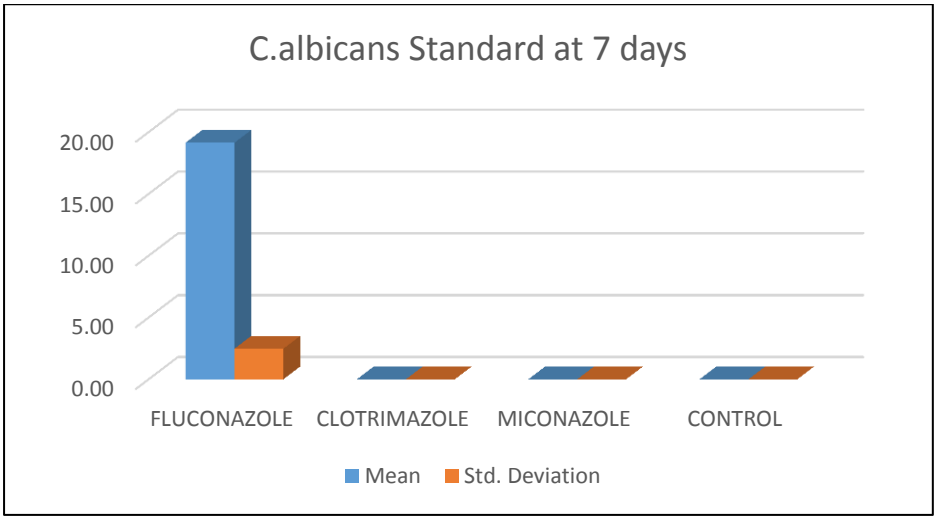


Graph 1: Graphical representation of mean and standard deviation

Inference: *Candida albicans* standard strain is most sensitive to fluconazole followed by miconazole and is resistant to clotrimazole.

Descriptive Analysis				
C.albicans standard (7 days)				
	N	Mean	Std. Deviation	Std. Error
Fluconazole	15.00	19.13	2.47	0.64
Clotrimazole	15.00	0.00	0.00	0.00
Miconazole	15.00	0.00	0.00	0.00
Control	15.00	0.00	0.00	0.00
Total	60.00	4.78	8.44	1.09

Table 2: Descriptive analysis for *candida albicans* standard strain on 7th day of inoculation.

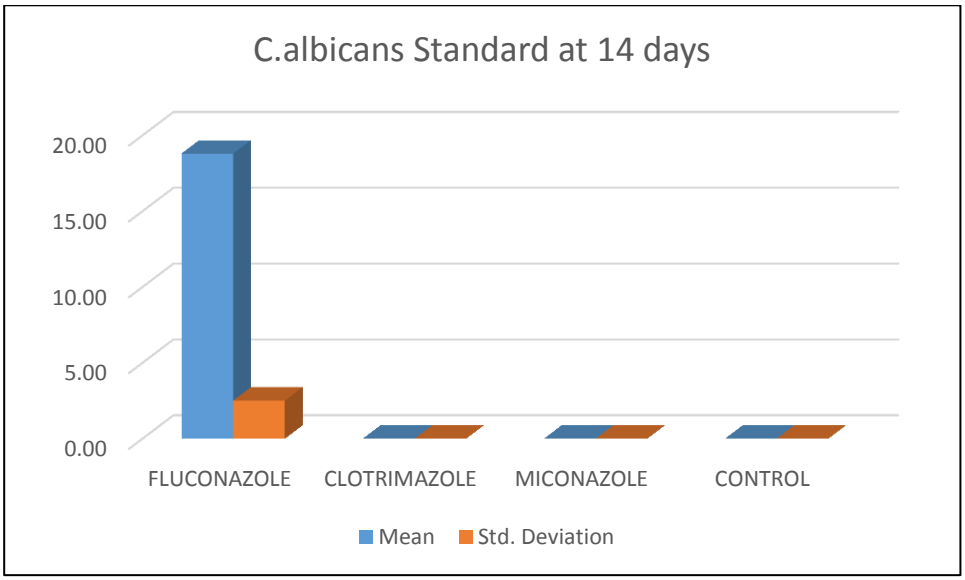


Graph 2: Graphical representation of mean and standard deviation.

Inference: *Candida albicans* standard shows highest sensitivity for fluconazole at 7 days.

Descriptive Analysis				
C.albicans standard (14 days)				
	N	Mean	Std. Deviation	Std. Error
Fluconazole	15.00	18.80	2.51	0.65
Clotrimazole	15.00	0.00	0.00	0.00
Miconazole	15.00	0.00	0.00	0.00
Control	15.00	0.00	0.00	0.00
Total	60.00	4.70	8.30	1.07

Table 3: Descriptive analysis for *Candida albicans* standard strain on 14th day of inoculation.

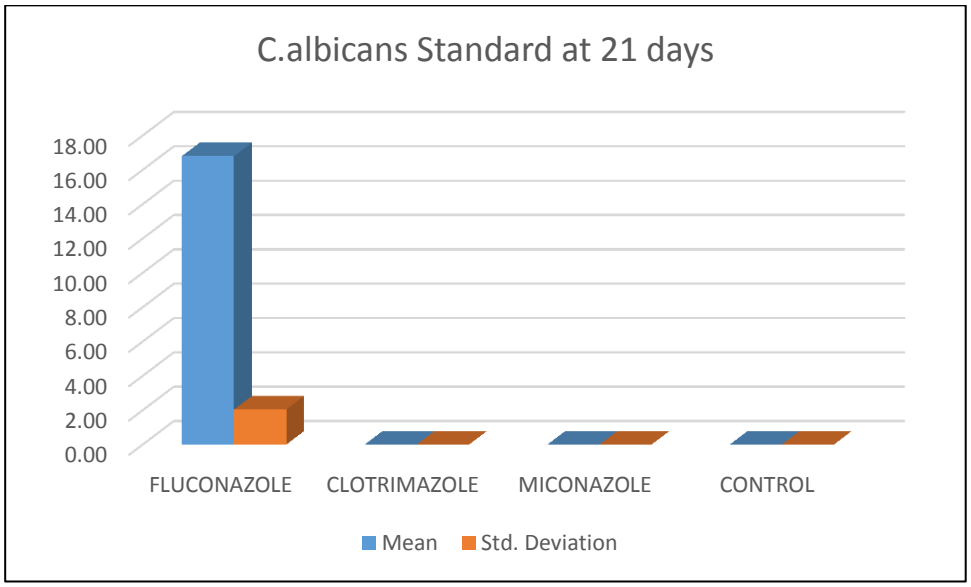


Graph 3: Graphical representation of mean and standard deviation.

Inference: *Candida albicans* standard strain shows highest sensitivity for fluconazole at 14 days.

Descriptive Analysis				
C. albicans (21 days)				
	N	Mean	Std. Deviation	Std. Error
Fluconazole	15.00	16.80	2.04	0.53
Clotrimazole	15.00	0.00	0.00	0.00
Miconazole	15.00	0.00	0.00	0.00
Control	15.00	0.00	0.00	0.00
Total	60.00	4.20	7.40	0.96

Table 4: Descriptive analysis for *Candida albicans* standard strain on 21st day of inoculation.



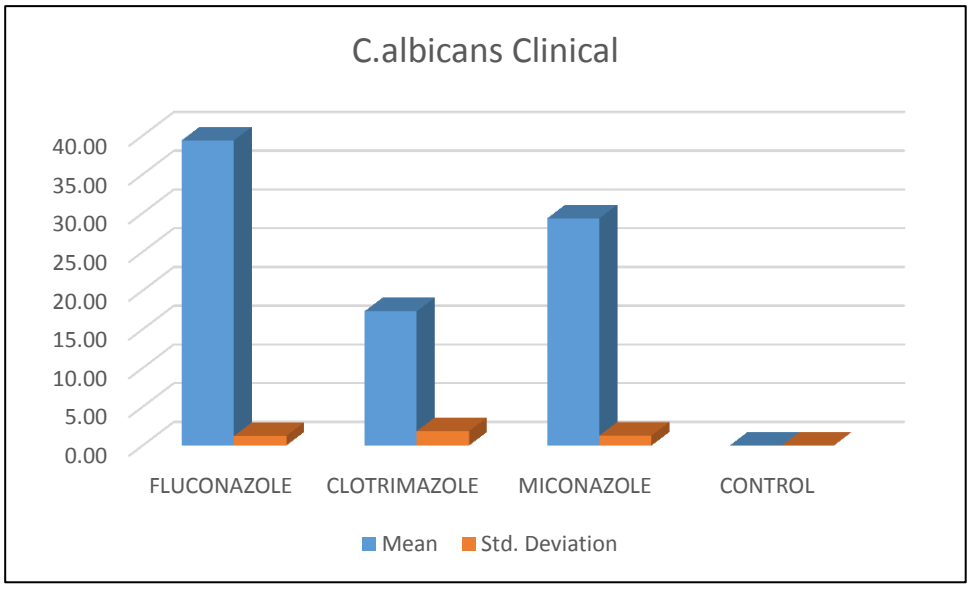
Graph 4: Graphical representation of mean and standard deviation.

Inference: *Candida albicans* standard strain is most sensitive to fluconazole at 21 days.

***CANDIDA ALBICANS* CLINICAL**

Descriptive Analysis				
C.albicans (Clinical)				
	N	Mean	Std. Deviation	Std. Error
Fluconazole	15.00	39.40	1.24	0.32
Clotrimazole	15.00	17.33	1.88	0.48
Miconazole	15.00	29.33	1.29	0.33
Control	15.00	0.00	0.00	0.00
Total	60.00	21.52	14.85	1.92

Table 5: Descriptive analysis for *Candida albicans* on 1st day of inoculation.

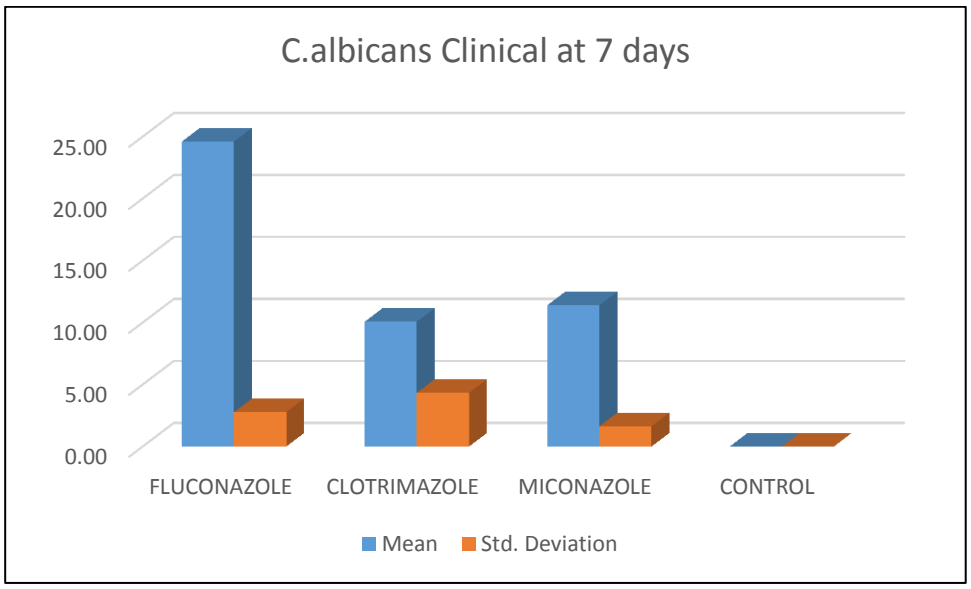


Graph 5: Graphical representation of mean and standard deviation.

