A COMPARATIVE EVALUATION OF THE ANTIFUNGAL ACTIVITY OF THE ESSENTIAL OILS *OF CINNAMOMUM ZEYLANICUM* AND *SYZYGIUM AROMATICUM* INCORPORATED INTO DENTURE SOFT LINER : AN *IN VITRO* STUDY

By

Dr.ANN GEORGE

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, CROWN & BRIDGE

Under the guidance of

Prof. Dr. GEORGE FRANCIS

Department of Prosthodontics, Crown & Bridge

St Gregorios Dental College, Chelad

Kothamangalam

2019-2022

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled " **A COMPARATIVE EVALUATION OF THE ANTIFUNGAL ACTIVITY OF THE ESSENTIAL OILS OF *CINNAMOMUM ZEYLANICUM* AND *SYZYGIUM AROMATICUM* INCORPORATED INTO DENTURE SOFT LINER : 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, Crown & Bridge, St Gregorios Dental College, Chelad, Kothamangalam.

Date: 12/1/2022

Kothamangalam

Dr. ANN GEORGE

St. Gregorios Dental College
Chelad - 686 681

CERTIFICATE BY THE GUIDE

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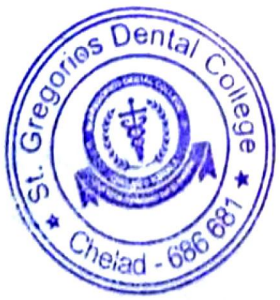
Signature of the Guide

Prof. Dr. GEORGE FRANCIS

(Dept. of Prosthodontics, Crown & Bridge)

Date: 12/1/2022

Kothamangalam



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Seal & Signature of HOD

H.O.D.
Dept. of Prosthodontics
St. Gregorios Dental College
Chelad

Prof. Dr. GEORGE FRANCIS

Professor & HOD

Date: 12/1/2022

Kothamangalam

Seal & Signature of Principal

PRINCIPAL
St. Gregorios Dental College
Chelad, Kerala - 686 681

Prof. Dr. JAIN MATHEW

Principal

St. Gregorios Dental College Chelad - 686 681

Date: 12/1/2022

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Dr. ANN GEORGE

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.

This is the opportunity I had waited for to thank my guide **Prof. Dr. George Francis, MDS,** Professor and Head of Department, Department of Prosthodontics, St. Gregorios dental college, Chelad with all my heart, who was not only a guide, but a mentor and a reputed academician with a treasure trove of knowledge.He was there to clarify my doubts at any time with his scholarly eminence. I was also given the freedom to express my own little views. Right from the selection of topic to completion of work he was the greatest motivation. I feel privileged to have worked under his esteemed guidance.

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, **Prof. Dr. Mathew M. Alani**, Professor, Department of Prosthodontics for conditioning my mind to always aim at excellence, **Dr. Paul Kariyatty**, Reader, Department of Prosthodontics, Chelad whose incessant effort and care was a matter of great inspiration.

Next, I would like to thank **Dr. Arun K Joy**, Reader, Department of Prosthodontics, who always with his academic brilliance and research mind instilled a factor of reverse thinking in many instances and was always willing to clarify any doubts.

I would like to express my gratitude to **Dr. Merin K. Joseph, Dr. Reshma Raju, Dr. Silpa Abraham** Senior Lecturers, Department of Prosthodontics whose unparalleled support was of great help in my work .

I also thank a staff who left us to join Government dental college, Kottayam **Dr. Reba P B** who was a great encouragement and strength during my entire post graduation period.

Words would not suffice to express my indebtedness to **Dr. Vidya K.C**, Professor and

Head of Department, Departmentof Microbiology, Mar Baselios Dental College, Kothamangalam under whose support and guidance my research was executed.

Dr. Hareesh Kumar K.S, Professor and Head of Department, Department of Medical Microbiology, School of Medical Education, Gandhinagar, Kottayam for all his support and timely advices.

Mrs. Jancy Thomas, lab technician, Mar Baselios dental college, is one who also needs much applause. Her uncanny skill helped me a lot in obtaining precise samples.

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

I would like to take this opportunity to convey my sincere thanks to **Dr. Abraham George**, Reader, Department of Orthodontics for his endless cooperation, motivation and unparalleled support throughout the course.

I would like to convey my sincere thanks to **Dr. Eapen Cherian,** for his help and advice during the course of my post-graduation.

I express my heartfelt thanks to my batchmates **Dr. Harishankaran U** and **Dr. Raisa Mariam Jacob** as well as my seniors **Dr. Merlin, Dr. Godwin, Dr. Amrutha, Dr. Kiran, Dr. Jerin,** and juniors **Dr. Arya , Dr. Sr. Jasmin, Dr. Jinson, Dr. Aishwarya, Dr. Divya, Dr. Mebil** for being very supportive throughout my postgraduate course.

I also owe my gratitude to all the staff members in my department for their kindness, motivation and endless cooperation throughout my course.

Words may fail to express the gratitude I have for the sacrifices of my grandparents and my dear parents, **Late Prof. P.J.George, Mrs. Shiji George**, in unison with the prayers of my loving brother **Jacob George.** I am deeply indebted to them for being a constant source of light whenever I encountered difficulties in my life.

My life has been graced by the comfort and love of a very special person, my husband **Dr. Amal Paul Joy,** who listened attentively to my ideas, accommodated me with his time and space. He helped me to think and write and was always near to lift my spirit

whenever I felt down. I will always be grateful for his love, patience, encouragement and understanding whose staunch support and care helped me in the completion of dissertation.

I also take this opportunity to express my gratitude to my Parents in law, **Mr. Joy Paul** and **Mrs. Meena N Jacob** along with prayers of my brother in law **Dr. Akhil Jacob Joy** whose love and support made me accomplish this work.

Above all, I thank and praise God for his blessings, constant presence and guidance for bringing me this far.

Date: 12/1/2022

Kothamangalam

Dr. ANN GEORGE

St. Gregorios Dental College
Chelad - 686 681

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ABSTRACT

Aim

This in-vitro study is sought to compare antifungal activity of the essential oils of *Cinnamomum zeylanicum* and *Syzygium aromaticum* incorporated into denture soft liner.

Background and objectives:

Removable prosthesis placed in the oral cavity can produce profound changes in the oral environment. One of the most common sequelae of wearing dentures is chronic atrophic candidiasis, known as denture stomatitis or denture sore mouth prevalent in 11% to 67% of complete denture wearers. Soft liners are elastomeric polymers used in the prevention of chronic soreness from dentures and preservation of supporting tissues.

In denture stomatitis patients, antifungal agents can be applied directly to the affected mucosa, fitting surface of the denture or can be incorporated into soft liners for sustained release of antifungal agents. The indiscriminate use of antifungal agents has led to the emergence of Candida strains resistant to conventional drugs and this induces the search of new antimicrobial agents. Many herbal formulations including *Cinnamomum zeylanicum*, *Syzygium aromaticum* have been established as potent antifungal agents that can be used safely with minimal side effects.

This in-vitro study is to compare the antifungal activity of *Cinnamomum zeylanicum* and *Syzygium aromaticum* essential oils incorporated into denture soft liner.

The key objectives of this research are:

i. To evaluate the anti-fungal efficacy of *Cinnamomum zeylanicum* essential oil incorporated into soft liner.

ii. To evaluate the anti-fungal efficacy of *Syzygium aromaticum* essential oil incorporated into soft liner.

iii. To compare the antifungal efficacy of *Cinnamomum zeylanicum* and *Syzygium aromaticum* essential oils incorporated into soft liner.

iv. To compare the antifungal efficacy of *Cinnamomum zeylanicum* and *Syzygium aromaticum* incorporated into soft liner with Fluconazole incorporated into soft liner.

Methods:

In this study, 15 samples of each group were taken including the control group. GC denture soft liner was mixed according to manufacturer's instructions with various antifungal agents like *Cinnamomum zeylanicum, Syzygium aromaticum* and fluconazole. 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 *Cinnamomum zeylanicum* and *Syzygium aromaticum* essential oils incorporated are the test groups and the disc without incorporation of antifungal agents is the negative control group and soft liner discs incorporated with flucanozole is the positive control. 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.

Results and discussion:

Anova test or analysis of variance was performed to infer any differences that existed between the groups of antifungal agents. As per the tests it was found that the antifungal agents showed different mean values and the tests show that the difference was statistically highly significant ($P<0.01$). As Anova showed a significant overall difference between the antifungal agentss, Post Hoc test was performed to check the level of difference at individual level. On comparing the efficacy between the antifungal agents, one synthetic- fluconazole and two natural - *Cinnamomum zeylanicum* and *Syzygium aromaticum* essential oils, it was found that fluconazole showed higher activity than *Cinnamomum zeylanicum* and *Syzygium aromaticum* on*Candida albicans.* However it was found that the soft liner discs incorporated with fluconazole showed an exponential decrease in the antifungal activity from day 1 to

day 3 is 4.72 mm and even more decrease from day 3 to day 7 (5.4 mm).This may be attributed to the regrowth of fungus.

However, *Cinnamomum zeylanicum* EO incorporated soft liner disc also showed a decrease in antifungal activity from day 1 to day 3 (4.36 mm) but its value was almost steady afterwards till 7th day (difference of 1.8 mm). Also, *Syzygium aromaticum* EO incorporated soft liner discs though showed a reduced ZOI when compared to the other groups but its antifungal activity was found to be almost steady as there was only diiference of 1.12 mm from day 1 to day 3 followed by a difference of 1.32 mm from day 3 to day 7.This indicates the sustained and steady release of natural antifungal agents incorporated soft liner discs when compared with the synthetic drug -fluconazole incorporated soft liner discs. Soft liner discs without incorporation of any antifungal agent neither ceased the growth of fungus.

Conclusion:

Denture soft liners, when mixed with antifungal agents - synthetic and natural, showed satisfactory inhibition of *candida albicans* 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 achieved.

Keywords: Denture stomatitis, soft liner, antifungal agents, *Cinnamomum zeylanicum*, *Syzygium aromaticum*, Fluconazole, *Candida albicans*

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INTRODUCTION

INTRODUCTION

Increased life expectancy combined with improved socialization has made people conscious of their appearance and performance in the society. There is a greater need for preservation of the remaining teeth and also for replacement of the lost teeth by an artificial substitute either fixed or removable. There are many advancements in the dental rehabilitative options but the mainstay for the management of edentulous state remains to be soft tissue supported complete denture.

When the dentures are worn by the elderly for a longer time than needed with a minimum rest given to the bearing tissues leads to soft tissue irritation, depriving it from blood supply and also leading to resorption of the supporting alveolar bony foundation.[1] As a consequence, the dentures tend to loosen demanding for the use of materials like tissue conditioners and denture adhesives to improve retention.

Liners also offer a valuable solution in the management of painful or fragile mucosa or ulcerated tissues associated with the wearing of dentures and provide comfort for patients who cannot withstand occlusal pressures, such as in case of alveolar ridge resorption, chronic soreness, and knife edge ridges.[2, 3]

Liners act as a cushion for the denture-bearing tissues by absorbing and redistributing forces transmitted to the stress-bearing areas of the edentulous ridges.[4]

Thus,they are used to improve fit of ill fitting dentures, to prevent traumatic damage to the atrophic mucosa underlying denture base, as a cushion between denture bearing mucosa and denture, to retain over denture bar attachments, to retain extra oral prosthesis, to distribute occlusal forces, to increase serviceable life of prosthesis, to replace the fitting surface of conventional hard dentures, to relieve mucosal pain under hard dentures, improves the rhythm of chewing strokes, it also compensates for the volumetric shrinkage of acrylic resin. [5,6,7,8]

Denture-related stomatitis (also termed denture sore mouth, denture stomatitis, chronic atrophic candidiasis, and denture-associated erythematous stomatitis) is a common condition characterized by erythematous and edematous lesions of the oral mucosa which is usually seen beneath a denture. The prevalence of denture stomatitis among denture wearers varies from 15 to 66.7 % .[9] In about 90% of

the cases, Candida species are involved and among them *Candida albicans* being the principal causative agent.[10] That is the adhesion of *Candida albicans* species to a denture base with inadequate denture hygiene and denture wearing habits when worn continously over traumatized mucosa can predispose to denture stomatitis.[11]

Management of candida associated denture stomatitis is complex due to its multifactorial etiology.[12] Patients with denture associated erythematous candidiasis should be examined for the adequacy of the dentures. Thorough evaluation and correction of oral and denture hygiene measures should be done and nocturnal wearing of dentures should be discouraged. Denture-lining materials, which include tissue conditioners and soft denture liners, are widely used as adjuncts in the prosthodontic treatment and management of traumatized oral mucosa. Even though these materials exhibit excellent tissue tolerance, one of the problems is the colonization of Candida species on and within the material.

Denture stomatitis can be effectively managed with antifungal agents. Laser beam, cryosurgery, electrosurgery and scalpel surgery are also successfully practiced in treating the infection, especially for type II and type III infection.[13]

One of the initial steps in prevention and treatment of denture stomatitis is to improve denture adaptation and to allow the recovery of the irritated denture bearing tissues via use of tissue conditioners. Even though these materials exhibit excellent tissue tolerance, one of the problems is the colonization of Candida species on and within the material. Fungal growth is known to destroy the surface properties of the liner and this may lead to irritation of the oral tissues. This is due to a combination of increased surface roughness and high concentrations of exotoxins and metabolic products produced by the fungal colonies.[14]

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.[15] 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.So, it would therefore be advantageous to provide treatment that is easier to administer and that 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 can provide a slow continuous release and that results in a sustained therapeutic effect.

Denture stomatitis can be effectively managed with antifungal agents, and the condition shows complete resolution within 12–14 days. Topical antifungals like2% Miconazole (gel form) Nystatin in the form of liquid suspension, cream and pastille can be applied to the cleaned surface of denture base. Cases which do not respond well to topical antifungal therapy can be treated with systemic antifungal agent. Azoles such as Fluconazole and ketoconazole are the systemic antifungal agents.