Inference: *Candida albicans* clinical strain is most sensitive to fluconazole followed by miconazole and then clotrimazole at day 1.

Descriptive Analysis				
C. albicans clinical (7 days)				
	N	Mean	Std. Deviation	Std. Error
Fluconazole	15.00	24.60	2.77	0.72
Clotrimazole	15.00	10.07	4.33	1.12
Miconazole	15.00	11.40	1.64	0.42
Control	15.00	0.00	0.00	0.00
Total	60.00	11.52	9.20	1.19

Table 6: Descriptive analysis for *Candida albicans* clinical strain on 7th day of inoculation.

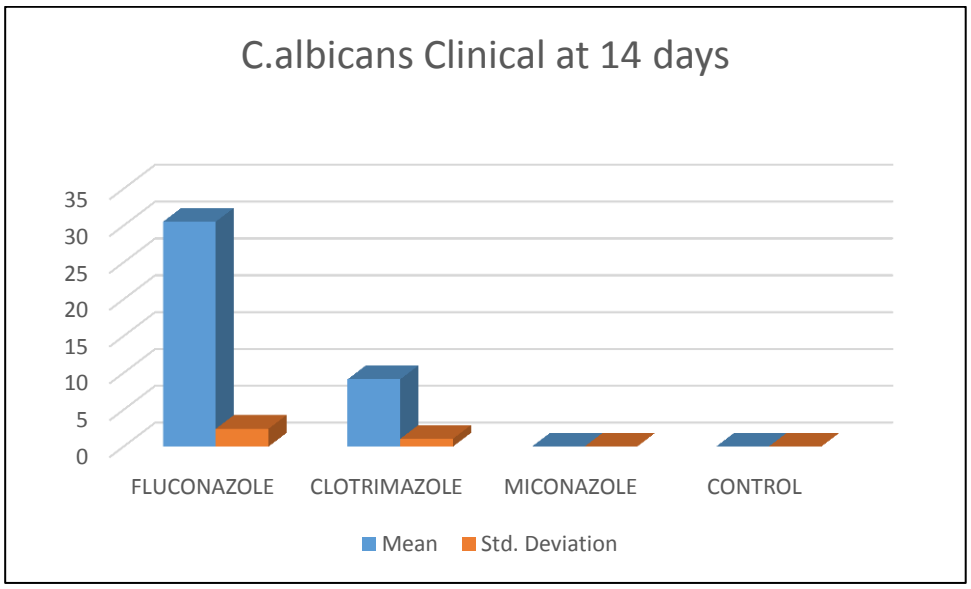


Graph 6: Graphical representation of mean and standard deviation.

Inference: *Candida albicans* clinical strain is most sensitive to fluconazole followed by miconazole and then clotrimazole at 7 days.

Descriptive Analysis				
C. albicans (14 days)				
	N	Mean	Std. Deviation	Std. Error
Fluconazole	15	30.5333	2.41622	0.62386
Clotrimazole	15	9.1333	1.0601	0.27372
miconazole	15	0	0	0
control	15	0	0	0
Total	60	9.9167	12.64414	1.63235

Table 7: Descriptive analysis for *Candida albicans* clinical strain on 14th day of inoculation.

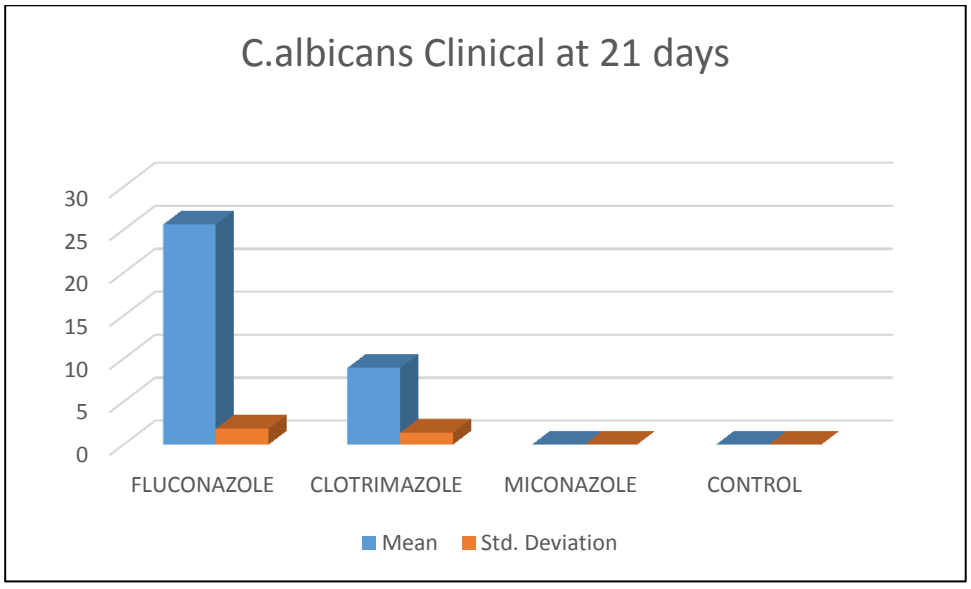


Graph 7: Graphical representation of mean and standard deviation.

Inference: *Candida albicans* clinical strain is most sensitive to fluconazole followed by clotrimazole at 14 days.

Descriptive Analysis				
C. albicans clinical (21 days)				
	N	Mean	Std. Deviation	Std. Error
Fluconazole	15	25.6	1.84391	0.4761
Clotrimazole	15	8.9333	1.38701	0.35813
Miconazole	15	0	0	0
Control	15	0	0	0
Total	60	8.6333	10.60055	1.36853

Table 8: Descriptive analysis for *Candida albicans* clinical strain on 21st day of inoculation.



Graph 8: Graphical representation of mean and standard deviation.

Inference: *Candida albicans* clinical strain is most sensitive to fluconazole followed by clotrimazole at 21 days.

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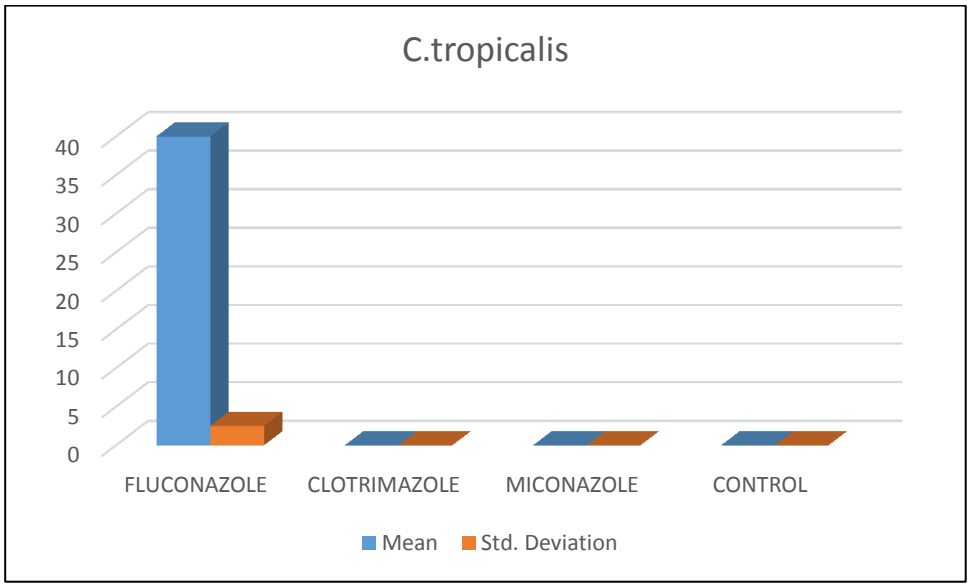
CANDIDA TROPICALIS

Descriptive Analysis				
C.tropicalis (Clinical)				
	N	Mean	Std. Deviation	Std. Error
Fluconazole	15	40	2.50713	0.64734
Clotrimazole	15	0	0	0
Miconazole	15	0	0	0
Control	15	0	0	0
Total	60	10	17.50932	2.26044

Table 9: Descriptive analysis for *Candida tropicalis* on day 1 of inoculation.



Graph 9: Graphical representation of mean and standard deviation.

Inference: *Candida tropicalis* is most sensitive to fluconazole on day 1 of inoculation.

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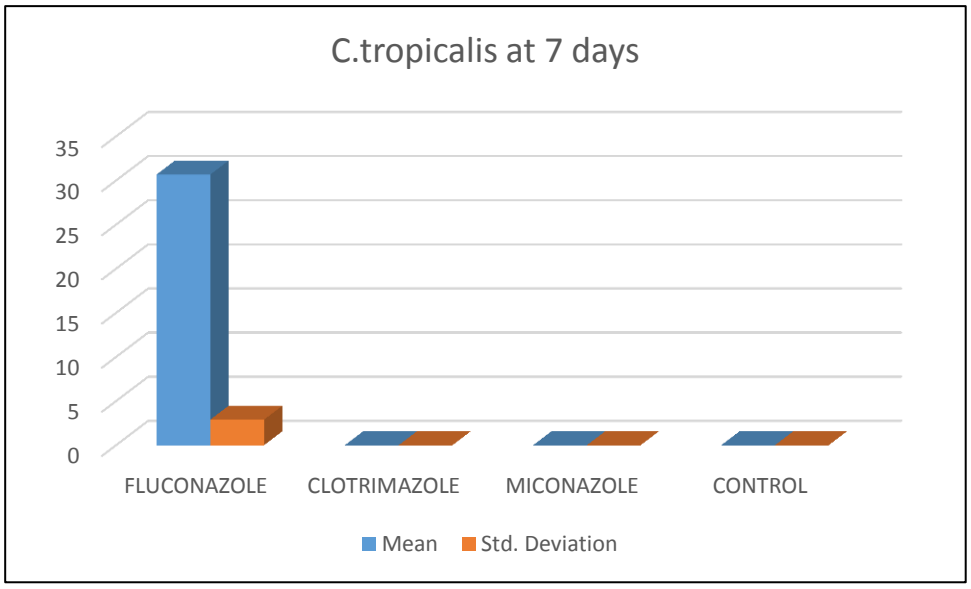
Descriptive Analysis				
C. tropicalis (7 days)				
	N	Mean	Std. Deviation	Std. Error
Fluconazole	15	30.6667	2.89499	0.74748
Clotrimazole	15	0	0	0
Miconazole	15	0	0	0
Control	15	0	0	0
Total	60	7.6667	13.46517	1.73835

Table 10: Descriptive analysis for *Candida tropicalis* on 7th day of inoculation.