However Fluconazole has been widely used because the drug is economic, has lower toxicity and has high bioavailability. Ketoconazole is also used systemically in a single dose of 200 mg during 14 days but the disadvantage is that, it is a hepatotoxic drug and can result in cardiac arrhythmias when used in combination with antihistamines or macrolide antibiotics. Amphotericin B was previously used in the treatment of Candida associated denture stomatitis. However, its use has declined because as it is extremely nephrotoxic and is administered intravenously.[16]

Systemic administration of drugs may not be as effective against candidal infection because the organism usually limits its activity only to the oral mucosa. Also, the success of topical application of drugs in the oral cavity may be compromised by the copious flow of saliva as well as by the lack of patient compliance. Therefore, antifungal agents can be incorporated in tissue conditioners to simultaneous treat injured peri-prosthetic tissues and Candidial infection.

Recently, azole antifungal compounds such as fluconazole, which have excellent efficacy toxicity profiles, is the principal drugs used in the treatment of candida infections.[17] However, fluconazole produces few side effects like nausea, vomiting and might lead to the development of drug resistance in *Candida albicans*.[18,19] So, to overcome these limitations of chemically synthetic antifungals,

novel and effective antifunal agents like some herbs with antifungal,antibacterial, and antiviral properties known as phytotherapeutic agents are of interest.[20] The broad spectrum of biological activities of natural products make them attrative phototypes as antifungal agents.

Anupama N. Devkatte et al conducted a study of the 38 oils of plant origin against *Candida albicans* growth. Among that 23 were found effective and 15, ineffective. Based on their Minimum Fungicidal Concentrations (MFC), plant oils were categorized into four categories-most effective, moderately effective, less effective and non-effective. Results of this study indicated that oils of plant origin can be used as potential anti-Candida agents.

Cinnamon oil (*Cinnamomum zeylanicum*) was the best, having fungicidal effect at 0.03% concentration in C. albicans. Clove oil (*Syzygium aromaticum*) was fungicidal at 0.12% concentration and there was no difference between MIC and MFC.[21]

Incorporation of an antifungal agent into soft liner serve dual purpose of sustained continous antifungal activity along with providing a cushioning effect to the traumatised inflamed tissues.This in vitro study is to compare the antifungal activity of *Cinnamomum zeylanicum* and *Syzygium aromaticum* essential oils incorporated into denture soft liner.

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The key objectives of this research are:

i. Anti-fungal efficacy of *Cinnamomum zeylanicum* essential oil incorporated into soft liner.

ii. Anti-fungal efficacy of *Syzygium aromaticum* essential oil incorporated into soft liner.

iii. Comparative antifungal efficacy of *Cinnamomum zeylanicum* and *Syzygium aromaticum* essential oils incorporated into soft liner.

iv. Comparative antifungal efficacy of *Cinnamomum zeylanicum* and *Syzygium aromaticum* incorporated into soft liner with Fluconazole incorporated into soft liner.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

- **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.[22]

- **Allison and Douglas in 1973** did a study on micro colonization of the denture fitting surface by *Candida albicans*. The researchers used light and scanning electron microscopy to analyse the denture fitting surfaces of two soft lining materials and one acrylic denture from three patients with denture stomatitis. The lining material had *Candida albicans* hyphae overgrowth on the fitting surfaceand the acrylic denture had a surface plaque with identifiable organisms.[23]

- **Masella, Dolan, and Laney in 1975** investigated the inhibition of Candida growth on silastic 390 soft denture liners in 1975. As per the findings of the study, silastic 390 does not supply nutrients for *C.albicans* to flourish. It was also proposed that routine daily immersion of a denture lined with silastic 390 in Pro-Kern denture cleaner, Zephiran, or water at 60^0C, after manually cleaning the denture with a brush, is an efficient technique in preventing candida species growth on silastic 390.[24]

- **Eino Makila et al in 1977** studied the mycotic flora of 39 people who wore soft-lined (Molloplast B) mandibular dentures and heat-cured acrylic resin maxillary dentures. Seven yeasts and two moulds were discovered in the samples. *Candida albicans* (86 percent), *Candida glabrata* (31 percent), and *Candida tropicalis* (11 percent) were the most prevalent fungi found (14 percent). In vitro, the uncured Molloplast material inhibited candida growth significantly, whereas the cured material had little effect.[25]

- **C.J Thomas et al in 1978** investigated the in vitro fungicidal properties of the tissue conditioner Visco-gel on *Candida albicans*, *Candida krusei*, and *Candida*

tropicalis with or without the addition of nystatin and amphotericin B. Visco-gel alone was fully inert, it should not be used without nystatin in the treatment of denture stomatitis caused by a yeast infection. When coupled with Visco-gel, Amphotericin B became completely ineffective, while it remained quite active in control experiments.[26]

- **L P Samaranayake et al in 1980** investigated the mechanisms that contribute to *Candida albicans* adhesion to acrylic surfaces, hypothesising that they may play a role in the aetiology of chronic atrophic candidiasis. It was demonstrated here that several intra-orally acting variables can alter *C. albicans* adhesion to acrylic surfaces, and that if these factors had a similar effect in vivo, yeast colonisation on denture surfaces could be regulated.[27]

- **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*.The effectiveness of miconazole and keteconazole as anti-fungal drugs is not affected when used in combination with any of the tissue conditioners studied, unlike amphotericin B being ineffective. According to the in vitro investigations, the period of localised antifungal and tissue conditioning therapy is two weeks.[28]

- **Wright et al in 1985** investigated the prevalence of various yeast species in a group of patients wearing denture soft lining materials, as well as the association between yeast presence and inflammatory changes in the mandibular denture bearing mucosa and the soft lining materials. When compared to the other species identified in the investigation, *C. albicans* was obviously and significantly ($p < 0.02$) associated with greater colonisation density.[29]

- **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.[30]

- **Schneid TR 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, low, medium, and high concentrations. In all experimental groups hardness increased with concentration and time, but remained within clinically acceptable limits (Shore A or = 49). For at least one concentration level of each drug, mean tensile strength improved in all experimental groups and was substantially stronger than the control, p or = 0.05.All tensile-strength-test failures were classified as cohesive. All of the medicines showed total, dose-related, or incubation time-related release from the tissue conditioner matrix and suppression of *C. albicans* growth prior to inoculation. All of the controls were in favour of growth.[1]

- **Nikawa et al in 1993** conducted a study to see how denture lining material, protein pellicle, and *Candida albicans* interact. Monitoring pH variations associated with protein-free and protein-coated lining material, as well as ultrastructural studies of yeast colonisation, were used to explore The results suggested that denture pellicle derived from saliva and/or serum may potentiate candidal colonization of denture lining materials.[31]

- **Paul S. Wright et al in 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 that the increased prevalence of yeast is linked to the availability of nutrients in the mouth, as well as the difficulty in maintaining and cleaning these materials.[32]

- **D. R. Radford et al in 1999** published a review to understand the mechanism and clinical importance of adhesion of *C. albicans* to denture base materials in relation to denture plaque and denture related stomatitis. In conclusion, the review significantly supports the hypothesis that *C.albicans* adhesion to denture-based materials in vitro is correlated to the organism's hydrophobicity.[33]

- **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.[34]

- **Bulad et al in 2004** studied the colonization and penetration of denture soft lining materials by *Candida albicans*. This study attempted to track this interaction by comparing *C. albicans* short-term adherence to six denture lining materials, as well as any longer-term material penetration by the yeast. Glass slides or dental stone were used to process denture lining materials (Molloplast B, Flexor, Permaflex, Luci-soft, Eversoft, and Ufi Gel hard C). When compared to the nystatin control, none of the materials created a zone of inhibition. On any of the smooth surfaces, there was no significant variation in cell counts (p>0.5). On roughened surfaces, a significantly larger number of cells (p0.001) was detected. When penetration was monitored, both hyphal and yeast forms were seen. Penetration was greatest into Ufi Gel hard C (no hyphae observed), but not at the acrylic-liner junction and least into Eversoft. [35]

- **Lamfon et al in 2005** conducted research into the composition of denture plaque biofilms and candida species susceptibility. Single drugs like Miconazole, fluconazole, or chlorhexidine were found to have no effect on candida species proliferation within these biofilms. For several days, however, a combination of miconazole and chlorhexidine, pulsed into the system to simulate patient use, reduced bacterial and candidal growth. As a result, dual therapy appeared to be effective in lowering viable organisms in the biofilm of denture plaque.[36]

- **Anupama N. Devkatte et al in 2005** conducted a study on the antifungal properties of 38 plant-based oils against *Candida albicans* growth. 23 were found to be effective, while fifteen were found to be ineffective. Plant oils were divided into four categories based on their Minimum Fungicidal Concentrations (MFC): most effective, moderately effective, less effective, and non-effective.

The findings of this study suggested that oils derived from plants could be employed as anti-Candida medications. Cinnamon oil (*Cinnamomum zeylanicum*) was the most effective, with a fungicidal impact on C. albicans at a concentration of 0.03 percent. At 0.12 percent concentration, clove oil (*Syzygium aromaticum*) was fungicidal, and there was no difference between MIC and MFC.[21]

- **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 frame. After each of the time periods studied, they found that the overall number of oral bacteria adhering to the soft liner material was increasing.[37]

- **Pinto E et al in 2009** studied the composition and antifungal activity of clove essential oil (EO), obtained from *Syzygium aromaticum*. Clove oil was purchased commercially and subjected to GC and GCMS analysis. The EO tested had a high eugenol concentration (85.3 percent). The antifungal activity of clove oil and its main component, eugenol, against Candida, Aspergillus, and dermatophyte clinical and American Type Culture Collection strains was evaluated using MICs and minimum fungicidal concentrations determined according to Clinical and Laboratory Standards Institute protocols. All of the strains tested demonstrated inhibitory efficacy against EO and eugenol. Flow cytometric and suppression of ergosterol synthesis investigations were done to understand its mode of action on yeasts and filamentous fungi. When yeast cells were treated with propidium iodide at concentrations just above the MICs, the majority of the cells were promptly pierced, indicating that the fungicidal action was caused by a widespread lesion of the cell membrane. Clove oil and eugenol also significantly reduced the amount of ergosterol, a particular component of fungal cell membranes. Oil and eugenol concentrations below the MIC values entirely or nearly completely suppressed the production of germ tubes by *Candida albicans*.The present study indicates that clove oil and eugenol have considerable antifungal activity against clinically relevant fungi, including fluconazole-resistant strains, deserving further investigation for clinical application in the treatment of fungal infections.[38]

- **Na Guo et al in 2009** conducted a study to determine the antifungal activity of thymol against clinical isolates of fluconazole -sensitive and resistant *Candida albicans* and thymol (THY) was found to have in vitro antifungal activity against 24 fluconazole (FLC)-resistant and 12 FLC-susceptible clinical isolates of *Candida albicans*, standard strain ATCC 10231 and one experimentally induced FLC-resistant C. albicans S-1. In addition, synergism was observed for clinical isolates of C. albicans with combinations of THY-FLC and THY-amphotericin B (AMB) evaluated by the chequerboard microdilution method.[18]

- **Marta Radnai et al in 2010** conducted a study to see how efficient antifungal gels mixed with tissue conditioner are at inhibiting *Candida albicans* development in vitro. To examine antifungal property over time, visco-gel discs containing chlorhexidine digluconate and 20% v/v miconazole were inoculated with *Candida albicans* and placed on SDA immersed in water for various periods of time. And it was determined that adding chlorhexidine digluconate gel had no influence on *C.albicans* growth. Miconazole has a dose-dependent inhibitory effect on candidal growth when it is incorporated.[39]

- **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.Molloplast B, GC Reline soft, Mollosil Plus, Silagum Comfort, and Palapress Vario were the materials employed in this investigation. In vitro, the number of cells adhered to saliva-coated surfaces was shown to be significantly lower than on non-treated surfaces. Oral Candida carriage was determined to be 78 percent. *Candida albicans*, *Candida glabrata*, *Candida intermedia*, and *Candida tropicalis* were all discovered. The growth of in vivo biofilms on the liners appeared to be a vast colonisation of microorganisms. The results of the in vitro tests imply that salivary film influences C. albicans strains early colonisation.[40]

- **Abbas Falah-Tafti et al in 2010** conducted a study to evaluate the efficacy of the two common antifungal agents mixed with tissue conditioner against *Candida albicans*. Tissue conditioner discs (Acrosoft) with a diameter of 5mm and a thickness of 1mm containing various concentrations of nystatin and fluconazole (1 percent, 3 percent, 5 percent, 10 percent wt/wt) as well as discs without antifungal agents (8 discs for each group) were prepared for

experimental biofilm formation by inoculation with *Candida albicans* cell suspensions. *Candida albicans* attachment and colonisation were totally blocked by a 1 percent to 10% mixture of nystatin and tissue conditioner, whereas fluconazole only required a 10% dosage to be completely inhibited.[41]

- **Jose Julian et al in 2010** did a study to evaluate the better drug delivery system. For the treatment of denture stomatitis, three alternative medication delivery systems were observed. The researchers discovered that tissue conditioner (Viscogel) may be used to administer antifungal medications such as Nystatin, Clotrimazole, and Ketoconazole. Drugs can be applied topically, however the objective is negated by the abundant flow of saliva. Because the bacterium normally confines its activities to the oral mucosa, systemic medication delivery may not be as effective against candidal infection. As a result, in the treatment of denture stomatitis, it is always preferable to maintain a therapeutic amount of drug release at the infection site.[42]

- **Nam et al in 2011** conducted a study to identify the in vitro antimicrobial activity of the tissue conditioner containing silver nanoparticles on microbial strains, Staphylococcus tissue conditioner displayed minimal bactericidal effect against Staphylococcus aureus and Streptococcus mutans strains, a 0.5% for fungal strain. Control group did not show any microbial inhibitory effect and there were no statistical difference between 24 hrs and extended 72 hrs incubation time ($P > .05$) suggesting the tissue conditioner containing silver nanoparticles as an effective antimicrobial agent in denture plaque control.[43]

- **Himanshu Gupta et alin 2011** did an in vitro study which 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*.Viscogel (VGC), GC Soft (GCC), ketoconazole (KTZ), and itraconazole were the four control groups studied (ITZ). Ketoconazole with GC Soft (KGC), ketoconazole with Viscogel (KV), itraconazole with Viscogel (IV), and itraconazole with GC Soft (IV) were the four combinations examined (IG). In VGC and GCC, there was no evidence of C albicans inhibition. All inhibition diameter values for KTZ and ITZ were within the standard permitted limits. In IG and IV, there was no inhibition at all.

KGC was shown to be substantially more inhibitory than KV in ketoconazole combinations (p value 0.001). The drug action was significantly inhibited by embedding the drug into tissue conditioners in all groups (p value.001). VGC and GCC do not have their own antifungal properties. The ineffectiveness of IG and IV was discovered. KGC was discovered to be much more inhibitory than KV.[44]

- **Dalirsani et al in 2011** investigated the antimicrobial effect of 10 medical plants on *Candida albicans*. The following plants were selected: thyme, mint, garlic, cinnamon, chamomile, tea tree, clove, spearmint, sage and rosemary. These plants had been selected according to the medical traditional usage and previous researches. C. albicans was cultured in Sabouraud dextrose Agar containing Chloramphenicole. On each plate, one plant extract disc, one chlorhexidine disc and one nystatin disc; as positive controls; and one methanol and one blank disc; as negative controls; were placed. After 24 hours, the mean diameter of non-growth halo around every plant extract was compared with the mean diameter of non-growth halo of positive control disks by T test statistical analyze.The results showed a non-growth halo in disks containing chamomile, garlic, clove, cinnamon, sage and thyme was observed. Cinnamon, garlic, chamomile, sage, clove and thyme had inhibitory effects on C.albicans. If similar results are confirmed in clinical trials, theses plant extracts can be used to produce new antifungal products.[45]

- **Hema Kanathila et al in 2011** conducted a study to test the efficacy of magnesium oxide combined with two tissue conditioners (Viscogel and GC Soft), in inhibiting the growth of *Candida albicans* and concluded that Magnesium oxide in combination with tissue conditioners are effective against *Candida albicans*; GC soft with magnesium oxide showed a better result than Viscogel with magnesium oxide; Increasing the concentration of magnesium oxide increases the zone of inhibition of *Candida albicans*.[46]

- **Chopde N et al in 2012** did a study to determine and compare the antifungal activity of two tissue conditioners coupled with nystatin, miconazole, and fluconazole against *Candida albicans*.The fluconazole groups showed the most inhibition, followed by miconazole, while the nystatin group showed the least

inhibition.Hence proved that the tissue conditioners combined with antifungal agents were found to inhibit *Candida albicans* effectively.[47]

- **Altarawneh et al in 2012**investigated the link between candida, dentures, and mucosal tissue,by exfoliative cytologyas well as candida levels in saliva, mucosal surfaces, and denture surfaces, as well as salivary flow rate and xerostomic circumstances.The presence of candida in the denture and saliva was discovered to be the most common etiological factor for denture stomatitis in this population. As a result, it was decisively proven that in healthy people, the treatment of denture stomatitis should initially focus on sanitising a current denture and/or fabricating a new denture.[48]

- **Srivastava et al in 2013**investigated the antifungal activity and properties of a tissue conditioner by incorporating origanum oil.Origanum oil was mixed into a tissue conditioner (Visco-gel) based on poly (methyl methacrylate), and its antifungal efficacy against *Candida albicans* was tested using the agar punch well method at 1 day and 1 week. *Candida albicans* adherence, surface roughness, tensile strength, and bond strength of the tissue conditioner were all evaluated using an optimised origanum oil concentration. Origanum oil can be used as an additive to tissue conditioner to reduce *Candida albicans* adherence without affecting its bond strength to heat-polymerized acrylic resin.[49]

- **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 mixed with three antifungal agents (fluconazole, clotrimazole, and neem) and inserted into the metal mould according to the manufacturer's instructions. T1 – Viscogel, T2 – Coe-soft were created from a total of 240 samples, which were divided into two groups of 120 samples each. The Shore-A-Durometer was used to measure the hardness of these on the first, seventh, and fourteenth days. The introduction of antifungal agents in tissue conditioners resulted in an increase in hardness levels in all 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.[50]

- **Sunanda Sharma et al in 2014** conducted an in vitro study to compare the antifungal activity of melaleuca alternifolia oil and fluconazole mixed with a tissue conditioner and reported that thirty percent w/w melaleuca oil was found to be the most effective and superior to 5%fluconazole in Visco-gel, as it retained substantial antifungal activity, even on day 7 when fluconazole had lost its antifungal effect completely as evidenced by regrowth of *Candida albicans* by day7.[17]

- **Choonharuangdej et al in 2014** studied the in vitro anti-candida effect of thai herbs (Clinacanthus nutans, Caesalpinia sappan Linn. and Cymbopogon citratus (DC.) when supplemented in tissue conditioner.The antifungal activity of each plant against *Candida albicans* was tested using the agar disc diffusion method. Micro-broth dilution and culture procedures were used to determine the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of individual and combination herbs. As directed by the manufacturer, an individual or combination herb was added to the liquid part of COE-COMFORT TM tissue conditioner before being produced.Antifungal activity against C. albicans ATCC 10231 was detected in varying degrees. C. citratus essential oil has the strongest anti-candida action, followed by C. sappan Linn. and C. nutans, in that order. Anti-candida action was established in mixed formulations containing all three plants. Only C. citratus essential oil at high concentrations (4-time MIC and higher) incorporated in the tissue conditioner had antifungal action against both reference and clinical C. albicans strains.It is concluded that C. citratus essential oil, both soluble and supplement forms, has demonstrated a strong anti-Candida action. The tissue conditioner treated with C. nutans and C. sappan Linn had no antifungal action.[20]

- **Amornvit et al in 2014** conducted a study to determine the anti - candida efficacy of COE-COMFORT tissue conditioner incorporated with lemongrass essential oil.The antifungal activity of lemongrass (Cymbopogon citratus) essential oil against *Candida albicans* ATCC 10231 and the MIC (minimum inhibitory concentration) value were measured using agar disk diffusion and broth microdilution techniques, respectively. Lemongrass essential oil was found to have significant antifungal action against C. albicans ATCC 10231,

with a MIC of 0.06 percent (v/v). The tissue conditioner containing the oil at the MIC level, on the other hand, did not stop the fungus from growing. After being exposed to a tissue conditioner containisng at least 0.25 percent (v/v) of the oil, both reference and clinical isolates of *Candida albicans* were completely inhibited (approximately 4-time MIC). As a negative and positive control, tissue conditioners without herb and with nystatin were used. COE-COMFORT tissue conditioner supplemented with lemongrass essential oil clearly demonstrated desirable property as in vitro anti-Candida efficacy to reduce the risk of Candida infection, according to the findings.[51]

- **Koteswara Rao Pachava et al in 2014** investigated and compared the antifungal activity of two soft liners in combination with Clotrimazole against *Candida albicans*. The agar disc diffusion method was used to test two soft liners, Coe soft (Acrylic) and GC Reline soft (Silicone), in combination with Clotrimazole pure powder form and microsphere form at varied concentrations (0.5, 1 and 1.5 percent w/v). At 1.5 and 1 percent W/V concentrations, the GC Reline soft with pure Clotrimazole showed the most inhibition, followed by the GC microsphere form, then the coe soft pure and microsphere forms.Finally, it was concluded that denture soft liners mixed with antifungal agents inhibited *Candida albicans* effectively, implying that the incorporation of antifungal agents into soft liners can be recommended for clinical use.[52]

- **Ginjupalli Kishore et al in 2014** aimed to determine and compare antifungal efficacy of two soft liners combined with Clotrimazole against *Candida albicans*. At 1.5 and 1 percent W/V concentrations, the GC Reline soft with pure clotrimazole showed the most inhibition, followed by the GC microsphere form, then the coe soft pure and microsphere forms. It was discovered that denture soft liners containing antifungal agents inhibited *Candida albicans* effectively, implying that antifungal drugs should be incorporated into soft liners for therapeutic use.[53]

- **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.[54]

- **Bharathiprakash et al in 2015** conducted a research to determine the prevalence of Candida species in healthy denture wearers and nondenture wearers, as well as their age and hygiene status. Swabs from 50 denture wearers and 50 non-denture wearers were collected and processed on Sabouraud's dextrose agar. The information gathered was correlated with age and oral hygiene, and statistical analysis was carried out. *Candida albicans*, *Candida tropicalis*, *Candida dubliensis*, and *Candida glabrata* were found to be the most common *Candida species* in denture wearers. Among nondenture wearers, C. albicans and *C. tropicalis* were isolated. Prevalence of Candida increased with increasing age among denture wearers.[55]

- **Lima et al.in 2016** investigated the porosity of a tissue conditioner (Softone) and a temporary resilient liner (Trusoft) for *Candida albicans* biofilm using minimum inhibitory concentrations (MICs) of antifungal drugs. Except for Chlorhexidine and Nystatin in Softone and Chlorhexidine in Trusoft at 14 days, adding antifungals at MICs had no adverse impacts on the porosity of both soft liners in varied periods of water immersion.[56]

- **Sushma Krishnamurthy et al in 2016** conducted a study on*Candida albicans* on the retention, colonisation, and penetration of four denture lining materials: Molloplast B, Permaflex, GC Soft Liner, and Ufi Gel Hard C. Smoother surfaces were found to maintain fewer cells than rough surfaces. Denture lining materials allow Candida to infiltrate their structure and have negligible antifungal properties.[57]

- **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.Efficacy and effectiveness of adding conventional organic antifungal medicines (nystatin, azole group derivatives, and chlorhexidine), non-organic antimicrobials/antifungals (silver zeolite, silver nano-particles, photo-catalysts, and metallic oxides), and natural and herbal antimicrobials (tea tree oil, lemongrass essential oil, and origanum oil) to various tissue conditioners have been reported in several studies. According to the review literature, incorporating antifungal drugs into tissue conditioners is beneficial, with little or no impact on the physical and mechanical properties of

the tissue conditioners and can be recommended for the management of denture induced stomatitis. [58]

- **Neppelenbroeke et al in 2017** investigated the ultimate tensile strength of temporary soft denture liners affected by *Candida albicans* biofilm minimum inhibitory concentrations (MICs) of antifungal agents. The addition of nystatin, chlorhexidine, and ketoconazole at MICs for C. albicans biofilm had no adverse impacts on the tensile strength and elongation % of the temporary soft denture liner materials for up to 14 days, according to the findings.[59]

- **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.Except for the miconazole in Softone, all antifungals had no detrimental effects on the materials' hardness during the assessment period. Up to 14 days, the MICs of nystatin in both temporary soft lining materials, ketoconazole in Softone, and chlorhexidine in Tru soft had no adverse impacts on roughness.[60]

- **Pragati Rawat et al in 2017** did a study that compares the anti-fungal and viscoelastic properties of tissue conditioner containing different antifungal agents, one synthetic - fluconazole, and two natural - oregano oil and virgin coconut oil. After 24 hours, 3 days and 7 days, the antifungal property and viscoelasticity of Viscogel containing antifungal agents were evaluated. Viscogel containing fluconazole has the best antifungal action, according to the results. Although Viscogel alone and in combination with fluconazole exhibited a decrease in viscoelasticity over a seven-day period, Viscogel in conjunction with natural agents showed no significant changes. Finally, it was discovered that using natural antifungal agents in tissue conditioners can be a viable alternative to systemic or topical synthetic antifungal drugs.[61]

- **Khadka S et al in 2017** studied about antifungal susceptibility pattern of Candida species to antifungal agents. *Candida albicans* (56%) was the most prevalent species among 100 Candida isolates. *Candida tropicalis* (20%) was the most common Candida isolate among non-albicans *Candida species*, followed by *Candida glabrata* (14%). *Candida species* were more responsive to

clotrimazole (82 percent), fluconazole (64 percent), and miconazole (44 percent) in terms of antifungal susceptibility. *Candida albicans* was shown to be the most common Candida species responsible for numerous Candidal infections. Clotrimazole, miconazole, and fluconazole were the most potent antifungal medications .[62]

- **Gujjari et al in 2017** conducted a study to assess the candidial carriage in type II diabetes patients with chronic periodontitis according to the duration of diabetes, gender and age also to evaluate the effect of cinnamon mouthwash on *Candida albicans* in type II diabetic patients with chronic periodontitis.Exfoliative cytology was used as a screening tool for detecting *Candida albicans*. 30 patients were divided into 2 groups that is diabetic type II patients with chronic periodontitis (test) and non-diabetic patients with chronic periodontitis (control). Glycosylated haemoglobin, questionnaire regarding duration of diabetes and medications were recorded and exfoliative cytology was carried out to detect *Candida albicans*. Patients with *Candida albicans* positive were taken up for the study. Both groups underwent scaling and root planing.On completion freshly prepared cinnamon mouthwash was dispensed in 100ml bottles with instruction for usage. At end of 3months exfoliative cytology was done and patients were advised to stop usage of mouthwash one week after cessation of mouthwash patients were recalled for clinical examination and exfoliative cytology. Candidal carriage was more in diabetics than non diabetics with chronic periodontitis. There was no difference between gender and the candidal carriage was more in patients with longer duration of diabetes over 10 years and severity of candidal carriage was more according to the duration. Candidal carriage was found to be more in uncontrolled diabetics. There was significant reduction in candidal carriage between baseline and 3 weeks in diabetic patients and in non-diabetics. After cessation of mouthwash recolonisation of *Candida albicans* was observed in both the groups. Results of the study proved the potential of cinnamon as an anti-fungal agent.[63]

- **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 species, which included five different Candida species, were isolated from 89 percent of

the patients. The most common species found was *Candida albicans* (78 percent of cases), followed by Candida famata and Candida tropicalis.[64]

- **Seenivasan et al in 2018** conducted a study to evaluate the in vitro growth inhibition of *Candida albicans,* in the soft-liner material and Shore A hardness from resin-based denture soft lining materials modified by neem or garlic incorporation. Resin discs were prepared with poly methyl methacrylate (PMMA) and soft liners incorporated with varying concentrations of neem or garlic. For antifungal activity, resin discs were placed on agar plates inoculated with C. albicans and were evaluated after 2, 4, and 7 days using the streaking method. The hardness of the PMMA was evaluated with the use of Shore A at 2, 4 and 7 days.Neem and garlic added to PMMA soft liner had an inhibitory effect on C. albicans. Both the neem and garlic when added showed positive results against C. albicans when compared to the control group. The soft liner hardness increased statistically by time but not for the different plant extract concentrations.It was found that neem and garlic can be used as an additive to tissue conditioner to reduce the adherence of C. albicans without significantly affecting the hardness of the heat-polymerized acrylic resin.[65]