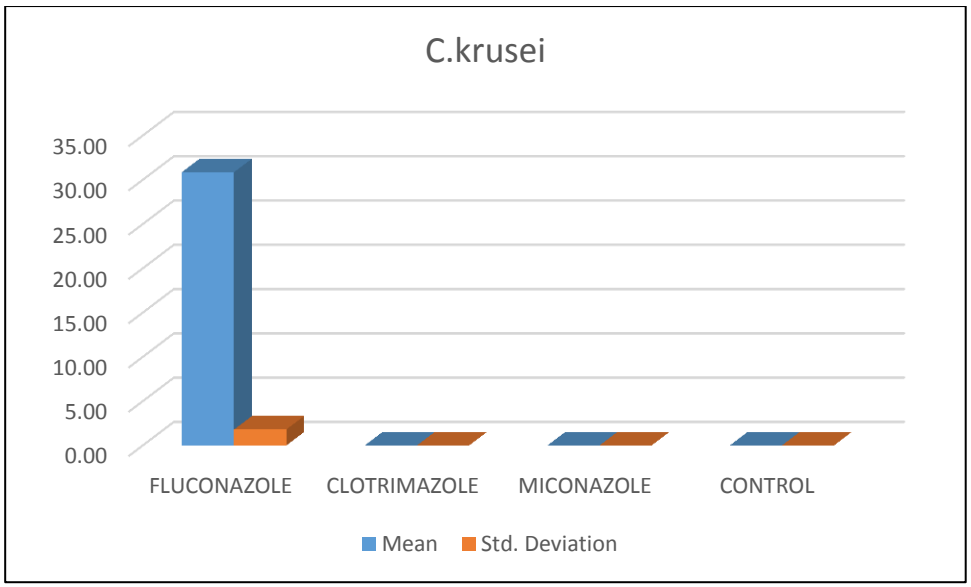
Graph 10: Graphical representation of mean and standard deviation.

Inference: *Candida tropicalis* is most sensitive to fluconazole at 7 days.

CANDIDA KRUSEI

Descriptive Analysis				
C. krusei (Clinical)				
	N	Mean	Std. Deviation	Std. Error
Fluconazole	15.00	30.87	1.81	0.47
Clotrimazole	15.00	0.00	0.00	0.00
Miconazole	15.00	0.00	0.00	0.00
Control	15.00	0.00	0.00	0.00
Total	60.00	7.72	13.51	1.74

Table 11: Descriptive analysis for *Candida krusei* on day 1 of inoculation.

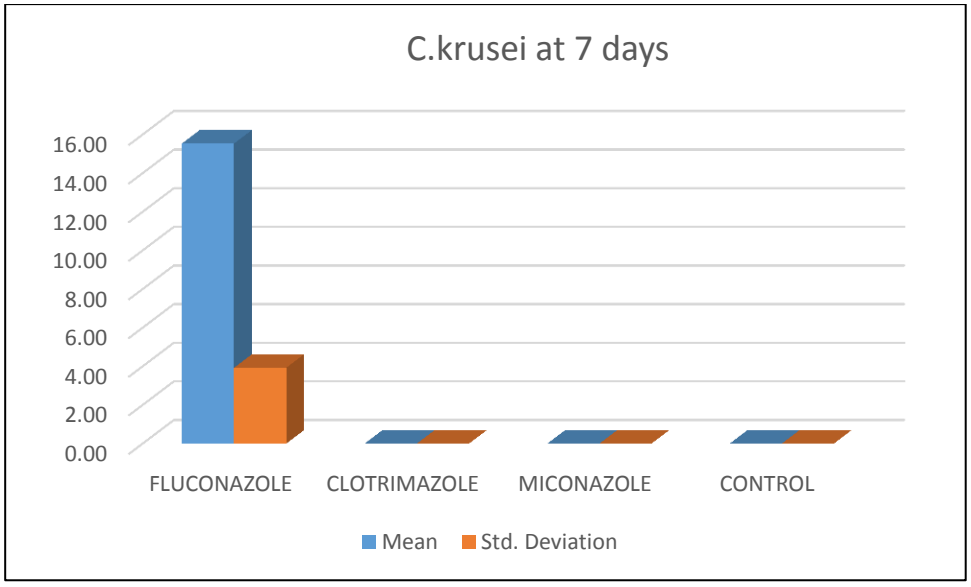


Graph 11: Graphical representation of mean and standard deviation.

Inference: *Candida krusei* is most sensitive to fluconazole on day 1 of inoculation.

Descriptive Analysis				
C. krusei (7 days)				
	N	Mean	Std. Deviation	Std. Error
fluconazole	15.00	15.53	3.93	1.01
clotrimazole	15.00	0.00	0.00	0.00
miconazole	15.00	0.00	0.00	0.00
control	15.00	0.00	0.00	0.00
Total	60.00	3.88	7.05	0.91

Table 12: Descriptive analysis for *Candida krusei* on 7th day of inoculation.



Graph 12: Graphical representation of mean and standard deviation.

Inference: *Candida krusei* is most sensitive to fluconazole on 7th day of inoculation.

TEST METHODS

ANOVA:

Analysis of variance (ANOVA) is a statistical technique that is used to check if the means of two or more groups are significantly different from each other. ANOVA checks the impact of one or more factors by comparing the means of different samples.

Another measure to compare the samples is called a t-test. When we have only two samples, t-test and ANOVA give the same results. However, using a t-test would not be reliable in cases where there are more than 2 samples. If we conduct multiple t-tests for comparing more than two samples, it will have a compounded effect on the error rate of the result.

POST HOC TUKEY/HONEST SIGNIFICANT DIFFERENCE:

The Tukey Test (or Tukey *procedure*), also called Tukey's Honest Significant Difference test, is a post-hoc test based on the range distribution. An ANOVA test can tell if the results are significant overall, but not where the differences lie. After ANOVA results are obtained, Tukey's HSD should be done to find out which specific group's means (compared with each other) are different. The test compares all possible pairs of means. To test all pairwise comparisons among means using the Tukey HSD, calculate HSD for each pair of means using the following formula:

$$HSD = \frac{M_i - M_j}{\sqrt{\frac{MS_w}{n_h}}}$$

$M_i - M_j$ is the difference between the pair of means.

MS_w is the Mean Square Within, and n is the number in the group or treatment.

ANOVA RESULTS:

Anova test or analysis or variance was performed to infer any differences that existed between the groups of drugs. Results are tabulated from table 13 to table 24. As per the tests it was found that the drugs showed different mean values and the tests show that the difference was statistically significant.

***Candida albicans* standard**

ANOVA				
C. albicans (Std)				
	Sum of Squares	Df	Mean Square	F
Between Groups	9128.05	3.00	3042.68	764.31
Within Groups	222.93	56.00	3.98	
Total	9350.98	59.00		

Table 13: Anova results indicate significant difference between the groups.

ANOVA				
C.albicans standard (7 days)				
	Sum of Squares	Df	Mean Square	F
Between Groups	4118.45	3.00	1372.82	896.71
Within Groups	85.73	56.00	1.53	
Total	4204.18	59.00		

Table 14: Anova results indicate significant difference between the groups.

ANOVA				
C.albicans standard (14 days)				
	Sum of Squares	Df	Mean Square	F
Between Groups	3976.20	3.00	1325.40	839.62
Within Groups	88.40	56.00	1.58	
Total	4064.60	59.00		

Table 15: Anova results indicate significant difference between the groups.

ANOVA				
C.albicans standard (21 days)				
	Sum of Squares	df	Mean Square	F
Between Groups	3175.20	3.00	1058.40	1014.90
Within Groups	58.40	56.00	1.04	
Total	3233.60	59.00		

Table 16: Anova results indicate significant difference between the groups.

***Candida albicans* clinical**

ANOVA					
C.albicans (Clinical)					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12920.72	3.00	4306.91	2558.56	0.00
Within Groups	94.27	56.00	1.68		
Total	13014.98	59.00			

Table 17: Anova results indicate significant difference between the groups.

ANOVA					
C.albicans clinical (7 days)					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4588.85	3.00	1529.62	209.88	0.00
Within Groups	408.13	56.00	7.29		
Total	4996.98	59.00			

Table 18: Anova results indicate significant difference between the groups.

ANOVA					
C.albicans clinical (14 days)					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	9335.117	3	3111.706	1787.847	0
Within Groups	97.467	56	1.74		
Total	9432.583	59			

Table 19: Anova results indicate significant difference between the groups.

ANOVA					
C.albicans clinical (21 days)					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6555.4	3	2185.133	1641.782	0
Within Groups	74.533	56	1.331		
Total	6629.933	59			

Table 20: Anova results indicate significant difference between the groups.

Candida tropicalis

ANOVA					
C.tropicalis (Clinical)					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	18000	3	6000	3818.182	0
Within Groups	88	56	1.571		
Total	18088	59			

Table 21: Anova results indicate significant difference between the groups.

ANOVA					
C.tropicalis (7 days)					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10580	3	3526.667	1683.182	0
Within Groups	117.333	56	2.095		
Total	10697.33	59			

Table 22: Anova results indicate significant difference between the groups.

Candida krusei

ANOVA					
C.krusei (Clinical)					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10718.45	3.00	3572.82	4374.88	0.00
Within Groups	45.73	56.00	0.82		
Total	10764.18	59.00			

Table 23: Anova results indicate significant difference between the groups.

ANOVA					
C.krusei (7 days)					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2714.45	3.00	904.82	234.87	0.00
Within Groups	215.73	56.00	3.85		
Total	2930.18	59.00			

Table 24: Anova results indicate significant difference between the groups.

P**OST HOC TUKEY TEST**:

As Anova showed a significant overall difference between the drugs, post hoc test was performed to check the level of difference at individual level.

***Candida albicans* standard**

Multiple Comparisons				
Dependent Variable: C. albicans (Std)				
Tukey HSD				
(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.
Fluconazole	Clotrimazole	29.33333*	0.73	0.00
	Miconazole	12.73333*	0.73	0.00
	Control	29.33333*	0.73	0.00
Clotrimazole	Fluconazole	-29.33333*	0.73	0.00
	Miconazole	-16.60000*	0.73	0.00
	Control	0.00	0.73	1.00
Miconazole	Fluconazole	-12.73333*	0.73	0.00
	Clotrimazole	16.60000*	0.73	0.00
	Control	16.60000*	0.73	0.00
Control	Fluconazole	-29.33333*	0.73	0.00
	Clotrimazole	0.00	0.73	1.00
	Miconazole	-16.60000*	0.73	0.00
* The mean difference is significant at the 0.05 level.				

Table 25: Post hoc test to evaluate the level of difference between the groups.

Multiple Comparisons				
Dependent Variable: C. albicans standard (7 days)				
Tukey HSD				
(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.
Fluconazole	Clotrimazole	19.13333*	0.45	0.00
	Miconazole	19.13333*	0.45	0.00
	Control	19.13333*	0.45	0.00
Clotrimazole	Fluconazole	-19.13333*	0.45	0.00
	Miconazole	0.00	0.45	1.00
	Control	0.00	0.45	1.00
Miconazole	Fluconazole	-19.13333*	0.45	0.00
	Clotrimazole	0.00	0.45	1.00
	Control	0.00	0.45	1.00
Control	Fluconazole	-19.13333*	0.45	0.00
	Clotrimazole	0.00	0.45	1.00
	Miconazole	0.00	0.45	1.00
* The mean difference is significant at the 0.05 level.				

Table 26: Post hoc test to evaluate the level of difference between the groups.

Multiple Comparisons				
Dependent Variable: C. albicans standard (14 days)				
Tukey HSD				
(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.
Fluconazole	Clotrimazole	18.80000*	0.46	0.00
	Miconazole	18.80000*	0.46	0.00
	Control	18.80000*	0.46	0.00
Clotrimazole	Fluconazole	-18.80000*	0.46	0.00
	Miconazole	0.00	0.46	1.00
	Control	0.00	0.46	1.00
Miconazole	Fluconazole	-18.80000*	0.46	0.00
	Clotrimazole	0.00	0.46	1.00
	Control	0.00	0.46	1.00
Control	Fluconazole	-18.80000*	0.46	0.00
	Clotrimazole	0.00	0.46	1.00
	Miconazole	0.00	0.46	1.00
* The mean difference is significant at the 0.05 level.				

Table 27: Post hoc test to evaluate the level of difference between the groups.

Multiple Comparisons				
Dependent Variable: C. albicans standard (21 days)				
Tukey HSD				
(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.
Fluconazole	Clotrimazole	16.80000*	0.37	0.00
	Miconazole	16.80000*	0.37	0.00
	Control	16.80000*	0.37	0.00
Clotrimazole	Fluconazole	-16.80000*	0.37	0.00
	Miconazole	0.00	0.37	1.00
	Control	0.00	0.37	1.00
Miconazole	Fluconazole	-16.80000*	0.37	0.00
	Clotrimazole	0.00	0.37	1.00
	Control	0.00	0.37	1.00
Control	Fluconazole	-16.80000*	0.37	0.00
	Clotrimazole	0.00	0.37	1.00
	Miconazole	0.00	0.37	1.00
* The mean difference is significant at the 0.05 level.				

Table 28: Post hoc test to evaluate the level of difference between the groups.

***Candida albicans* clinical**

Multiple Comparisons				
Dependent Variable: C. albicans (Clinical)				
Tukey HSD				
(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.
Fluconazole	Clotrimazole	22.06667*	0.47	0.00
	Miconazole	10.06667*	0.47	0.00
	Control	39.40000*	0.47	0.00
Clotrimazole	Fluconazole	-22.06667*	0.47	0.00
	Miconazole	-12.00000*	0.47	0.00
	Control	17.33333*	0.47	0.00
Miconazole	Fluconazole	-10.06667*	0.47	0.00
	Clotrimazole	12.00000*	0.47	0.00
	Control	29.33333*	0.47	0.00
Control	Fluconazole	-39.40000*	0.47	0.00
	Clotrimazole	-17.33333*	0.47	0.00
	Miconazole	-29.33333*	0.47	0.00
* The mean difference is significant at the 0.05 level.				

Table 29: Post hoc test to evaluate the level of difference between the groups.

Multiple Comparisons				
Dependent Variable: C. albicans clinical (7 days)				
Tukey HSD				
(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.
Fluconazole	Clotrimazole	14.53333*	0.99	0.00
	Miconazole	13.20000*	0.99	0.00
	Control	24.60000*	0.99	0.00
Clotrimazole	Fluconazole	-14.53333*	0.99	0.00
	Miconazole	-1.33	0.99	0.53
	Control	10.06667*	0.99	0.00
Miconazole	Fluconazole	-13.20000*	0.99	0.00
	Clotrimazole	1.33	0.99	0.53
	Control	11.40000*	0.99	0.00
Control	Fluconazole	-24.60000*	0.99	0.00
	Clotrimazole	-10.06667*	0.99	0.00
	Miconazole	-11.40000*	0.99	0.00
* The mean difference is significant at the 0.05 level.				

Table 30: Post hoc test to evaluate the level of difference between the groups.

Multiple Comparisons				
Dependent Variable: C. albicans clinical (14 days)				
Tukey HSD				
(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.
Fluconazole	Clotrimazole	21.40000*	0.48173	0
	Miconazole	30.53333*	0.48173	0
	Control	30.53333*	0.48173	0
Clotrimazole	Fluconazole	-21.40000*	0.48173	0
	Miconazole	9.13333*	0.48173	0
	Control	9.13333*	0.48173	0
Miconazole	Fluconazole	-30.53333*	0.48173	0
	Clotrimazole	-9.13333*	0.48173	0
	Control	0	0.48173	1
Control	Fluconazole	-30.53333*	0.48173	0
	Clotrimazole	-9.13333*	0.48173	0
	Miconazole	0	0.48173	1
* The mean difference is significant at the 0.05 level.				

Table 31: Post hoc test to evaluate the level of difference between the groups.

Multiple Comparisons				
Dependent Variable: C. albicans clinical (21 days)				
Tukey HSD				
(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.
Fluconazole	Clotrimazole	16.66667*	0.42126	0
	Miconazole	25.60000*	0.42126	0
	Control	25.60000*	0.42126	0
Clotrimazole	Fluconazole	-16.66667*	0.42126	0
	Miconazole	8.93333*	0.42126	0
	Control	8.93333*	0.42126	0
Miconazole	Fluconazole	-25.60000*	0.42126	0
	Clotrimazole	-8.93333*	0.42126	0
	Control	0	0.42126	1
Control	Fluconazole	-25.60000*	0.42126	0
	Clotrimazole	-8.93333*	0.42126	0
	Miconazole	0	0.42126	1
* The mean difference is significant at the 0.05 level.				

Table 32: Post hoc test to evaluate the level of difference between the groups.

Candida tropicalis

Multiple Comparisons				
Dependent Variable: C. tropicalis (Clinical)				
Tukey HSD				
(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.
Fluconazole	Clotrimazole	40.00000*	0.45774	0
	Miconazole	40.00000*	0.45774	0
	Control	40.00000*	0.45774	0
Clotrimazole	Fluconazole	-40.00000*	0.45774	0
	Miconazole	0	0.45774	1
	Control	0	0.45774	1
Miconazole	Fluconazole	-40.00000*	0.45774	0
	Clotrimazole	0	0.45774	1
	Control	0	0.45774	1
Control	Fluconazole	-40.00000*	0.45774	0
	Clotrimazole	0	0.45774	1
	Miconazole	0	0.45774	1
* The mean difference is significant at the 0.05 level.				

Table 33: Post hoc test to evaluate the level of difference between the groups.

Multiple Comparisons				
Dependent Variable: C. tropicalis (7 days)				
Tukey HSD				
(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.
Fluconazole	Clotrimazole	30.66667*	0.52855	0
	Miconazole	30.66667*	0.52855	0
	Control	30.66667*	0.52855	0
Clotrimazole	Fluconazole	-30.66667*	0.52855	0
	Miconazole	0	0.52855	1
	Control	0	0.52855	1
Miconazole	Fluconazole	-30.66667*	0.52855	0
	Clotrimazole	0	0.52855	1
	Control	0	0.52855	1
Control	Fluconazole	-30.66667*	0.52855	0
	Clotrimazole	0	0.52855	1
	Miconazole	0	0.52855	1
* The mean difference is significant at the 0.05 level.				

Table 34: Post hoc test to evaluate the level of difference between the groups.

Candida krusei

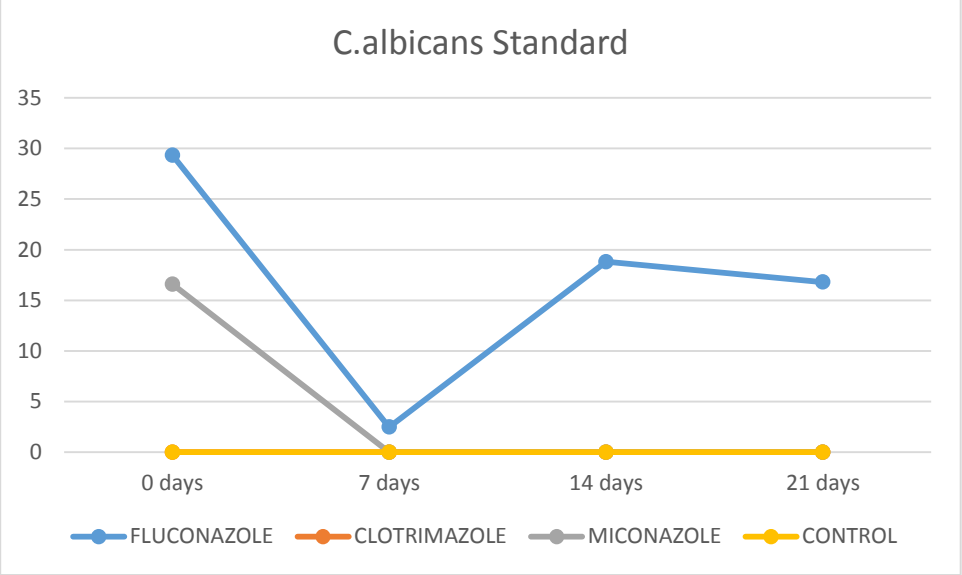
Multiple Comparisons				
Dependent Variable: C. krusei (Clinical)				
Tukey HSD				
(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.
Fluconazole	Clotrimazole	30.86667*	0.33	0.00
	Miconazole	30.86667*	0.33	0.00
	Control	30.86667*	0.33	0.00
Clotrimazole	Fluconazole	-30.86667*	0.33	0.00
	Miconazole	0.00	0.33	1.00
	Control	0.00	0.33	1.00
Miconazole	Fluconazole	-30.86667*	0.33	0.00
	Clotrimazole	0.00	0.33	1.00
	Control	0.00	0.33	1.00
Control	Fluconazole	-30.86667*	0.33	0.00
	Clotrimazole	0.00	0.33	1.00
	Miconazole	0.00	0.33	1.00
* The mean difference is significant at the 0.05 level.				

Table 35: Post hoc test to evaluate the level of difference between the groups.

Multiple Comparisons				
Dependent Variable: C. krusei (7 days)				
Tukey HSD				
(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.
Fluconazole	Clotrimazole	15.53333*	0.72	0.00
	Miconazole	15.53333*	0.72	0.00
	Control	15.53333*	0.72	0.00
Clotrimazole	Fluconazole	-15.53333*	0.72	0.00
	Miconazole	0.00	0.72	1.00
	Control	0.00	0.72	1.00
Miconazole	Fluconazole	-15.53333*	0.72	0.00
	Clotrimazole	0.00	0.72	1.00
	Control	0.00	0.72	1.00
Control	Fluconazole	-15.53333*	0.72	0.00
	Clotrimazole	0.00	0.72	1.00
	Miconazole	0.00	0.72	1.00
* The mean difference is significant at the 0.05 level.				