- **Laura de Fatima et al in 2019** conducted a study to investigate the anti-Candida activity and the Shore A hardness of a tissue conditioner (Softone™) modified by incorporation of terpinen-4-ol and cinnamaldehyde. The agar diffusion antimicrobial effect of the experimental tissue conditioner resulted C. albicans growth complete inhibition for concentration of 20, 30and 40% cinnamaldehyde or terpinen-4-ol. Cinnamaldehyde incorporated experimental liners at concentrations of 10–40% prevented C. albicans biofilm formation. Shore A hardness of control and all test groups increased during a 7-day period. However, this increase is within the clinical parameters of the ISO standards.[66]

- **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*. When compared to natural neem extract, Fluconazole had a much improved elution profile and antifungal

efficacy against *Candida albicans*. Permanent silicone soft liner, followed by auto polymerising acrylic resin and heat polymerising acrylic resin, had much greater elution and better antifungal action in terms of colony inhibition of *Candida albicans*. Fluconazole was found to be more effective against *Candida albicans* than natural neem extract. The most effective polymeric technology for prolonged release of antifungal drugs for up to 21 days was found to be permanent silicone soft liners.[67]

- **Hiba et al in 2020** investigated the efficacy of adding the kappa-carrageenan powder to heat-cured, acrylic-based soft lining material against *Candida albicans* adherence.Different percentage (control: 0wt.%; experimental: 1.5wt.% and 2wt.%). of powder of kappa-carrageenan added to the heat-cured acrylic-based soft lining material. When compared to the control group, the findings of the *Candida albicans* adherence test demonstrated a highly significant decrease in the values of attached *Candida albicans* cells following integration of 1.5wt. percent and 2wt. percent kappa-carrageenan powder . The results of this investigation reveal that adding kappa-carrageenan powder to the surface of a heat-cured acrylic-based soft lining material is an effective medication against *Candida albicans* adhesion, and that adding 2wt. percent is more effective than 1.5wt. percent. [68]

- **Habibzadeh et al in 2021** evaluated the in vitro antifungal efficacy of addition of silver nanoparticles to Mucopren silicone soft liner material. Twenty disc samples (8 × 2 mm) of Mucopren silicone soft liner containing 0wt% (control), 0.5wt%, 1wt%, 2wt%, and 3wt% silver nanoparticles were fabricated. Samples were powdered and added to 150 mL of Sabouraud dextrose agar culture medium and placed on separate culture dish plates. According to the CLSI procedure, each plate was inoculated with 106 colony forming units per millilitre (CFUs/mL) of *Candida albicans* (PTCC 5027) and incubated at 37°C. The antifungal effect of the samples was assessed based on the percentage of viable cells in the two subgroups with and without thermocycling after 24 hours. All experimental groups showed higher antifungal activity than the control group, and this effect was dose-dependent ($P<0.05$). The lowest colony count

was recorded in the 3wt% group. Thermocycling had no significant effect on the antifungal efficacy, except in 0.5wt% concentration of silver nanoparticles.[69]

- **Godil et al in 2021** investigated the *In Vitro* activity of incorporated antifungal agents like Fluconazole and *Ocimum sanctum* oil (Tulsi) in the denture soft liners to reduce the risks associated with the biofilms of *Candida albicans.* In this study, the minimum inhibitory concentration (MIC) of two antifungal agents, Fluconazole and O. sanctum (Tulsi oil), was assessed against *Candida albicans* (ATCC 10231) to see how effective they were at reducing candida counts and biofilms. Physical parameters of soft denture liners (test and control) such as surface roughness and hardness were also examined. Using scanning electron microscopy, the effect of both antifungal drugs on the morphology of candida cells was examined (SEM). Fluconazole and O. sanctum oil have MIC values of 600 and 400 g/ml, repectively. The hardness and roughness of the surface of the soft denture material (test) remained unchanged. Finally, SEM tests showed that the antifungal agents integrated in the cell morphology of C. albicans at their respective MIC values were efficacious. This method enables for sustained drug release in the oral cavity, treating both wounded denture-bearing tissues and infection, as well as candida biofilms, without damaging their physical qualities. These investigations are important and have a lot of medicinal and therapeutic implications.[70]

- **Choonharuangdej et al in 2021** conducted an in vitro study to determine the efficacy of cinnamon and lemongrass essential oils in eradicating *Candida albicans* biofilm on heat-polymerized PMMA specimens and to determine whether they retard the formation of fungal biofilm. To establish the minimal inhibitory doses of cinnamon and lemongrass essential oils, researchers used agar disc diffusion and broth microdilution procedures. Before being individually treated with varied concentrations (12, 1, 2, 4, 8, 16 times minimum inhibitory concentration) of each tested oil for varying exposure durations, a mature C albicans biofilm (48 hours) was pre-established on PMMA specimens (1, 2, 4,8, and 24 hours). In a separate experiment, fungal biofilm was formed on PMMA specimens that had been primed with varied amounts of the tested oils for varying periods of time.The 2,3-bis-(2-methoxy-4-

nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT)-reduction assay was used to quantitate biofilm viability in both experiments.In conclusion cinnamon and lemongrass essential oils can eliminate pre-established C albicans biofilm and restrain the formation of fungal biofilm on heat-polymerized PMMA specimens. Both effects of the tested essential oils depended on dose and exposure or priming time. [71]

RELEVANCE

RELEVANCE

Candida albicans is a frequent opportunistic pathogen in humans that is able to colonize several surfaces, including oral epithelia and denture prostheses.The prolonged adherence of *Candida albicans* on to the denture surface is a major causative factor for denture stomatitis. Use of antifungals agents is the usual mode of treatment.

Knowledge of a prosthetic material or technique which can minimize this association would be beneficial towards a better and long-term outcome for a patient.One approach is, incorporation of an antifungal agent into a soft liner, which has the primary goal of improving denture fit and reducing trauma by cushioning tissues against applied loads along with sustained antifungal activity.

The traditional system of medicine in India have used various herbal and spice extracts and essential oils to treat numerous diseases because of their proven therapeutic effects.But the newer synthetic drugs have replaced the use of such agents against infectious diseases.

However , in recent times more experiments on the antimicrobial activity of plant essential oils due to the increasing development of drug - resistant strains towards antifungal drugs.The focal points of these natural agents is that they have multiple microbial targets enabling them to show wide range of antimicrobial action with little or no event of antimicrobial resistance

Of all the strategies taken to overcome resistance to antifungal drugs, the search of new and effective natural products showing antifungal activity against biofilm cells with low cytotoxicity is important.

This study aims to comparatively evaluate the antifungal activity of the essential oils of *Cinnamomum zeylanicum* and *Syzygium aromaticum* when incorporated into denture soft liner.

METHODOLOGY

METHODOLOGY

Materials used:

1. Soft liner : GC soft liner (GC Corporation, Japan).
2. Antifungal agents:
    a. *Cinnamomum zeylanicum* essentialoil (Synthite Industries Private Limited)
    b. *Syzygium aromaticum* essential oil (Synthite Industries Private Limited)
    c. Fluconazole (Himedia Laboratories private Limited, Mumbai)
3. *Candida albicans* standard strain (MTCC 227).
4. Microbiological culture media used: Mueller Hinton Agar (Himedia) with methylene blue.

Sampling

Sample Size:

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

$$n = \left[2 \frac{(Z\alpha + Z\beta)^2 (sd)^2}{d^2} \right]$$

Where, sd is the standard deviation

d is the expected difference

Zα=1.96 and Zβ=0.84

$$n = \left[\frac{2(1.96 + 0.84)^2 (1.3)^2}{(1.3)^2} \right]$$

=15 / group

The sample size has been calculated as 15 specimen in each group.There are 4 groups attributing to a total of 60 samples.

Sampling Procedure:

The Candida species chosen were *Candida albicans* standard strain MTCC 227.The antifungal agents chosen were *Cinnamomum zeylanicum* essential oil, *Syzygium aromaticum*essential oil, fluconazole as they have proven to be efficient against the *Candida* species.

A total of 60 samples comprising of 15 samples were taken for each group of antifungal incorporated soft liner (cinnamon bark essential oil, clove bud essential oil, fluconazole) 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 of 1^{st}, 3^{rd} and 7^{th} day .

Simple Random sampling was the sampling technique used. There are 4 groups with each group consisting of 15 samples.

Groups	**Description**	**Sample Number**
Group I	Plain soft liner discs	15
Group II	*Cinnamomum zeylanicum* essential oil incorporated soft liner discs	15
Group III	*Syzygium aromaticum* essential oil incorporated soft liner discs	15
Group IV	Soft liner discs incorporated with Fluconazole	15

This is an in-vitro study that was conducted in the Department of Prosthodontics and Crown & Bridge, St. Gregorios Dental College, Chelad in collaboration with Department of Microbiology, Mar Baselios Dental College, Kothamangalam

PREPARATION OF SAMPLES

GROUP 1

Soft denture liner was mixed according to manufacturer's instructions.

The standard powder liquid ratio is 2.2gms/1.8gms as given by manufacturer.

Soft liner powder was added at the manufacturer's recommended ratio and mixed for 30 seconds.

The mixture was then left to gel on a pre-fabricated stainless-steel template having disc size of 5mm diameter and 1 mm thickness.

The mixture was left to gel for 30minutes.

Sample discs of 5.0 mm diameter were then cut out.

A total of 15 such specimens were made designated as group 1 and this served as control disc.

GROUP 2

To the soft-liner liquid, 150 µl/ml of 75% *Cinnamomum zeylanicum* essential oil was added and mixed for 20 seconds.

Then, soft liner powder was added at the manufacturer's recommended ratio and mixed for 30 seconds.

The mixture was then left to gel on a pre-fabricated stainless-steel template having disc size of 5 mm diameter and 1 mm thickness.

The mixture was left to gel for 30 minutes.Sample discs of 5.0 mm diameter were then cut out.

Total of 15 such specimens were made designated as group 2 was the test discs.

GROUP 3

To the soft-liner liquid, 150 µl/ml of 75 % Syzygium *aromaticum* essential oil was added and mixed for 20 seconds.

Then, soft liner powder was added at the manufacturer's recommended ratio and mixed for 30 seconds.

The mixture was then left to gel on a pre-fabricated stainless-steel template having disc size of 5 mm diameter and 1 mm thickness.

The mixture was left to gel for 30 minutes.Sample discs of 5.0 mm diameter were then cut out.

Total of 15 such specimens were made designated as group 3 served as the test discs.

GROUP 4

Fluconazole was added into the powder of soft liner based on their minimum inhibitory concentration (16µg/ml) as per CLSI M44-A2. [72]

Then, soft liner powder was added at the manufacturer's recommended ratio and mixed for 30 seconds. The mixture was then left to gel on a pre-fabricated stainless-steel template having disc size of 5mm diameter and 1 mm thickness.

The mixture was left to gel for 30 minutes. Sample discs of 5.0 mm diameter were then cut out.

Total of 15 such specimens were made designated as group 4 and this served as positive control.

Preparation of *Candida albicans* inoculum

Standard *Candida albicans* strain MTCC 227 was used (procured from MTCC Chandigarh)

Cells were harvested by centrifugation at 3000 rpm for 10 min, washed by brief vortexing for 1 minute and spreaded onto Meuller Hinton agar with methylene blue uniformly by pipetting 0.5 ml Candida species suspension.

Test and control discs were placed onto Meuller Hinton agar with methylene blue and incubated aerobically at 37°C for 24 h.

After incubation, the diameter of the zones of inhibition of Candida species were measured using vernier caliper on 1st day, on day 3 and on day 7.

Evaluating the effect of antifungal incorporated soft liner on *Candida albicans*

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

Antifungal agents - Cinnamon essential oil: 150 µl/ml, Clove essential oil: 150 µl/ml and Fluconazole: 16 µg/ml, was separately added into the soft liner. 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 the antifungal agent is the control group. Routine antifungal susceptibility was done on all isolates by CLSIM44-A2. [73] 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.[39 Test] and control discs were 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.

Once the measurements were made, the results were calculated and the statistical analysisof the data was carried out using MS Excel and IBM SPSS 16 with one-way ANOVA

The descriptive analysis was performed by the calculation of Mean, Standard Deviation and percentages.

The significance was defined as $p<0.05$.