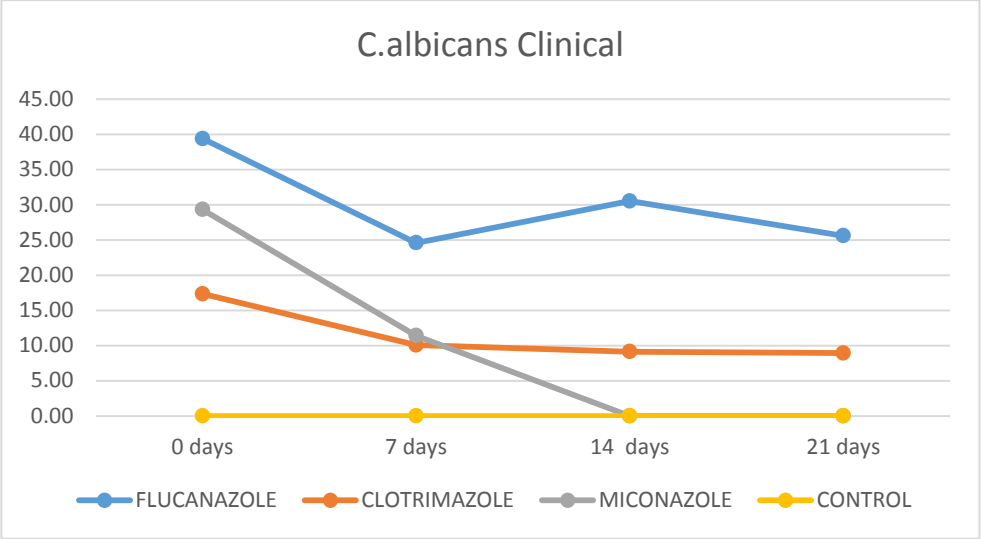
Table 36: Post hoc test to evaluate the level of difference between the groups.

INFERENCE: Based on the Post hoc Tukey test, the differences between the various groups can be interpreted as follows



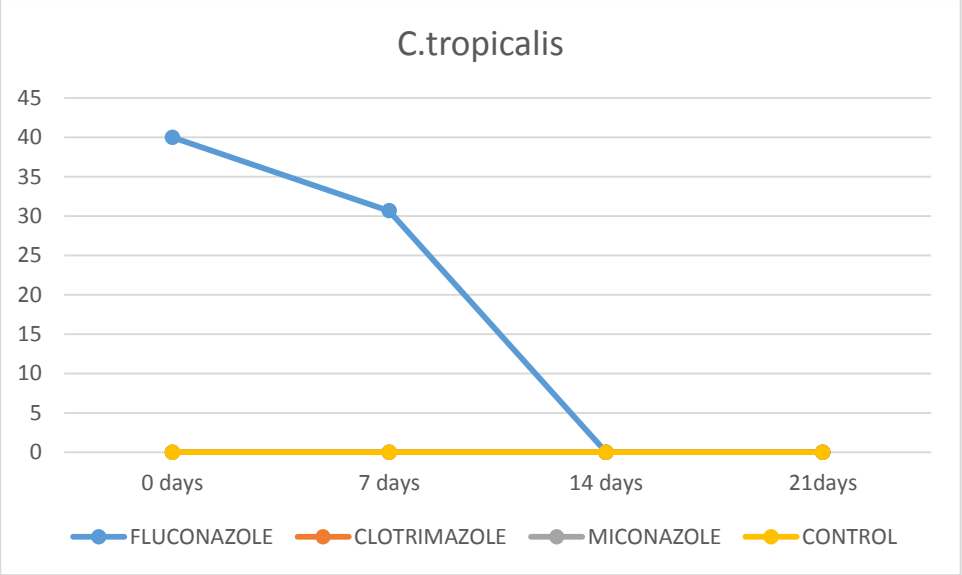
Graph 13: Comparison of the efficacy of drugs at various time intervals for C.albicans standard strain.

Inference : *Candida albicans* standard strain shows highest value for fluconazole at day 1 followed by a decrease at day 7 and then an increase at day 14, eventually declining at day 21. It shows its activity with miconazole for 7 days. However it is resitant to clotrimazole.



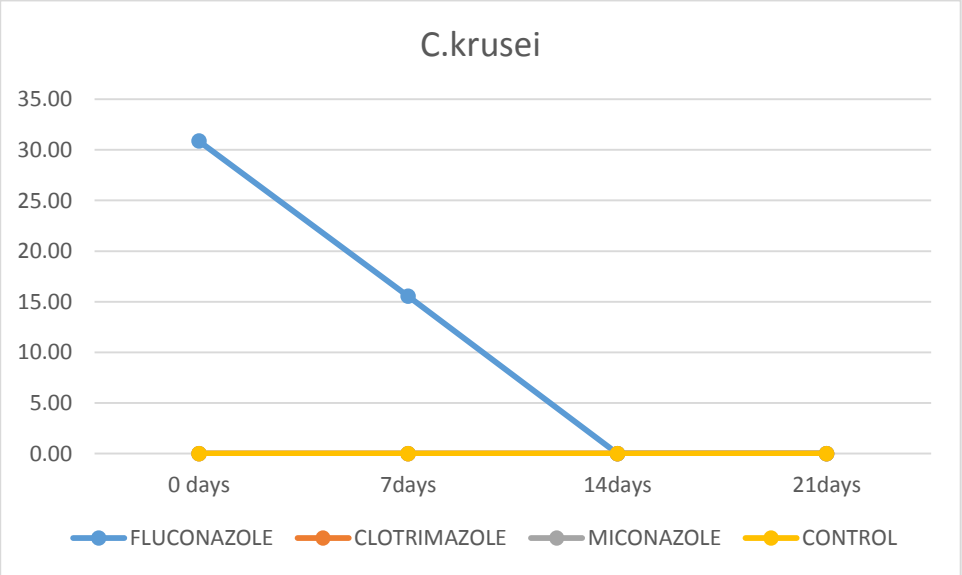
Graph 14: Comparison of the efficacy of drugs at various time intervals for *C.albicans* clinical strain.

Inference: *Candida albicans* clinical strain is most sensitive to fluconazole on day 1 of inoculation followed by decrease at 7 days. A slight rise is observed on day 14 followed by a decrease on day 21. At a lower value it shows sensitivity to miconazole which gradually decreases by day 14. It shows sensitivity to clotrimazole, whose value remains higher than miconazole at day 14 and 21.



Graph 15: Comparison of the efficacy of drugs at various time intervals for *C.tropicalis*

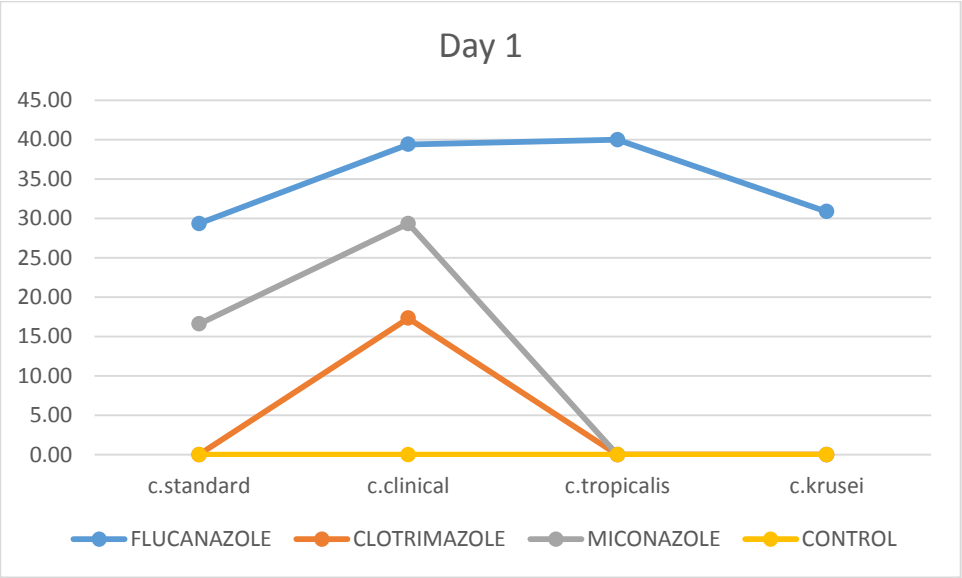
Inference: *Candida tropicalis* is highly sensitive to fluconazole and the activity reduces by 14 days. However it is resistant to the other drugs.



Graph 16: Comparison of the efficacy of drugs at various time intervals for *C.krusei.*

Inference: *Candida krusei* is highly sensitive to fluconazole and the activity reduces by 14 days. However it is resistant to the other drugs.

GRAPHICAL REPRESENTATION OF THE EFFICACY OF DRUGS AT VARIOUS TIME INTERVALS



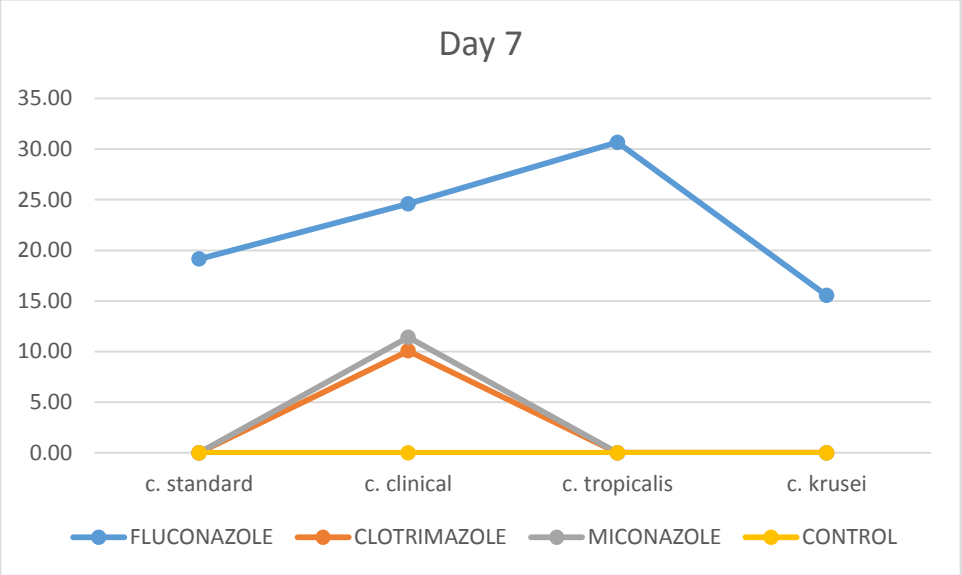
Graph 17: Comparison of the efficacy of drugs on *candida species* on day 1

Inference: On the day 1 of inoculation *Candida albicans* clinical strain shows highest sensitivity towards fluconazole followed by miconazole and then clotrimazole.

Candida tropicalis shows similar value of sensitivity for fluconazole (as that of *Candida albicans* clinical strain) and shows resistance to miconazole and clotrimazole.

Candida albicans standard strain shows highest sensitivity towards fluconazole followed by miconazole and it is resistant to clotrimazole.

Candida krusei shows highest sensitivity towards fluconazole and resistance to miconazole and clotrimazole.