Figure 1: GC soft liner

Figure 2: Cinnamon bark essential oil and clove bud essential oil

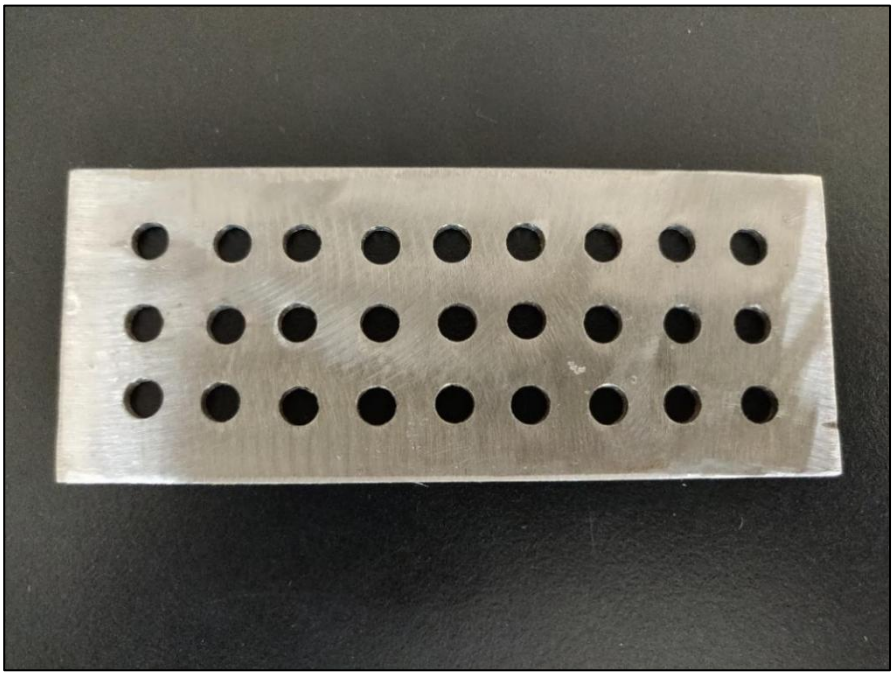


Figure 3: Pre-fabricated stainless steel template

Figure 4 : Pre fabricated stainless steel template filled with the soft liner to which the antifungal agent is added

Figure 5 : Mueller Hinton agar with Methylene blue

Figure 6: Colonies of *Candida albicans*- Standard strain

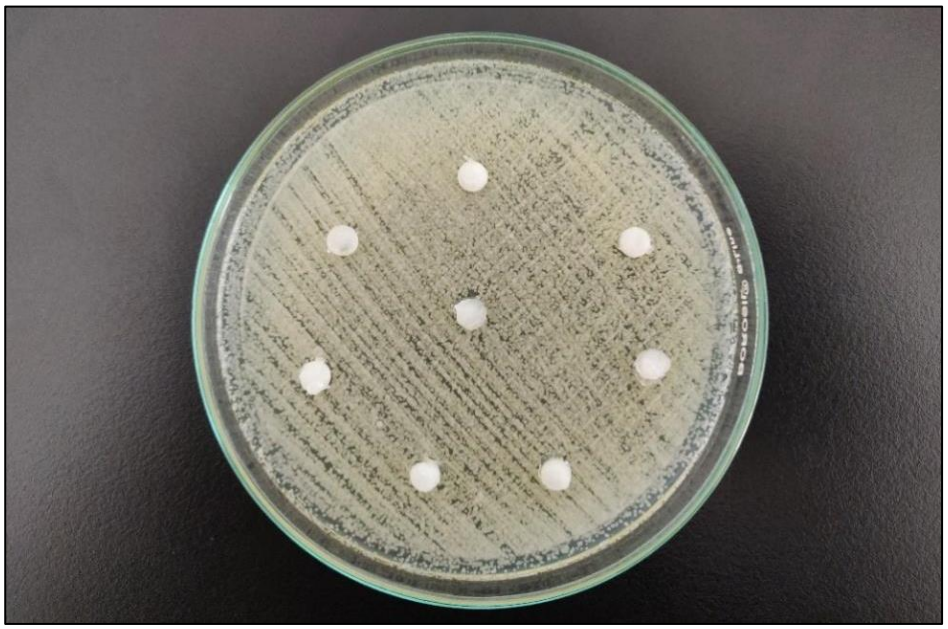


Figure 7: Agar plate inoculated with *candida albicans* standard strain having soft liner discs

Figure 8: Soft liner disc incorporated with Cinnamon (*Cinnamomum zeylanicum)* showing its activity against *candida albicans* standard strain at 24 hours

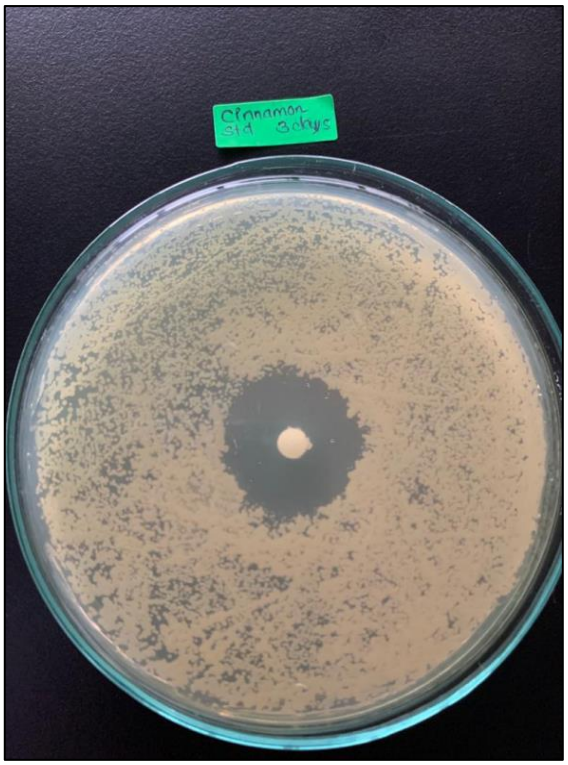


Figure 9: Soft liner disc incorporated with Cinnamon (*Cinnamomum zeylanicum)* showing its activity against C*andida albicans* standard strain at 3 days.

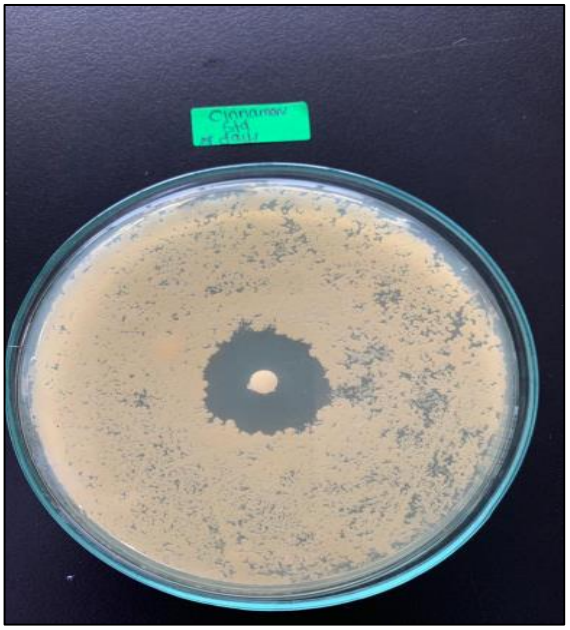


Figure 10: Soft liner disc incorporated with Cinnamon (*Cinnamomum zeylanicum)* showing its activity against C*andida albicans* standard strain at 7 days

Figure 11: Soft liner disc incorporated with clove (*Syzygium aromaticum*) showing its activity against C*andida albicans* standard strain at 24 hours

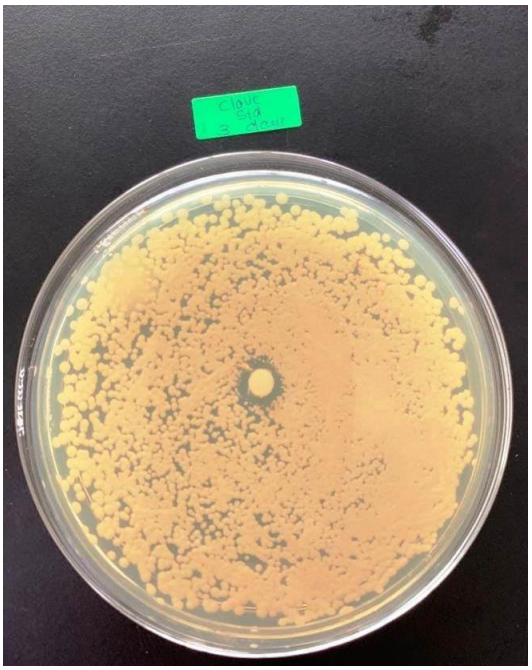


Figure 12: Soft liner disc incorporated with clove (*Syzygium aromaticum*) showing its activity against C*andida albicans* standard strain at 3 days

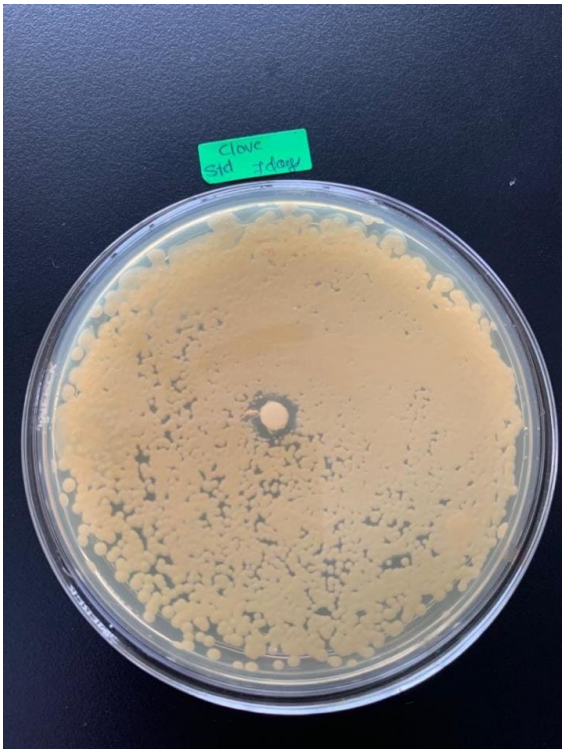


Figure 13: Soft liner disc incorporated with clove (*Syzygium aromaticum*) showing its activity against C*andida albicans* standard strain at 7 days

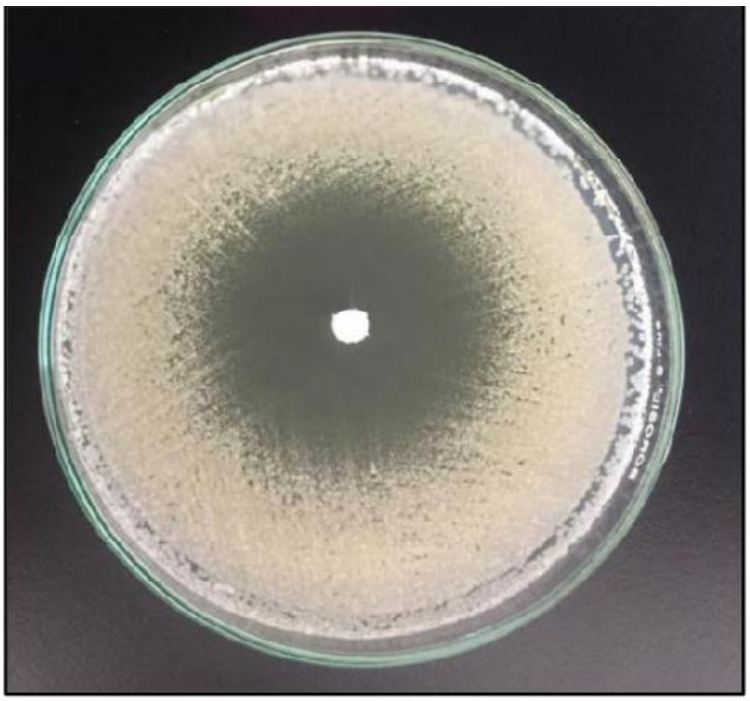


Figure 14: Soft liner disc incorporated with fluconazole showing its activity against *candida albicans* standard strain on at 24 hours

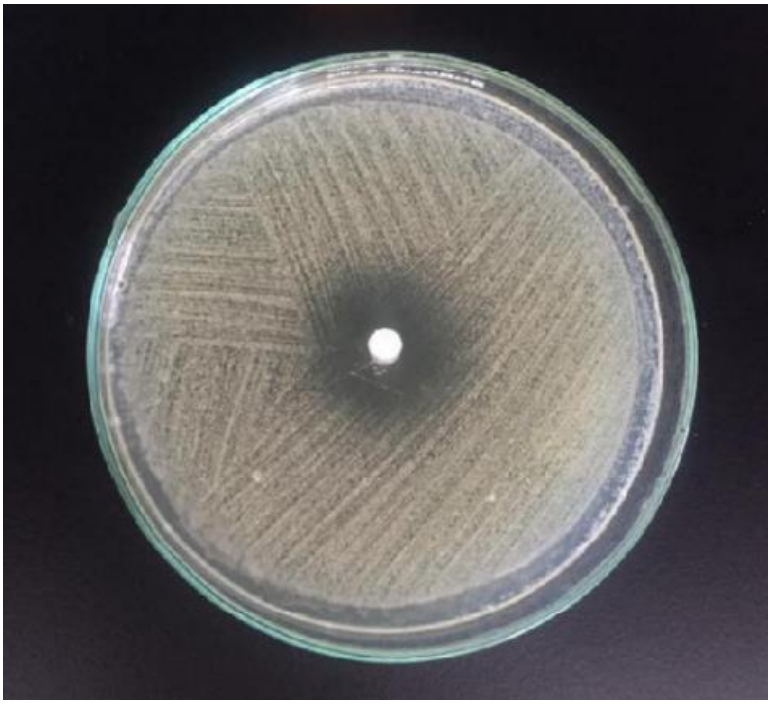


Figure 15: Soft liner disc incorporated with fluconazole showing its activity against C*andida albicans* standard strain at 3 days

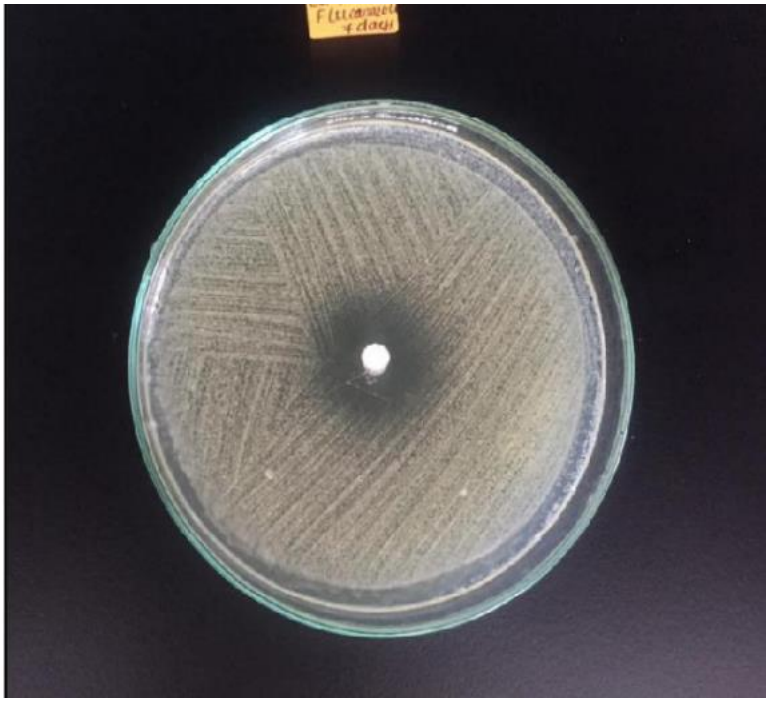


Figure 16: Soft liner disc incorporated with fluconazole showing its activity against C*andida albicans* standard strain at 7 days

RESULTS

RESULTS

The data were gathered and then processed in response to the problems posed in the aims and objectives of this dissertation.This in-vitro study is to compare the antifungal activity of *Cinnamomum zeylanicum* and *Syzygium aromaticum* essential oils incorporated into denture soft liner. It drove the collection of the data and the subsequent data analysis objectives were fulfilled. The findings presented in this study 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, 3 days, 7 days with 15 samples for each group.