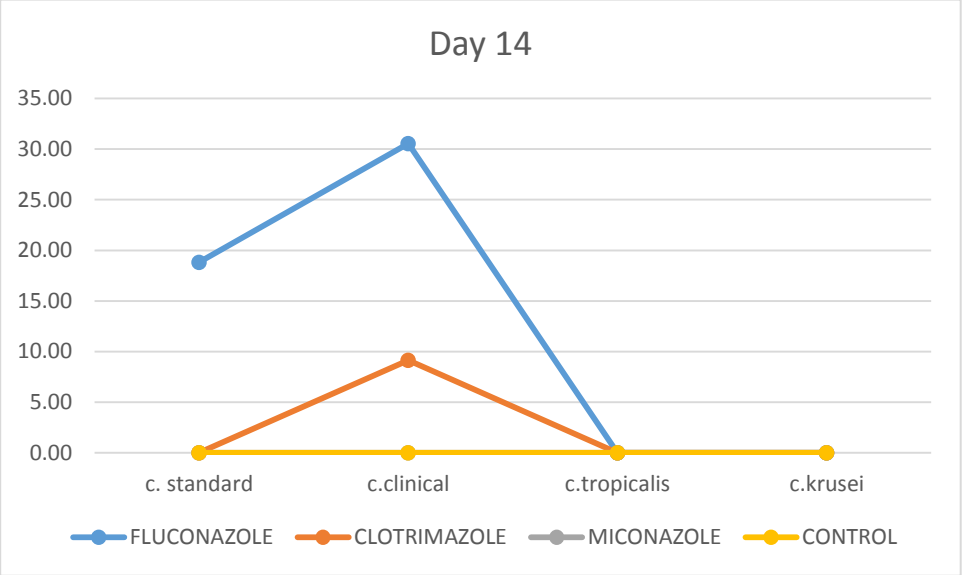
Graph 18: Comparison of the efficacy of drugs on *candida species* on day 7.

Inference: On the day 7 of inoculation *C.tropicalis* strain shows highest sensitivity towards fluconazole and it is resistant to miconazole and clotrimazole.

The next fungi to show sensitivity is *Candida albicans* clinical strain which is sensitive to fluconazole followed by miconazole and then clotrimazole.

Candida albicans standard strain is sensitive to fluconazole followed by *C.krusei* which is also sensitive to fluconazole. Both are resistant to the other drugs.

However the values are lesser than that of day 1.

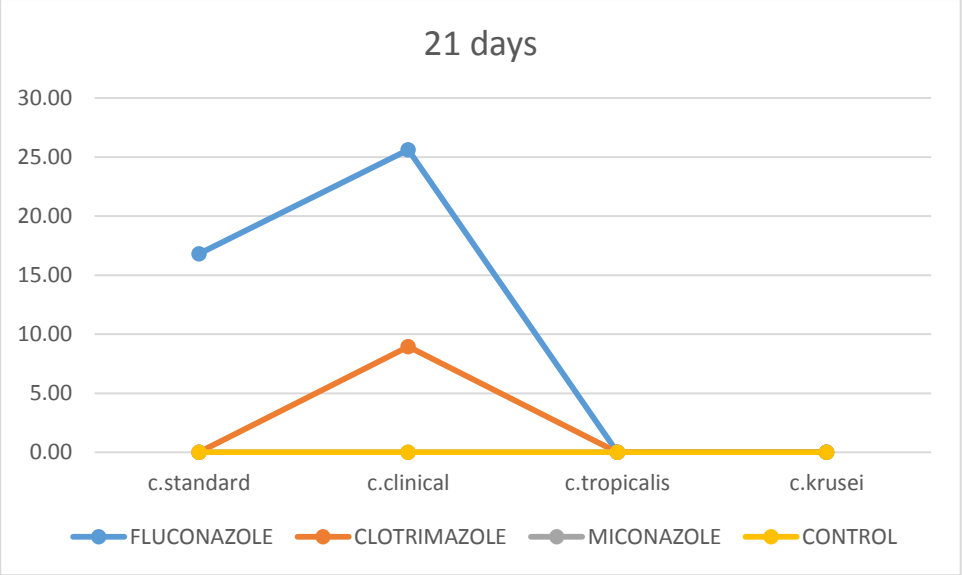


Graph 19: Comparison of the efficacy of drugs on candida species at day 14.

Inference: On the day 14 of inoculation *Candida albicans* clinical strain shows highest sensitivity towards fluconazole followed by clotrimazole and is resistant to miconazole.

The next fungi to show sensitivity is *Candida albicans* standard strain to fluconazole.

C. topicalis and *C.krusei* shows resistance to all the drugs.



Graph 20: Comparison of the efficacy of drugs on candida species at day 21

Inference: On the day 21 of inoculation *Candida albicans* clinical strain shows highest sensitivity towards fluconazole followed by clotrimazole and is resistant to miconazole.

The next fungi to show sensitivity is *Candida albicans* standard strain to fluconazole

C. topicalis and *C.krusei* shows resistance to all the drug.

DISCUSSION

Discussion

Denture-related stomatitis (also termed denture sore mouth, denture stomatitis, chronic atrophic candidiasis, and denture-associated erythematous stomatitis) is a common condition characterized by mild inflammation and redness of the oral mucous membrane which is usually seen beneath the denture surface.

The cause of denture stomatitis is believed to be multifactorial, and the most reported factors include denture trauma, wearing dentures at night, denture hygiene, predisposing systemic conditions and microbial pathogens. In about 90% of cases, *Candida species* are involved, which are normal commensals of the oral microbiota in many people.

The presence of *C. albicans* on the fitting surfaces of dentures is a major causative factor in denture associated chronic atrophic candidiasis (denture stomatitis), the most common form of oral candidiasis. The relatively acidic and anaerobic environment under the denture provides an ideal and favorable habitat for fungal growth.

Different classification have been proposed, but the reference classification for denture stomatitis is the one suggested by Newton in 1962, based exclusively on clinical criteria. [68]

- Newton's type I: pin-point hyperemic lesions (localized simple inflammation) classified as mild
- Newton's type II: diffuse erythema confined to the mucosa contacting the denture (generalized simple inflammation) classified as moderate
- Newton's type III: granular surface (inflammatory papillary hyperplasia) classified as severe

Type III often is seen in association with types I and II. Type III denture stomatitis involves the epithelial response to chronic inflammatory stimulation secondary to yeast colonization and possibly, low grade local trauma resulting from an ill-fitting denture

In 1970, another classification by Budtz-Jorgensen and Bertram, different terminology for the same changes were given [69]:

- Type I – simple localized inflammation,
- Type II – simple diffused (generalized) inflammation,
- Type III – Granular inflammation

Denture-induced candidiasis is a common disease in elderly denture wearers with *Candida albicans* as the principal causative agent. The problem is aggravated for elderly patients with limited motor skills who fail to follow a precise antifungal drug regime. Denture-induced candidiasis recurrence rate is high due to the medications poor penetration into the microbial biofilm on the porous denture material and also because of their quick clearance by saliva and tongue movements. Usually a mixture of Candida species such as *Candida albicans, Candida tropicalis, Candida krusei, Candida guilliermondii, Candida parapsilosis, and Candida glabrata* can be isolated from oral lesions [70]

Gauch et al [71] identified *Candida species* from the oral cavity of denture wearers with denture-related stomatitis. *Candida spp*. were isolated from 89% of the cases and included five different *Candida species*. *C. albicans* was the most frequently recovered species (78% of the cases), followed by *C. famata* and *C. tropicalis*

The results of a study by **Wright et al** [72] to evaluate different species of yeasts in a group of patients wearing denture soft lining materials indicated that *C. albicans* was clearly and significantly ($p<0.02$) associated with greater density of colonization when compared with the other species isolated in the study.

Denture stomatitis is a chronic condition that challenges treatment. Treatment of the same involves local and systemic antifungal therapies, reduction or eradication of denture related biofilm, laser treatment of the affected mucosa, or combination approaches.

Treatment of denture stomatitis has mainly focused on the use of antifungal medications. Topical antifungal therapy is the corner stone of treatment of localised candidiasis. Topical agents are effective on fungi that invade superficial tissues but topical therapy is frequently associated with poor patient compliance due to unpleasant taste and frequency of dosage. They are available in many forms like pastilles, creams, ointments and oral suspensions.

The antifungal treatments used are antifungal suspensions based on nystatin, amphotericin-B, miconazole, fluconazole. Almost all drugs usually produce a complete remission of symptoms by 12-14 days. A nystatin suspension of 100,000 unit per ml is generally prescribed. Clotrimazole (1% cream) is used topically, because of gastrointestinal and neurological toxicity. Miconazole (2-4%) can also be used topically.

Antifungal agents are either polyenes (nystatin and amphotericin B) or azoles which are classified into: imidazoles (clotrimazole, econazole, fenticonazole, ketoconazole, miconazole) and triazole (fluconazole, itraconazol). These act by inhibiting pathways necessary for cell membrane synthesis or by altering the permeability of the cell membrane of the fungal cells. It may also alter RNA and DNA metabolism or create an intracellular accumulation of peroxide that is toxic to the fungal cell. The effect of the anti-fungal agent depends on its concentration, susceptibility of the strain and the source of mucosal surface.

The imidazole compounds such as clotrimazole, miconazole, fluconazole are broad spectrum antifungal agents which bind to the ergosterol on the candida cell membranes and causes changes in the permeability of the cell membrane, which leads to the penetration of the drug into the cell finally causing cell death

MacFarlane and Samaranayake [73] have emphasized that the treatment of denture stomatitis involves strict denture hygiene measures and the use of antifungal agents. In particular, patients should be discouraged from wearing their dentures at night and the dentures should be soaked overnight in an antiseptic solution followed by topical treatment with amphotericin B.

Treatment options require good patient compliance which can be difficult to achieve when the patient is hospitalized or lacking independence. Institutionalized patients may find it difficult to follow the prescription for application of the antifungal agent onto the denture and consequently rely on nursing or other healthcare professionals. It would therefore be advantageous to provide treatment that is easier to administer and requiring less frequent application.

Tissue conditioning materials were introduced about 50 years ago. These are soft, resilient, temporary relining materials which by reducing and evenly distributing

stresses on the mucosa of the basal seat, have a rehabilitating effect on unhealthy tissue and allow the condition to return back to normal.

Soft lining materials can be divided into acrylic-and silicon based groups and both groups are offered in auto-or heat-cured systems. Most of the currently used materials are based on plasticized acrylic resins, which, when applied to the fitting surface of the denture, absorb the impact of mastication and distribute the force widely hence helping to reduce the mucosal pain

CLASSIFICATION OF TISSUE CONDITIONERS [74]

I. Based on curing
   a. Self-cure resins
   b. Heat cure resins
   c. Light cure resins

II. Based on composition
   a. Silicone elastomers
   b. Soft acrylic compounds
   c. Phthalate liners
   d. Polyolefin liners
   e. Fluoride containing liners

III. Based on duration
   a. Temporary/short term liners
   b. Definitive/ long term liners

IV. Based on consistency
   a. Hard denture liners
   b. Soft denture liners
      - Silicone based and resin based
      - Auto cure and heat cured

V. Based on availability

a. Home reliners
b. Tissue conditioners

VI. Based on water sorption property
a. Hydrophilic
b. Hydrophobic

Composition:

1. Powder of cold cure acrylic based material consists of polyethyl methacrylate/copolymer, polymethy methacrylate/copolymer, benzoyl peroxide, pthalyl butyl gluconate, pigments, and fillers.
2. Liquid contains methyl methacrylate, ethylene glycol dimethacrylate, ester plasticizer mixture like dibutyl phthalate, butyl pthalybutylglycolate, benzyl butyl phthalate, dibutylsebacate, ethyl alchohol
3. Heat cure liquid has in addition benzoyl peroxide initiator
4. Home reliners consist of polyvinyl acetate, ethyl alchohol, calcium carbonate, polypropylene glycol, white bees wax and alkyl methacrylate copolymers
5. Polypropylenene glycol and wax allows for easy peeling of conditioners from dentures and these along with alkyl methacrylate copolymer prevents adhesion to fingers
6. White bees wax also acts as plasticizer
7. Calcium carbonate increases elasticity of polymer
8. Liquid consists – acrylic, triacetyl citrate, tris methoxyvinylsilane
9. Silica consists of 2 MDX (silastic MDX – 4210) RTV silicones, fumed silica with high surface area, hemamethyldisilanazane surface treatment to repel water, vinyl terminated polydimethylsiloxane, adhesive like 3-methacryloxypropyl trimethoxysilane and silicic acid
10. Light cured material consists of urethane acrylate oligomers, benzoylperoxide, camphoroquinone. [74]

Gelation reaction:

When the powder and liquid are mixed, plasticizer dissolves polymer. This reaction is responsible for chain entanglement and thus formation of gel. Since monomer is absent, it is made of non-cross linked amorphous polymers.

These tissue liners have been used clinically to improve adaptation of the denture base to supporting tissue. Acrylic or silicone soft liners act as shock absorber and are used as a therapeutic measure for patients who cannot tolerate stresses induced by dentures. These materials are often used in the management of edentulous patients who suffer from chronic pain, traumatized oral mucosa due to prolonged contact between the rigid denture base materials and the underlying tissues.

However, despite their vast clinical benefits, the most challenging factor in the use of long and short-term soft liners is their tendency to support the growth of *C. albicans* due to material porosity, water absorption and diffusion of nutrient materials. This is further complicated by the difficulty of cleaning most of the liners with routine mechanical or chemical methods.

Gruber et al showed that silicone and methacrylate soft denture liners would support the growth of *C. albicans*. [75]

In vitro studies covering longer time periods show that the use of soft liners might intensify the formation of fungal biofilms. Colonization of soft liners by *Candida albicans* is favored by the presence of saliva and serum pellicles. Fungal adhesion to material surfaces is the first step of colonization. Fungi can then penetrate into the material. Adhesion of candida albicans to soft liners involves microbial attachment, cell proliferation, matrix production and detachment.

These materials are porous, difficult to keep clean and may act as reservoirs for *Candida albicans.* Therefore, prevention of the growth of *C. albicans* has focused on the use of antifungal medications. Topical application of antifungal agents has not been encouraged as saliva leaves an insufficient concentration at the site of action and washes the medication off. Systemic administration requires large doses of drugs with a serious risk of side effects. To overcome these disadvantages antifungal agents have been incorporated into denture liners.

A soft liner with antifungal activity could be a great advantage for patients with high risk of denture stomatitis. Several attempts have been made to incorporate

additives and antifungals to soft liner as a drug delivery method for controlling microbial attachment and colonization. An antifungal agent incorporated into a soft liner can provide a slow continuous release resulting in a sustained therapeutic effect. [76]

Local drug carriers have been suggested to prolong the efficiency of oral treatment in order to maintain ideal therapeutic drug levels at the site of infection over the required period by release of the drug as stated by **Brook IM et al.** [77] These are convenient for the patients as they do not require compliance to frequent application regimes. In addition to this, direct delivery of the drug to the site of infection reduces the risk of systemic side effects.

Although denture induced candidiasis could be treated by various treatment modalities directed toward the oral mucosa, treatment modalities directed toward the denture base is advantageous. Favorable results for incorporation of antifungal agents in different polymeric systems have been reported in studies done by **Amin WM et al** [78]. Therefore, a local delivery system is an alternative option to maintain therapeutic drug levels at the site of pathology.

Odds [79] considered that denture liners alone usually have no effect on *candida species* and antifungals need to be incorporated.

Hence in this study, combining antifungal agents and soft liners was considered a therapeutic approach. By reducing trauma to the denture bearing tissue in conjunction with sustained release of antifungal agents to destroy the organism primarily involved in oral mycotic infections, two prominent etiological factors are addressed simultaneously. This would be easier for the patient to keep clean.

Thomas and Nutt [80] showed that Viscogel (tissue conditioner) combined with nystatin powder were successful in inhibiting the growth of *Candida albicans, Candida krusei* and *Candida tropicalis.*

In this study, a total of 60 samples of each of the *candida* species (i.e 15 samples for *Candida albicans* standard strain, 15 samples for *Candida albicans* clinical strain, 15 samples for *Candida krusei*, 15 samples for *Candida tropicalis*) were taken for each group of antifungal incorporated soft liner(fluconazole, clotrimazole, miconazole) including control group.

The soft liner used in this study is commercially available (GC corporation, Japan). In the present study an agar based medium (SDA) was devised to investigate the fungicidal effects of antifungal agent and soft liner combinations.

The selection of GC soft liner for this experiment is sufficed by the previous studies on tissue conditioners by **Kanathila et al** [81] in India and by **Thomas et al**, [80] who observed that tissue conditioners would not be beneficial without antifungal agents in the treatment of denture stomatitis. Also, in an in vitro study conducted by **Chow et al [26]** to know the efficacy of antifungal agents in tissue conditioners in inhibiting *C. albicans*, samples containing only tissue conditioners did not exhibit significant fungicidal activity as compared to combinations of antifungal agents and tissue conditioners.

Denture soft liners, when mixed with antifungal agents showed satisfactory inhibition of *candida albicans* suggesting that incorporation of antifungal agents into soft liners can be recommended for clinical use.

The drugs used in this study were fluconazole, miconazole, clotrimazole as they showed high potency towards *candida* species. Group type azoles were used in this study.

The experiments were carried out in two stages:

- Stage I – effect of antifungal incorporated soft liner on *Candida species*
- Stage II – effect of antifungal incorporated soft liner on *Candida species* after immersion in water for varying periods of 7,14 and 21 days

All the samples were tabulated and analysed for statistical significance using ANOVA (analysis of variance) and Post hoc test.

Fluconazole incorporated soft liner specimen.

The zone of inhibition for every interval was calculated. The mean zone of inhibition for *Candida albicans* standard strain was found to be 29 mm for day 1, 19 mm for day 7, 18 mm for day 14, 16mm for day 21

The mean zone of inhibition for *Candida albicans* clinical strain was found to be 39 mm for day 1, 24 mm for day 7, 30 mm for day 14 and 25 mm for day 21.

The mean zone of inhibition for *Candida tropicalis* was found to be 40 for day 1 and 30 for day 7. No activity was recorded on day 14 and 21

The mean zone of inhibition for *Candida krusei* was found to be 30 mm for day 1 and 15 mm for day 4. No activity was recorded on day 14 and 21.

Clotrimazole incorporated soft liner specimen

The zone of inhibition for *Candida albicans* standard strain was found to be 0 proving no activity.

The zone of inhibition for *Candida albicans* clinical strain was 17 mm at day 1, 10 mm at day 7, 9 mm at day 14, 8 mm at day 21

No activity was seen for *Candida tropicalis* and *Candida krusei*

Miconazole incorporated soft liner specimen

The zone of inhibition for *Candida albicans* standard strain was found to be 16mm and day 1 and No further activity was recorded

The zone of inhibition for *candida albicans* clinical strain was found to be 29mm at day 1 and 11 mm at day 7. No further activity was recorded

Anova test or analysis of variance was performed to infer any differences that existed between the groups of drugs. As per the tests it was found that the drugs showed different mean values and the tests show that the difference was statistically significant ($P<0.005$). As Anova showed a significant overall difference between the drugs, post hoc test was performed to check the level of difference at individual level. Thus an inter group comparison was done using post hoc which tells which group is more significant.

The results of this study proved that fluconazole had the strongest inhibiting action against the candida genus followed by miconazole and clotrimazole. On comparing the efficacy between the drugs it was found that **fluconazole showed higher activity** than clotrimazole and miconazole and the ***Candida albicans*** **clinical strain was the most sensitive** showing mean ZOI of 39mm on the 1st day followed by a decrease of 24mm on 7th day, then a rise by 30mm on 14th day , and 25mm on day 21. This was similar to the study by **Chopde N et al** [40] which compared the antifungal activity of two tissue conditioners combined with nystatin, miconazole and

fluconazole against *Candida albicans*. The results showed maximum inhibition in the fluconazole groups followed by miconazole and nystatin.

The activity on *Candida tropicalis* showed highest value on day 1 followed by a gradual decrease till day 7.*Candida albicans* standard strain also showed positive values with a gradual decrease till 21 days. *Candida krusei* showed its efficacy till 7 days only.

A study done by **Darwish RM** et al, demonstrated that fluconazole incorporated into auto polymerising acrylic resin leached out steadily over the time period of 28 days and had significant *Candida albicans* inhibitory activity in terms of colony inhibition. It was shown that the released drug demonstrated antifungal activity against both standard and resistant *C. albicans*. The findings of this investigation have a clinical value in terms of their significant contribution to the treatment of fungal infections of the oral cavity. [81]

Within the limitation of this study fluconazole showed a peak on the 1st day followed by a decrease and then slight increase on day 14. This can be attributed to the internal configuration of the matrix of the material which completely matures on day 14 showing a sustained release of fluconazole. Also there can be changes in the mechanical and physical properties. Schneid showed that chlorhexidine, clotrimazole, fluconazole and nystatin can be released from the tissue conditioner matrix, demonstrating in-vitro growth inhibition of *C. albicans* by these agents. This study has also proved that these antifungal drugs can bring about a change in the mechanical and physical properties of the tissue conditioner.

The change in the rate of drug release is attributed to the fact that the leaching behavior of fluconazole into water is governed by a concentration dependent diffusion process. In the presence of fluconazole, the rapid elution phase probably indicates surface release process. The subsequent slow phase of sustained release may be due to complex processes involving formation of fluid clusters around the drug molecules and the interaction of these clusters with the mechanism of fluid absorption of the resin. Similar behavior has been reported for the release into distilled water from a methacrylate-based polymeric system but of a different drug. [82]

It has been clearly established that methacrylate-based polymers absorb up to 30% water depending on the osmolarity of the external solution or the formulation of

the particular polymer. The mechanism of elution consists of two phases, a rapid linear behavior obeying Fick's law, followed by the development of discrete clusters of the immersion liquid of an unidentified osmotic activity.

The elution behavior of fluconazole may also be enhanced by crazes and surface porosity, formed in the brittle PMMA by the osmotic forces formed by inclusion of the antifungal drug. This is consistent with previously reported finding of a study that used the same polymeric system for delivery of hydrocortisone. [77]

Clotrimazole showed its effect on only *Candida albicans* clinical strain from day 1, and then decreasing up till day 21. The other strains proved resistance to it.

Differences in the susceptibilities of various yeasts to drugs have been reported. In fact, a few *C. albicans* strains exhibiting resistance have been encountered, and concern has been expressed that primary imidazole resistance in *C. albicans* may not be rare [83].

However the potent activity of clotrimazole can be compared to the study by **Koteswara Rao Pachava et al** [46] which studied the antifungal activity of two soft liners combined with Clotrimazole against *Candida albicans*. Two soft liners Coe soft (Acrylic) and GC Reline soft (Silicone) combined with Clotrimazole pure powder form and microsphere form at different concentrations (0.5, 1 and 1.5% w/v) were tested against *Candida albicans* by agar disc diffusion method. Maximum inhibition was seen in the GC Reline soft with pure Clotrimazole followed by GC microsphere form which suggested that incorporation of antifungal agents into soft liners can be recommended for clinical use.