Null hypothesis

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

Alternate hypothesis

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

Level of significance, $\alpha=0.05$

CANDIDA ALBICANS STANDARD

Table 1: Descriptive analysis table for plain soft liner disc on *Candida albicans* standard strain on day 1,day 3 and day 7 of inoculation.

	Mean±SD
Day 1	0
Day 3	0
Day 7	0

Inference: There is no antifungal activity for plain soft liner discs.

Table 2: Descriptive analysis table for *Cinnamomum zeylanicum* essential oil incorporated soft liner disc on *Candida albicans* standard strain on day 1,day 3 and day 7 of inoculation.

	Mean±SD	P value
Day 1	21.21±1.34	<0.01
Day 3	16.85±1.03	
Day 7	15.05±1.32	

Inference: There is antifungal activity and the values are highly significant.The ZOI on day 1,3 and 7 is 21.21±1.34 ,16.85±1.03,15.05±1.32 respectively.The values are gradually deterioting from day 1 to day 7 showing a gradual decrease in the antifungal activity .

Table 3: Descriptive analysis table for*Syzygium aromaticum* essential oil incorporated soft liner discon *Candida albicans* standard strain on day 1, day 3 and day 7 of inoculation.

	Mean±SD	P value
Day 1	8.53±0.78	<0.01
Day 3	7.41±0.46	
Day 7	5.82±0.39	

Inference: There is antifungal activity and the values are highly significant.The ZOI on day 1,3 and 7 is 8.53±0.78 ,7.41±0.46,5.82±0.39 respectively.The values are gradually deterioting from day 1 to day 7 showing a gradual decrease in the antifungal activity .

Table 4: Descriptive analysis table forsoft liner discs incorporated with Fluconazoleon *Candida albicans* standard strain on day 1,day 3 and day 7 of inoculation.

	Mean±SD	P value
Day 1	29.24±0.98	<0.01
Day 3	24.52±0.81	
Day 7	19.12±1.01	

Inference: There is antifungal activity and the values are highly significant.The ZOI on day 1,3 and 7 is 29.24±0.98,24.52±0.81,19.12±1.01 respectively. The values are gradually deterioting from day 1 to day 7 showing a gradual decrease in the antifungal activity.

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 of variance was performed to infer any differences that existed between the groups of antifungal agents. Results are tabulated below. As per the tests it was found that the antifungal agents showed different mean values and the tests showed that the difference was statistically highly significant.

***Candida albicans* standard**

Table 5: Anova results indicate significant difference between the groups on day 1

Group	Mean±SD	P value
1	0	<0.01
2	21.21±1.34	
3	8.53±0.78	
4	29.24±0.98	

Table 6: Pair wise comparison on day 1

	1	2	3
2	<0.01	-	-
3	<0.01	<0.01	-
4	<0.01	<0.01	<0.01

Table 7: Anova results indicate significant difference between the groups on day 3

Group	Mean±SD	P value
1	0	<0.01
2	16.85±1.03	
3	7.41±046	
4	24.52±0.81	

Table 8: Pairwise comparison on day 3

	1	2	3
2	<0.01	-	-
3	<0.01	<0.01	-
4	<0.01	<0.01	<0.01

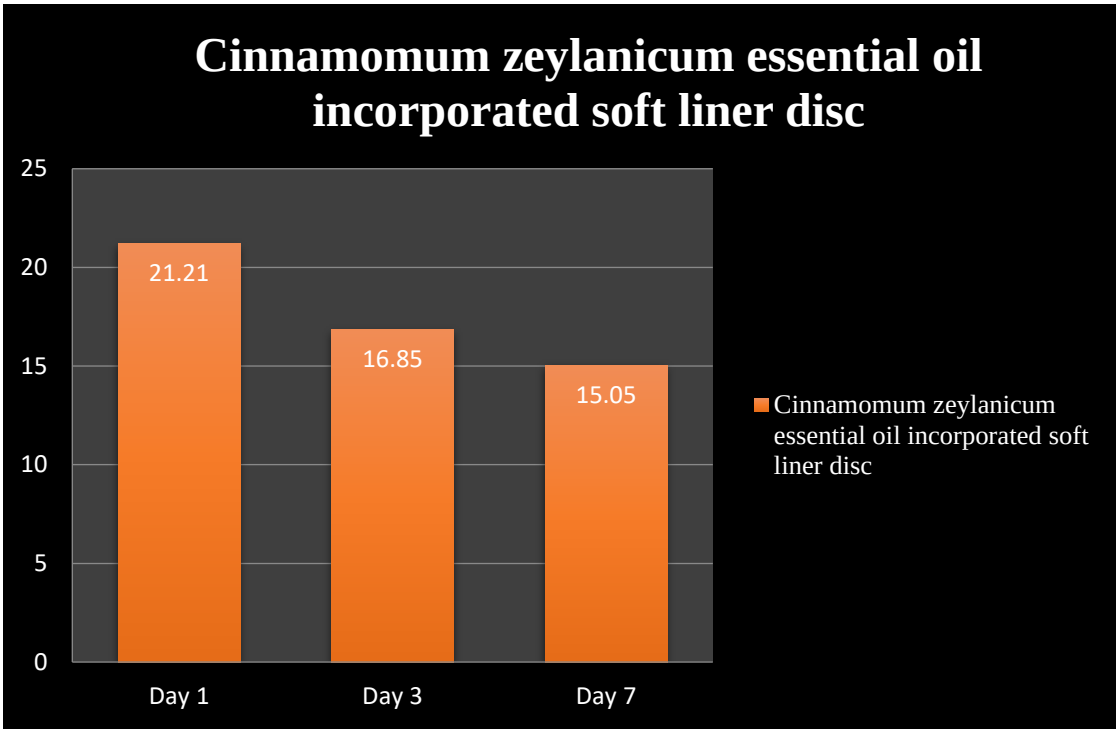
Table 9: Anova results indicate significant difference between the groups on day 7

Group	Mean±SD	P value
1	0	<0.01
2	15.05±1.32	
3	5.82±0.39	
4	19.12±1.01	

Table 10: Pairwise comparison on day 7

	1	2	3
2	<0.01	-	-
3	<0.01	<0.01	-
4	<0.01	<0.01	<0.01

GRAPHICAL REPRESENTATION OF THE EFFICACY OF DIFFERENT ANTIFUNGAL AGENTS AT VARIOUS TIME INTERVALS



Graph 1: Comparison of the antifungal efficacy of *Cinnamomum zeylanicum* at various time intervals for *C.albicans* standard strain.

Inference: The ZOI on day 1,3 and 7 is 21.21±1.34 ,16.85±1.03,15.05±1.32 respectively.The values are gradually deterioting from day 1 to day 7 corresponding to a gradual decrease in the antifungal activity .However, *C.albicans* standard strain exhibit sensitivity to *Cinnamomum zeylanicum* essential oil incorporated soft liner disc.

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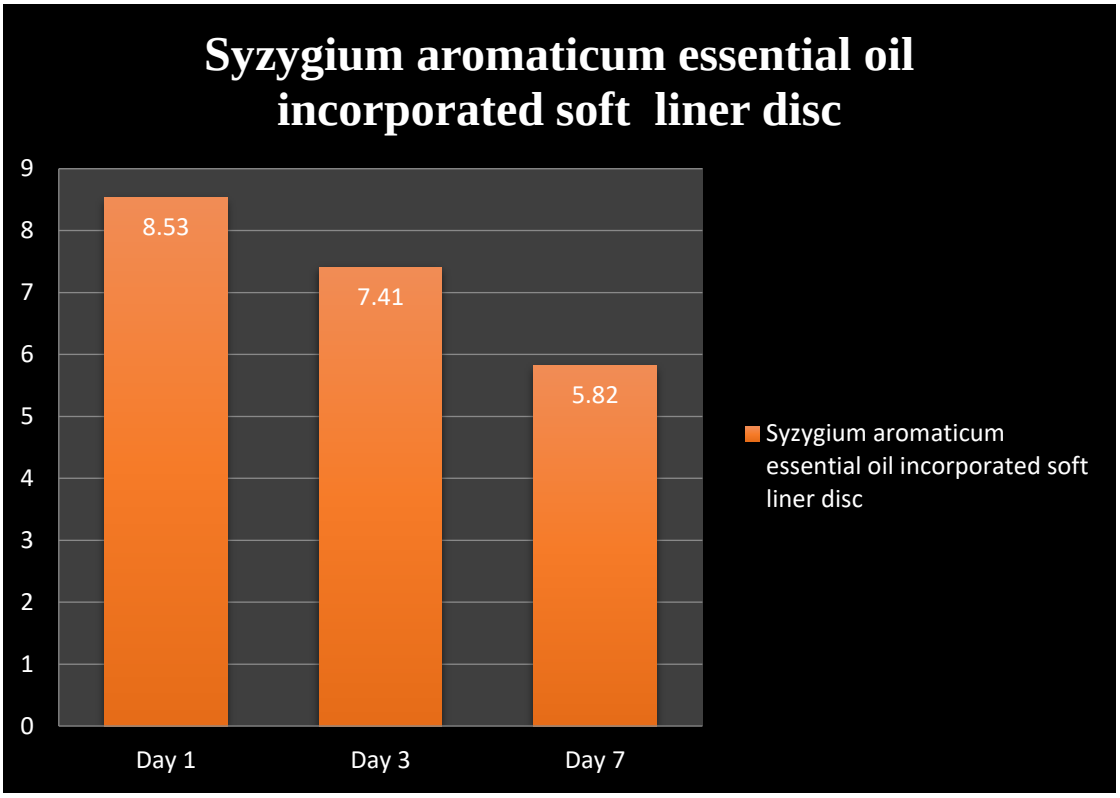
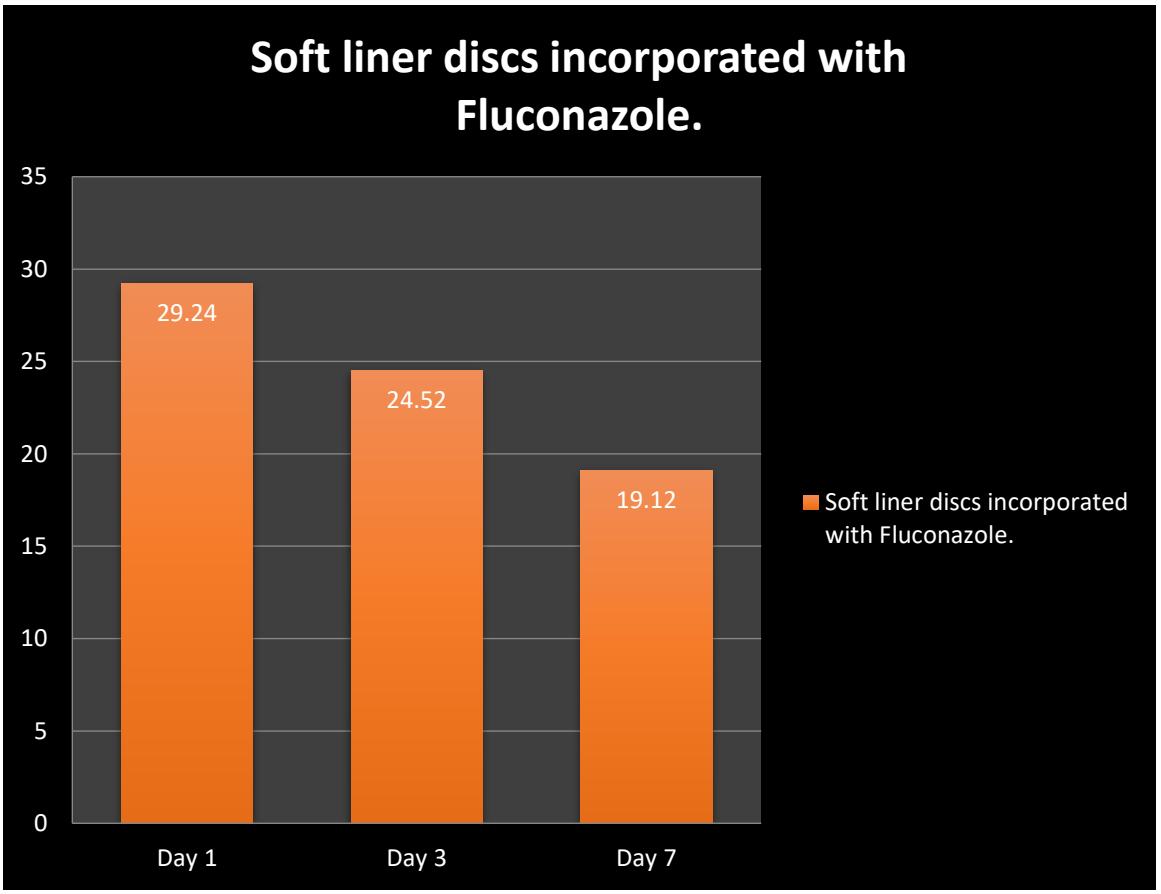


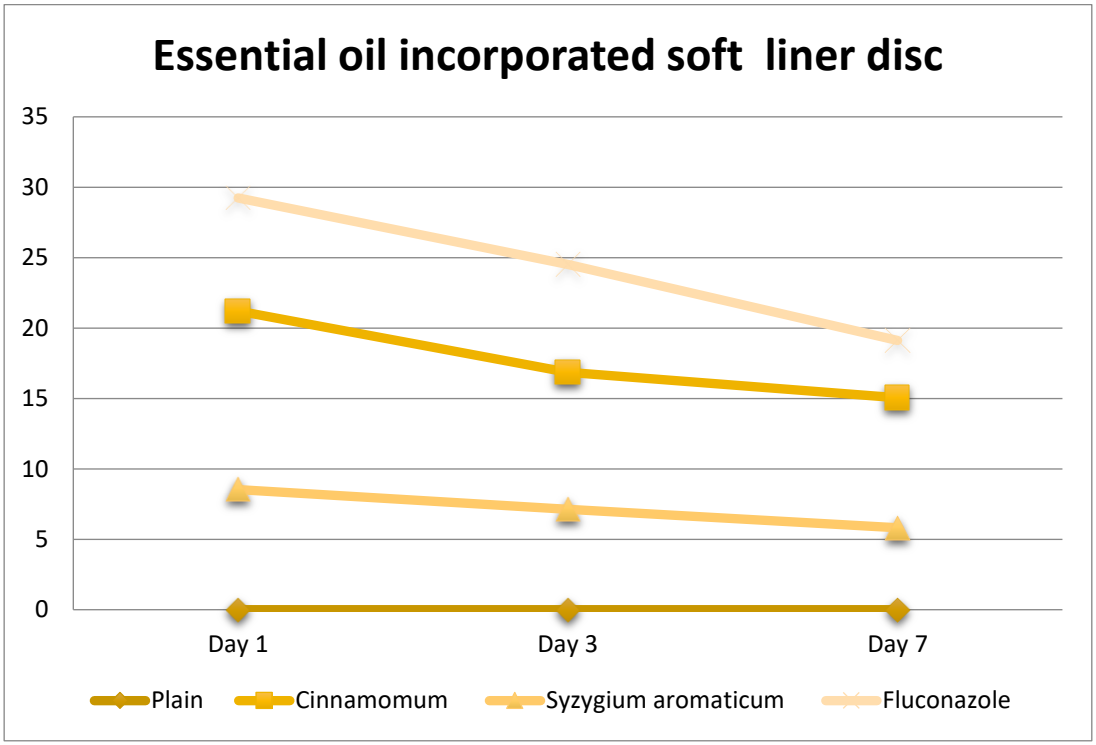
Graph 2: Comparison of the efficacy of *Syzygium aromaticum* at various time intervals for *C.albicans* standard strain.

Inference:.C.albicans standard strain is most sensitive to *Syzygium aromaticum* essential oil incorporated soft liner disc on day 1 of inoculation. The ZOI on day 1, 3 and 7 is 8.53±0.78, 7.41±0.46, 5.82±0.39 respectively.The values are gradually deterioting from day 1 to day 7 corresponding to a gradual decrease in the antifungal activity



Graph 3: Comparison of the antifungal efficacy of fluconazole incorporated soft liner discs at various time intervals for *C.albicans* standard strain.

Inference: C.albicans standard strain is most sensitive to fluconazole incorporated soft liner disc on day 1 of inoculation. The ZOI on day 1,3 and 7 is 29.24±0.98, 24.52±0.81,19.12±1.01 respectively.The values are gradually deterioting from day 1 to day 7 corresponding to a gradual decline in the antifungal activity.



Graph 4: Comparison of the antifungal efficacy of different groups on C.albicans standard strain at day 1, 3 and 7.

Inference: On inoculation *C.albicans* standard strain is most sensitive to fluconazole followed by *Cinnamomum zeylanicum* and then *Syzygium aromaticum* incorpoated soft liner discs on all the tested days - day 1 ,3 and 7.However, in group 2,3 and 4 there is a gradual decrease in the actifungal activiy on day 3 and eventually declining at day 7.

The values of day 3 particularly of group 2 and group 4 seeks special attention to the fact that though there was a mean difference of approximately 8.03 mm between the 2 groups on day 1, it has reduced to about 4.07 mm on 7th day indicating that effectiveness of *Cinnamomum zeylanicum* was comparable to synthetic drug, fluconazole as days passby.

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 frequently visible beneath the surface of the denture.

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. [74]

- 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 characterised by 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: [75]

- 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.[76]

Gauch et al identified *Candida species* from the oral cavity of denture wearers with denture-related stomatitis. *Candida species* 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.*[77]

The results of a study by **Wright et al** 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.[78]

Denture related 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 like fluconazole, nystatin etc. Topical antifungal therapy is the corner

stone of treatment of localized 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, but can cause 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).Ergosterol, a 5, 7-diene oxysterol, is the most abundant sterol in fungal cell membranes, where it regulates permeability and fluidity of the cell. Because of its crucial functions, unique structural properties, and particular biosynthetic steps, ergosterol is the target of the majority of clinically available antifungals. Polyenes, interact with fungal membrane sterols physicochemically and azoles, act by inhibiting the synthesis of ergosterol (the main fungal sterol).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 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.[79]

The azole drug, Fluconazole is widely prescribed against various *Candida albicans* infections.[80-84] Even though very widely acclaimed for their efficacy, these drugs are known to have side effects.[85,86] Fluconazole is fungistatic in nature and there are reports of emergence of Fluconazole resistance among clinical isolates of C. albicans.[87-91] Thus there is a need for better, novel antifungal agents against infections caused by *C. albicans* which are effective and have lesser side effects. Many natural agents like plant products including extracts, oils, etc. are traditionally used against various ailments which are proven to have antimycolic activity.[92–94]

The increased drug resistance ,high production cost and enormous side effects of chemical materials, paved way for use of alternative medicinal plant extract having antifungal activity.Because of the side effects, emerging resistance ,cost and difficulty of therapeutic-chemical materials production, using medical plants which have less side effects and are economically cost effective, have been recently taken into consideration.Plants have been used in medicine for a long time as they are economic,being natural, easily available and applicable in various diseases. Briefly, about some plants that showed antimicrobial activity, clinical trial is necessary to determine the usefulness of these plant extracts for the treatment of oral candidiasis.

If similar results are confirmed in clinical trials, theses plant extracts can be used to produce new, useful and economic antifungal products.

In previous research antimicrobial effects of some plant extracts have been studied with different methods. In a previous study by Saekhi et al in 1989, in vitro antimicrobial activity of different natural agents against to a few mouth bacteria has been evaluated and it was found among them, Cinnamon, Clove oil and spices extract have inhibitory effects on mouth bacteria.[95]

In 2001 Ahmad et al investigated about the effect of 45 Indian plants on human resistant pathogens to different drugs, 40 plants showed antimicrobial activity against one or more bacteria and 24 plants showed antifungal activity against Candida.[96]

Similarly various previous studies by Mau, et al., (2001), De, et al (1999), Saeki, et al., (1989), Quale, et al., (1996) studies, confirmed the inhibitory effect of cinnamon on different microorganisms, such as E.coli, Sacharomyces cerevisiae, Bacillus subtilis, and oral bacteria, such as Streptococcus sp, Actinomyces sp, Actinobacillus sp, Bacteroides sp, Capnocytophaga sp, Eikenella sp, Fusobacterium sp, propionibacterium sp and a few species of Candida respectively. [95,97-99]

Singh et al in 1995 identified cinnamon aldehyde as the active fungitoxic constituent of cinnamon.[100]

Soliman et al in 2002 conducted study on 12 plant species with therapeutic effect and found that cinnamon, spearment, and thyme had more antifungal activity.[101]

Ahmad N et al in 2005 showed strong antifungal activity of the clove oil against different fungi such as *Candida albicans*. The achieved result was similar to the result which was observed in the present research. [102]

Suhr et al confirmed the inhibitory effect of cinnamon, thyme and clove against a few different fungi such as *Candida albicans*.[103]

Ahmad N et al in 1998 studied effect of 82 Indian plants against pathogenic and opportunist microorganisms. Among them, 56 plants had the antimicrobial activity against one or more pathogen. Five plants showed strong antibacterial. Also, they discovered that alcoholic extract of the plants had higher activity than water and Hexan extracts.[104]

Cervenka et al in 2006 conducted a research on methanol and chloroform extracts of 17 plants such as rosemary, sage, chamomile and cinnamon, showed that methanol extract of these plants had higher antimicrobial effects than their chloroform extracts .[105]

Dalirsani et al in 2011 investigated the antimicrobial effect of 10 medical plants on *Candida albicans*. The following plants were selected: thyme, mint, garlic, cinnamon, chamomile, tea tree, clove, spearmint, sage and rosemary. These plants had been selected according to the medical traditional usage and previous researches. C. albicans was cultured in Sabouraud dextrose Agar containing Chloramphenicole. On

each plate, one plant extract disc, one chlorhexidine disc and one nystatin disc; as positive controls; and one methanol and one blank disc; as negative controls; were placed. After 24 hours, the mean diameter of non-growth halo around every plant extract was compared with the mean diameter of non-growth halo of positive control disks by T test statistical analyze. Non-growth halo in disks containing chamomile, garlic, clove, cinnamon, sage and thyme was observed. Cinnamon, garlic, chamomile, sage, clove and thyme had inhibitory effects on C.albicans.[106]

Devkatte et al 2005 tested the in vitro efficacy of 38 plant oils against four isolates of C. albicans. Minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) of the effffective oils are reported. Based on their effIcacy (MFC), the oils are classified into three categories.

In general, it was observed that the fungicidal concentration was higher than MIC.Seven oils were found to be most effective. Cinnamon oil was the best, having fungicidal effect at 0.03% concentration in all four isolates of C. albicans. Clove oil was fungicidal at 0.12% concentration and there was no diffffference between MIC and MFC.[21]

Gujjari et al conducted a clinical study by evaluating the effect of cinnamon mouthwash on *Candida albicans* in type II diabetics with chronic periodontitis and the results showed that cinnamon could be a promising alternative antifungal agent.[107]

Cinnamon, a commonly used spice which has been used since ancient times, has been found to have medicinal benefits in addition to its flavouring property as a condiment. The bark of C. zeylanicum and C. cassia are the only two approved medicinal herbs of the genus Cinnamomum. It has been found to have anti-inflammatory, antibacterial, antioxidant properties as well as antifungal. Though there are reports of cinnamon being given as an antifungal in HIV patients

Figure 16: Cinnamon bark

Plant Profile

Habitat: The spice is derived from the brown bark, which forms quills with longitudinal striations. The plant is native to Sri Lanka, South eastern India, Indonesia, South America, and the West Indies.

Plant Hierarchy

Kingdom Plantae – Plants

Subkingdom Tracheobionta – Vascular plants

Superdivision Spermatophyta – Seed plants

Division Magnoliophyta – Flowering plants

Class Magnoliopsida – Dicotyledons

Subclass Magnoliidae

Order Laurales

Family Lauraceae – Laurel family

Genus Cinnamomum Schaeff. – cinnamon P

Species Cinnamomum zylinicum J. Presl – cinnamon P

Scientific Names

Cinnamomumzylinicum, Cinnamomum cassia, Cinnamomumzeylanicum, Cinnamomumloureirii

Common name(s)

Cinnamon, Cinnamomon, Ceylon cinnamon, Chinese cinnamon, Chinese cassia, Saigon cinnamon

It contains a number of compounds, including essential oils that provide the spice's flavor. Other compounds which are present in lesser percentages those are Cinnamic acid, Hydroxyl Cinnamaldehyde, Cinnamyl alcohol, Coumarin, Cinnamyl acetate, Borneol etc.

Cloves are the aromatic flower buds of a tree in the family Myrtaceae, *Syzygium aromaticum*. They are native to the Maluku Islands (or Moluccas) in Indonesia, and are commonly used as a spice. Cloves are available throughout the year owing to different harvest seasons in different countries.

Figure 17: Clove buds

Plant hierarchy

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order: Myrtales

Family: Myrtaceae

Genus: Syzygium

Species: S. aromaticum

Binomial name

Syzygium aromaticum (L.) Merr. & L.M.Perry

Synonyms

- Caryophyllus aromaticus L.
- Eugenia aromatica (L.) Baill.
- Eugenia caryophyllata Thunb.
- Eugenia caryophyllus (Spreng.)

Eugenol comprises 72–90% of the essential oil extracted from cloves, and is the compound most responsible for clove aroma.Complete extraction occurs at 80 minutes in pressurized water at 125 °C (257 °F). Ultrasound-assisted and microwave-assisted extraction methods provide more rapid extraction rates with lower energy costs.

Other important essential oil constituents of clove oil include acetyl eugenol, beta-caryophyllene, vanillin, crategolic acid, tannins such as bicornin, gallotannic acid, methyl salicylate (painkiller), the flavonoids eugenin, kaempferol, rhamnetin, and eugenitin, triterpenoids such as oleanolic acid, stigmasterol, and campesterol and several sesquiterpenes. Eugenol has not been classified for its potential toxicity.

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 [108]

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. [108]

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 abscent, 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*. [109]

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.[110]

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.** [111] These are convenient for the patients as they do not require any compliance to frequent application regimes. In addition to this, direct delivery of the drug at 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.[112]** Therefore, a local delivery system is an alternative option to maintain therapeutic drug levels at the site of pathology.

Odds considered that denture liners alone usually have no effect on *candida species* and antifungals need to be incorporated.[113]

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 showed that Viscogel (tissue conditioner) combined with nystatin powder were successful in inhibiting the growth of *Candida albicans*, *Candida krusei* and *Candida tropicalis.*[114]

In this in -vitro study, antifungal efficacy of 15 samples of plain soft liner ,15 samples for *cinnamomum zeylanicum*, 15 samples for *Syzygium aromaticum and* 15 samples for fluconazole when .incorporated soft linerwere evaluated for *Candida albicans* standard strain (MTCC 227).

The soft liner used in this study is commercially available (GC corporation, Japan). In the present study an agar based medium Sabourand dextrose agar (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** [46] in India and by **Thomas et al**, who observed that tissue conditioners would not be beneficial without antifungal agents in the treatment of denture stomatitis.[114] Also, in an in vitro study conducted by **Chow et al** [34]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 antifungal agents used in this study is - natural antifungal agents like *Cinnamomum zeylanicum* and *Syzygium aromaticum* as they have proven potent antifungal effect and the synthestic drug ,fluconazole was added as the positive control as they showed high potency towards *candida* albicans

This in-vitro study is to compare the antifungal activity of *Cinnamomum zeylanicum* and *Syzygium aromaticum* essential oils incorporated into denture soft liner.