Miconazole showed its activity on *Candida albicans* clinical strain till 21 days. It also showed its effect on *Candida albicans* standard strain upto 7 days. **Marta Radnai et al** [4] examined the incorporation of miconazole and stated that it has dose related inhibitory effect on candidal growth.

In the present study fluconazole showed the highest antifungal activity. This observation could be because of the fact it's a more potent antifungal and also its MIC values are higher than clotrimazole and miconazole. [40]

It was observed that there was an initial high rate of release followed by a sustained release phenomenon over the three week duration. The initial high release is a surface phenomenon where the molecules at the surface are released at an early stage. The later slow diffusion is likely to be due to the diffusion of the drug from the core of the polymer by water cluster formation around the drug particles which is controlled by concentration dependent diffusion.[78]

The tissue conditioner method of drug delivery is advantageous in that it is cheaper than conventional therapy and does not depend on patient cooperation. Tissue conditioners are drug release vehicles which are a promising method of drug delivery as supported by the inhibition diameter results found in this study. Peak antifungal activity at 1-7 days suggests that mixtures prepared for clinical study may be replaced soon after this time for maximum effectiveness. Generally the life of a tissue conditioner is one to two weeks, thus, a 21-day period was selected for the testing of fungicidal effects of different antifungal agents and tissue conditioner combinations [26, 63]

Immersion of the discs in water showed an inverse relationship between time of immersion and degree of inhibition. Immersion in water was done to evaluate the effect of the antifungal in soft liner when the patient immerses the denture in water when not in use. The results proved that the effect reduced significantly from day 7, hence repeated patient re-calls to change the material is advised.

However comparing results from different studies was difficult due to the lack of standardization of concentrations of antifungal agents used. This study did not include physical and mechanical properties of the soft liner material after addition of antifungal additives. Comparisons of the methodology of mixing antifungal agents into tissue conditioners versus conventional topical or systemic regimens can only be realized once in vivo tests are performed

CONCLUSION

CONCLUSION

The following conclusions were drawn from the study:

- Antifungal incorporation into soft liner is beneficial in preventing denture stomatitis
- Fluconazole incorporated soft liner was found to be the better antifungal agent with potent activity against *candida species*
- Miconazole and clotrimazole incorporated soft liner also showed significant activity against the species
- *Candida albicans* clinical strain was the most sensitive species
- *Candida tropicalis* and *Candida krusei* were sensitive only to fluconazole
- There is a gradual decrease in the activity following immersion in water from day 7 to day 21

To summarize, the present study indicates that incorporating antifungals into soft-liner showed positive results in inhibiting *candida albicans*, *candida tropicalis* and *candida krusei.* This would be beneficial in limiting candidiasis to an extent, considering the repeated change of soft liner over a period of 2 weeks. However, the study does not give an insight to the possible changes in mechanical and physical properties of the material. Also, the clinical approach would be confirmed only on conducting in vivo studies.

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ANNEXURES

Annexures

Zone of inhibition of control group

Control	**Zone of inhibition (mm)**														
C. albicans (Std)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. albicans (7 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. albicans (14 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. albicans (21 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. albicans (Clinical)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. albicans (7 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. albicans (14 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. albicans (21 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. tropicalis (Clinical)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. tropicalis (7 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. tropicalis (14 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. tropicalis (21 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. krusei (Clinical)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. krusei (7 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. krusei (14 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. krusei (21 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Zone of inhibition for fluconazole incorporated soft liner discs

Fluconazole	**Zone of inhibition (mm)**														
C. albicans (Std)	32	28	32	33	30	33	30	30	24	30	32	24	22	30	30
C. albicans (7 days)	20	18	18	25	20	18	23	22	18	19	18	18	17	17	16
C. albicans (14 days)	22	25	22	20	18	19	18	18	17	19	18	17	16	16	17
C. albicans (21 days)	19	19	15	20	18	19	18	18	17	16	15	15	14	14	15
C. albicans (Clinical)	38	41	40	37	40	40	39	41	40	39	41	39	40	38	38
C. albicans (7 days)	20	28	23	20	27	27	23	20	27	27	26	25	26	25	25
C. albicans (14 days)	35	36	28	27	30	32	30	29	30	31	30	30	28	31	31
C. albicans (21 days)	23	22	25	26	28	28	24	24	25	26	26	25	27	27	28
C. tropicalis (Clinical)	35	38	40	39	41	39	42	45	42	39	38	44	40	39	39
C. tropicalis (7 days)	30	35	34	24	30	35	34	28	30	29	30	30	30	31	30
C. tropicalis (14 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. tropicalis (21 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. krusei (Clinical)	30	35	32	30	30	30	30	35	30	31	30	31	30	29	30
C. krusei (7 days)	20	10	20	20	15	18	20	15	15	12	12	10	10	18	18
C. krusei (14 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. krusei (21 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Zone of inhibition for miconazole incorporated soft liner discs

Miconazole	**Zone of inhibition (mm)**															
C. albicans (Std)	18	15	16	14	20	17	15	20	15	15	17	20	17	15	15	17
C. albicans (7 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. albicans (14 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. albicans (21 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. albicans (Clinical)	30	30	28	30	30	28	27	31	30	27	29	30	30	31	29	30
C. albicans (7 days)	14	12	10	14	10	10	12	14	10	12	11	12	9	10	11	10
C. albicans (14 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. albicans (21 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. tropicalis (Clinical)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. tropicalis (7 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. tropicalis (14 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. tropicalis (21 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. krusei (Clinical)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. krusei (7 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. krusei (14 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. krusei (21 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Zone of inhibition for clotrimazole incorporated soft liner discs

Clotrimazole	**Zone of inhibition (mm)**														
C. albicans (Std)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. albicans (7 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. albicans (14 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. albicans (21 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. albicans (Clinical)	20	19	20	15	18	18	20	17	18	15	15	17	17	16	15
C. albicans (7 days)	0	10	13	12	12	10	0	10	12	13	15	10	10	12	12
C. albicans (14 days)	10	10	10	10	10	8	7	8	9	8	10	8	9	10	10
C. albicans (21 days)	10	10	8	8	6	7	8	9	9	11	10	8	10	10	10
C. tropicalis (Clinical)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. tropicalis (7 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. tropicalis (14 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. tropicalis (21 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. krusei (Clinical)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. krusei (7 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. krusei (14 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. krusei (21 days)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

ST.GREGORIOS DENTAL COLLEGE

UNDER THE MANAGEMENT OF MJSCE TRUST, PUTHENCRUZ

CHELAD , KOTHAMANGALAM, ERNAKULAM DIST, KERALA - 686681

ETHICAL CLEARANCE CERTIFICATE

SGDC/152/2017/1733/7 Date:- 20-10-2017

To,

Dr. Merlin Riya Mathew
St. Gregorios Dental College
Chelad, Kothamangalam

Dear Dr. Merlin Riya Mathew,

Subject: - Ethics Committee Clearance Reg.

Protocol –Comparative evaluation of the effect of antifungal agents incorporated into denture soft liner on *Candida species*: An *in vitro* study

After the Institutional Ethics Committee (IEC) held on 20th of October, 2017, this study was examined and discussed. After the consideration, the committee had decided to approve and grant clearance for the aforementioned study.

The members who attended the meeting at which the protocol was discussed were:-

1. Dr.CKK Nair - Former BARC scientist
2. Dr.Ommen Aju Jacob - Dean, St. Gregorios Dental College, Chelad
3. Dr.Cinu Thomas A - Scientist, Senior Lecturer, Department of Pharmaceutical Sciences Centre for Professional and Advanced Studies
4. Rev. Fr. Shanu K. Paulose
5. Lissy Jose – Former Member Women's Welfare Association
6. Adv. Jose Aranjani - Advocate
7. Dr.Sauganth Paul - Senior Lecturer, Department of Biochemistry, St.Gregorios Dental College
8. Dr.Eapen Cherian - Secretary
9. Dr.Jain Mathew - Principal and Head of the Department, Department of Conservative Dentistry and Endodontics.
10. Dr.George Francis - Head of the Department, Department of Prosthodontics Crown & Bridge
11. Dr.Binnoy Kurian - Head of the Department, Department of Orthodontics & Dentofacial Orthopaedics

Dr.CKK Nair
Chairman Institutional Ethics Committee
St.Gregorios Dental College, Chelad

St. Gregorios Dental College ★ Chelad - 686 681 ★

Dr.Eapen Cherian
Secretary

Phone : 0485-2572529, 530, 531, 2571429, Fax : 0485-2572530,
Email : sgdc@rediffmail.com, Web : sgdc.ac.in

ACKNOWLEDGEMENT

I owe my first expression of deepest gratitude to the **God Almighty** in whose name I started the journey and who has always been on my side in all my work showering His favours upon me.

I express my heartfelt gratitude and indebtness to my esteemed guide, **Prof. Dr George Francis,** Professor and Head of Department, Department of Prosthodontics for patiently guiding and motivating me. His sense of humor and positivity has always helped me work at ease and inspires me to create a constructive work environment.

I would also like to thank our esteemed Principal **Dr Jain Mathew** for his encouragement and support

I would like to take this opportunity to thank **my beloved teachers, Dr. Mathew Alani** for conditioning my mind to always aim at excellence, **Dr. Donny** for his assistance in the selection of the study, **Dr. Susan** for consistently and lovingly propelling me to arrive at the completion, **Dr. Reba** for devoting her time to correcting and perfecting the flaws that were present in the study**, Dr. Paul** for the support and timely advices, and **Dr Arun** for constantly being my strong base at every academic decisions I have made.

Words would not suffice to express my indebtedness to **Dr Vidya K.C, Dr Hareesh Kumar K.S**, Department of Microbiology, Mar Baselios Dental College, Kothamangalam, under whose support and guidance my research was executed.

I gratefully acknowledge the help of **Dr Jacob Kuruvilla** for helping me prepare the statistical analysis of the study.

I would like to take this opportunity to convey my sincere thanks to **Dr. Eapen Cherian,** for his endless cooperation throughout the course.

I express my heartfelt thanks to my colleagues **Dr. Godwin George** and **Dr Reshma Raju** as well as my juniors **Dr. Amrutha, Dr. Kiran, Dr. Jerrin, Dr Hari, Dr. Raisa** and **Dr Ann** for being very supportive.

I cannot do away with the selfless and timely help given by my dear friends **Dr Jimmy George, Dr Merrin Joseph, Dr Deena Elizabeth, Dr Vincymol, Dr. Jessica Charles.** Their benevolent persona inspires me to emulate them, being given similar circumstances in life.

Words may fail to express the gratitude I have for the sacrifices of my parents, **Mr. Mathew Ninan, Mrs. Jenny Mathew**, in unison with the prayers of my loving brother **Mervin Mathew.**

Date:

Kothamangalam **Dr Merlin Riya Mathew**

LIST OF ABBREVIATIONS USED

C.spps	*Candida species*
C.albicans	*Candida albicans*
C.tropicalis	*Candida tropicalis*
C.krusei	*Candida krusei*
^{o}C	Degree Celsius
µm	Micrometer
%	Percentage
ZOI	Zone of Inhibition