The key objectives of this research are:

i. Anti-fungal efficacy of *Cinnamomum zeylanicum* essential oil incorporated into soft liner.

ii. Anti-fungal efficacy of *Syzygium aromaticum* essential oil incorporated into soft liner.

iii. Comparative antifungal efficacy of *Cinnamomum zeylanicum* and *Syzygium aromaticum* essential oils incorporated into soft liner.

iv. Comparative antifungal efficacy of *Cinnamomum zeylanicum* and *Syzygium aromaticum* incorporated into soft liner with Fluconazole incorporated into soft liner

All the samples were tabulated and analysed for statistical significance using ANOVA

(analysis of variance) and Post hoc test.

Plain soft liner discs

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

***Cinnamomum zeylanicum* incorporated soft liner specimen**

The zone of inhibition for *Candida albicans* standard strain was found to be 21.21 mm at day 1, 16.85 mm at day 3 and 15.05 mm at day 7

***Syzygium aromaticum* incorporated soft liner specimen**

The zone of inhibition for *Candida albicans* standard strain was found to be 8.53mm for day 1, 7.41 mm at day 3 and 5.82 mm at day 7. No further activity was recorded

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 29.24 mm for day 1, 24.52 mm.

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 antifungal agents demonstrated different mean values and the tests show that the difference was statistically highly significant ($P<0.01$). As Anova showed a significant overall difference between the antifungal agents, 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.

On comparing the efficacy between the antifungal agents , one synthetic -fluconazole and two natural - *Cinnamomum zeylanicum* and *Syzygium aromaticum* essential oils , it was found that fluconazole showed higher activity than *Cinnamomum zeylanicum* and *Syzygium aromaticum* on *Candida albicans*.However it was found that the soft liner discs incorporated with fluconazole showed an exponential decrease in the antifungal activity from day 1 to day 3 is 4.72 mm and even more decrease from day 3 to day 7 (5.4 mm).This may be attributed to the regrowth of fungus.

However ,*Cinnamomum zeylanicum* EO incorporated soft liner disc also showed a decrease in antifungal activity from day 1 to day 3 (4.36 mm) but its value was almost steady afterwards till 7th day (difference of 1.8 mm).Also, *Syzygium aromaticum* EO incorporated soft liner discs though showed a reduced ZOI when compared to the other groups but its antifungal activity was found to be almost steady as there was only diiference of 1.12 mm from day 1 to day 3 followed by a difference of 1.32 mm from day 3 to day 7.This indicates the sustained and steady release of natural antifungal agents incorporated soft liner discs when compared with the synthetic drug -fluconazole incorporated soft liner discs.Soft liner discs without incorporation of any antifungal agent neither ceased the growth of fungus.

On comparing the efficacy between the antifungal agents it was found that **fluconazole showed higher activity** showing mean ZOI of 29.24mm on the 1st day followed by a decrease of 24.52mm on 3rd day and 19.12 mm on 7th day compared to cinnamon and clove.

This was similar to the study by **Chopde N et al** [47] 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 results of this study proved that fluconazole had the strongest inhibiting action against the candida genus followed by natural antifungal agents *Cinnamomum zeylanicum* and *Syzygium aromaticum*.*Candida albicans* standard strain also showed positive values with a gradual decrease till 7 days.

A study done by **Darwish RM** et al, demonstrated that fluconazole incorporated into auto polymerizing 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. [115]

Within the limitation of this study fluconazole showed a peak on the 1st day followed by a gradual decrease till day 7. This can be attributed to the internal configuration of the matrix of the material 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.[1]

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. [116]

However the potent activity of clotrimazole can be compared to the study by **Koteswara Rao Pachava et al** [52] 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.

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 *Cinnamomum zeylanicum* and *Syzygium aromaticum* .[47]

It was observed that there was an initial high rate of release followed by a sustained release phenomenon over the one 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.[112]

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 7-day period was selected for the testing of fungicidal effects of different antifungal agents and tissue conditioner combinations.[26]The results proved that the effect reduced significantly from day 7, hence repeated patient recalls 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

Anova test or analysis of variance was performed to infer any differences that existed between the groups of antifungal agents. As per the tests it was found that the antifungal agents showed different mean values and the tests show that the difference

was statistically highly significant (P<0.01). 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 antifungal agents , one synthetic -fluconazole and two natural - *Cinnamomum zeylanicum* and *Syzygium aromaticum* essential oils , it was found that fluconazole showed higher activity than *Cinnamomum zeylanicum* and *Syzygium aromaticum* on*Candida albicans*.However it was found that the soft liner discs incorporated with fluconazole showed an exponential decrease in the antifungal activity from day 1 to day 3 is 4.72 mm and even more decrease from day 3 to day 7 (5.4 mm).This may be attributed to the regrowth of fungus.

However ,*Cinnamomum zeylanicum* EO incorporated soft liner disc also showed a decrease in antifungal activity from day 1 to day 3 (4.36 mm) but its value was almost steady afterwards till 7th day (difference of 1.8 mm).Also, *Syzygium aromaticum* EO incorporated soft liner discs though showed a reduced ZOI when compared to the other groups but its antifungal activity was found to be almost steady as there was only diiference of 1.12 mm from day 1 to day 3 followed by a difference of 1.32 mm from day 3 to day 7.This indicates the sustained and steady release of natural antifungal agents incorporated soft liner discs when compared with the synthetic drug -fluconazole incorporated soft liner discs.Soft liner discs without incorporation of any antifungal agent neither ceased the growth of fungus.

This study also proves the antifungal efficacy of natural spice essential oils like *Cinnamomum zeylanicum* and *Syzygium aromaticum* even after incorporating into denture soft liner.The result is similar to the previous experiment by **Choonharuangdej et al in 2021** proved that cinnamon and lemongrass essential oils can eliminate pre-established C albicans biofilm and restrain the formation of fungal biofilm on heat-polymerized PMMA specimens.

Though, fluconazole has been widely used in the treatment of Candida associated denture stomatitis.However, its use has declined because as it is extremely nephrotoxic and is administered intravenously.[16]

Recently, azole antifungal compounds such as fluconazole, which have excellent efficacytoxicity profiles, is the principal drugs used in the treatment of

candida infections.[17] However, fluconazole produces few side effects like nausea, vomiting and might lead to the development of drug resistance in *Candida albicans*.[18,19] So, to overcome these limitations of chemically synthetic antifungals,novel and effective antifunal agents like some herbs with antifungal,antibacterial, and antiviral properties known as phytotherapeutic agents are of interest.[20] The broad spectrum of biological activities of natural products make them attrative phototypes as antifungal agents.

Anupama N. Devkatte et al conducted a study of the 38 oils of plant origin against *Candida albicans* growth. Among that 23 were found effective and fifteen, ineffective. Based on their Minimum Fungicidal Concentrations (MFC), plant oils were categorized into four categories-most effective, moderately effective, less effective and non-effective. Results of this study indicated that oils of plant origin can be used as potential anti-Candida agents.

Cinnamon oil (*Cinnamomum zeylanicum*) was the best, having fungicidal effect at 0.03% concentration in C. albicans. Clove oil (*Syzygium aromaticum*) was fungicidal at 0.12% concentration and there was no difference between MIC and MFC.[21]

Incorporation of an antifungal agent into soft liner serve dual purpose of sustained continous antifungal activity along with providing a cushioning effect to the traumatised inflamed tissues.This invitro study is to compare the antifungal activity of *Cinnamomum zeylanicum* and *Syzygium aromaticum* essential oils incorporated into denture soft liner.

Denture soft liners, when mixed with antifungal agents - synthetic and natural, showed satisfactory inhibition of *Candida albicans* 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 achieved.

CONCLUSION

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The following conclusions were drawn from the study:

- Antifungal incorporation into soft liner whether natural (*Cinnamomum zeylanicum* and *Syzygium aromaticum)* or synthetic is beneficial in preventing denture stomatitis.
- Fluconazole incorporated soft liner was found to be the better antifungal agent with potent activity against *candida albicans* followed by soft liner incorporated with *Cinnamomum zeylanicum* essential oil and the least effect is seen with soft liner incorporated with *Syzygium aromaticum* essential oil
- There is a gradual decrease in the antifungal activity from day 1 to day 3 and to day 7.This decrease was more evident for Fluconazole incorporated soft liner discs when compared to those incorporated with essential oils.

To summarize, the present study indicates that incorporating antifungals into soft liner showed positive results in inhibiting *candida albicans* .This would be beneficial in limiting candidiasis to an extent, considering the repeated change of soft liner over a period of 2 weeks.The natural antifungal agents used in this study provide a cost effective treatment option and is highly relevant in the era with the increased number of developing drug resistant strains of any microorganism , in conjunction with the restricted number of commercially available antifungal drugs that still present many side effects , and the incresed cost of treatment. The focal points of these natural agents is that they have multiple microbial targets enabling them to show wide range of antimicrobial action with little or no event of antimicrobial resistance.So, incorporation of *Cinnamomum zeylanicum* EO and *Syzygium aromaticum* EO is a promising alternative for the treatment of denture stomatitis due to biofilm inhibition and anti -Candida effects. 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.

Because of the antimicrobial effects of some medical plants, which have minimal side effects in comparison with chemical drugs, more in vivo and in vitro investigations about oral cavity flora and antibacterial and antifungal effects of different plants on oral bacteria and fungi should be recommended.

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ANNEXURES

ANNEXURES

Species: *Candida albicans* standard strain

Type of soft liner disc: Group 1 - PLAIN SOFT LINER DISC

Zone of inhibition (mm)			
Sl.No:	**Day 1**	**Day 3**	**Day 7**
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	0	0	0
7	0	0	0
8	0	0	0
9	0	0	0
10	0	0	0
11	0	0	0
12	0	0	0
13	0	0	0
14	0	0	0
15	0	0	0

Species: *Candida albicans* standard strain

Type of soft liner disc: Group 2: *Cinnamomum zeylanicum* essential oil incorporated soft liner disc.

Zone of inhibition(mm)			
Sl.No:	**Day 1**	**Day 3**	**Day 7**
1	20.8	16.6	16.2
2	18.2	15.4	13.2
3	21.2	16.8	14.8
4	22.4	18	16.8
5	18.6	15.4	13.6
6	20.8	16.2	14.4
7	21.8	16.8	14.6
8	22.4	18	16.6
9	21.6	16.6	14.2
10	22.8	18.2	16.8
11	20.8	15.8	13.6
12	22.6	18.8	17.2
13	21.8	17.4	15.2
14	20.6	16.2	14.2
15	21.8	16.6	14.4

Species: *Candida albicans* standard strain

Type of soft liner disc: Group 3 - *Syzygium aromaticum* essential oil incorporated soft liner disc

Zone of inhibition(mm)			
Sl.No:	**Day 1**	**Day 3**	**Day 7**
1	8.4	7.6	6.2
2	8.6	7.8	6.2
3	7.8	7	6
4	9	7	5.2
5	8.8	7.2	5.4
6	7	6.8	5.2
7	8	7	6
8	8.8	8	6.2
9	10	7.8	5.8
10	8	7.2	5.4
11	7.8	7	5.4
12	9.8	8.2	6.2
13	8.2	7	6
14	8.8	7.6	6
15	9	8	6.2

Species: *Candida albicans* standard strain

Type of soft liner disc: Group 4 - Soft liner discs incorporated with Fluconazole.

Zone of inhibition(mm)			
Sl.No:	**Day 1**	**Day 3**	**Day 7**
1	30.2	25.2	20.4
2	28.8	23.8	19.8
3	32	25.6	20.8
4	28.6	23.6	19.6
5	28	24.2	18.4
6	29	24.8	18.6
7	29.2	24.6	18.4
8	29.6	24.8	18.6
9	28.6	23.6	18
10	28.2	24.8	18.2
11	29.4	24.4	18.6
12	29.2	26.2	21.2
13	28.8	24	18.2
14	30.2	25	19.2
15	28.8	23.2	18.8

ST. GREGORIOS DENTAL COLLEGE

UNDER THE MANAGEMENT OF MJSCE TRUST, PUTHENCRUZ

CHELAD , KOTHAMANGALAM, ERNAKULAM DIST, KERALA - 686681

SGDC/152/2019/3726

15/11/2019

ETHICAL CLEARANCE CERTIFICATE

To,

Dr.Ann George
St.Gregorios Dental College
Chelad, Kothamangalam

Dear Dr.Ann George,

Subject: Ethics Committee Clearance - reg.

Protocol: A Comparative evaluation of the antifungal activity of the essential oils of *Cinnamomum zeylanicum* and *Syzygium aromaticum* incorporated into denture soft liner: An in-vitro study.

At the Institutional Ethics Committee (IEC) held on 15th of November 2019, this study was examined and discussed. After consideration, the committee has 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.C.K.K Nair - Former BARC Scientist.
2) Dr.Cinu Thomas A - Scientist, Senior lecturer, Department of Pharmaceutical Sciences Centre for Professional and Advanced Studies.
3) Dr.Lissy Jose - Former member Women's Welfare Association.
4) Adv. Jose Aranjani - Advocate.
5) Dr.Sauganth Paul - Reader, Department of Biochemistry, St.Gregorios Dental College.
6) Dr.Eapen Cherian - Secretary.
7) Dr.Jain Mathew - Principal and Head of the Department, Department of Conservative Dentistry and Endodontics.
8) Dr.George Francis - Head of the Department, Department of Prosthodontics and Crown & Bridge.
9) Dr.Binoy Kurian - Head of the Department, Department of Orthodontics &Dentofacial Orthopaedics.

Dr. C.K.K 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

LIST OF ABBREVIATIONS USED

Abbreviations	Full Form
C.spps	*Candida species*
C.albicans	*Candida albicans*
^{o}C	Degree Celsius
µm	Micrometer
%	Percentage
ZOI	Zone of Inhibition
EO	Essential oil
µl	Microlitre
µg	Microgram
mm	Millimetre
Fig	Figure