"EVALUATION OF ANTIFUNGAL ACTIVITY AGAINST *CANDIDA ALBICANS*, WATER SORPTION AND SOLUBILITY OF A SOFT LINER INCORPORATED WITH ROOT EXTRACT OF LICORICE (*GLYCYRRHIZA GLABRA*) -AN IN VITRO STUDY"

BY

REG. NO- IM0221005

Dissertation

Submitted to
KLE Academy of Higher Education and Research
Belagavi, Karnataka
In partial fulfillment of the requirements for the degree of

MASTER OF DENTAL SURGERY

In

PROSTHODONTICS AND CROWN & BRIDGE
(BRANCH – I)

DEPARTMENT OF PROSTHODONTICS
AND CROWN & BRIDGE
KAHER V.K. INSTITUTE OF DENTAL SCIENCES,
BELAGAVI, KARNATAKA.

2021 – 2024

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH
KLE V.K. INSTITUTE OF DENTAL SCIENCES,
BELAGAVI, KARNATAKA

Endorsement by the HOD, Principal/ Head of the Institution

This is to certify that this dissertation entitled **"EVALUATION OF ANTIFUNGAL ACTIVITY AGAINST *CANDIDA ALBICANS*, WATER SORPTION AND SOLUBILITY OF A SOFT LINER INCORPORATED WITH ROOT EXTRACT OF LICORICE (*GLYCYRRHIZA GLABRA*) -AN IN VITRO STUDY"** is a bonafide research work done by **REG. NO- IM0221005.**

Head of Department

Dr. RAMESH P. NAYAKAR M.D.S.
Professor & Head,
Department of Prosthodontics
and Crown & Bridge,
KAHER Vishwanath Katti Institute
of Dental Sciences, Belagavi.
Date: 23/04/2024
Place: Belagavi

Dr. Ramesh Nayakar
Consultant : Regd.No. I
Prosthodontics Crown and
KLE VK IDS, Belgaum

Principal

Dr. ALKA D. KALE M.D.S., Ph.D.
Principal,
KAHER Vishwanath Katti Institute of
Dental Sciences,
Belagavi.

Date: 23/04/2024
Place: Belagavi

PRINCIPAL
KLE V.K. Institute of Dental Sciences
Nehru Nagar BELAGAVI-590010

BIOSTATISTICS CLEARANCE CERTIFICATE

K L E VISHWANATH KATTI
INSTITUTE OF DENTAL SCIENCES
(A Constituent unit of KLE Academy of Higher Education & Research
(Formerly known as KLE University) Deemed-to-be-University u/s 3 of the UGC Act, 1956)
J.N.M.C. Campus, Nehru Nagar, Belagavi-590 010, Karnataka, India
Accredited 'A+' grade by NAAC (3rd Cycle) Placed in Category 'A' by MHRD (GoI)
☎: 0831-2470362
FAX: 0831-2470640
Web: http://www.kledental-bgm.edu.in
E-mail : principal@kledental-bgm.edu.in

Biostatistics Clearance Certificate

This is to certify that Biostatistics aspect of the Dissertation/Research work of **REG. NO- IM0221005** **Graduate Student,** under the guidance of **Department of Prosthodontics and Crown and Bridge,** entitled **"Evaluation of antifungal activity against Candida albicans, water sorption and solubility of a soft liner incorporated with Root extract of Licorice (Glycyrrhiza glabra) - an in vitro study."** has been done under my guidance and considered satisfactory.

Place: Belagavi

Date: 01-04-2024

01/04/2024

Name & Signature of Biostatistician

Ms. Anjali J. Patil
Lecture in Statistics
Dept. of Community Medicine
J.N. Medical College, Belagavi

UNDERTAKING

I, **REG. NO- IM0221005,** Post-Graduate student in the subject of **Prosthodontics and Crown and Bridge** have completed research work on the topic **"EVALUATION OF ANTIFUNGAL ACTIVITY AGAINST *CANDIDA ALBICANS*, WATER SORPTION AND SOLUBILITY OF A SOFT LINER INCORPORATED WITH ROOT EXTRACT OF LICORICE (*GLYCYRRHIZA GLABRA*) -AN IN VITRO STUDY",** in the year **2021-2024**.

I have been given to understand that any research work I undertake for the purpose of dissertation, oral presentation or publication during my study course shall be the property of the *KAHER Vishwanath Katti Institute of Dental Sciences, Belagavi.* Hence, I hereby declare that the name of the Department, Institute and University shall be mentioned in my publications. The authorship shall be according to the guideline informed to me.

Date: **Signature of the Post-Graduate Student**

Place: Belagavi

UNDERTAKING

I, **REG. NO- IM0221005,** hereby declare that the information and the data mentioned in my thesis entitled **"EVALUATION OF ANTIFUNGAL ACTIVITY AGAINST *CANDIDA ALBICANS*, WATER SORPTION AND SOLUBILITY OF A SOFT LINER INCORPORATED WITH ROOT EXTRACT OF LICORICE (*GLYCYRRHIZA GLABRA*) -AN IN VITRO STUDY",** belongs to me and is original. I am aware of the definition of plagiarism as detailed below:

- An act or instance of using or closely imitating the language and thoughts of another author without authorization and the representation of that author's work as one's own, as by not crediting the original author.
- A piece of writing or other work reflecting such unauthorized use or imitation.
- The deliberate or reckless representation of another's words, thoughts or ideas as one's own without attribution in connection with submission of academic work, whether graded or otherwise.

I hereby declare that the thesis prepared by me is original one and does not involve plagiarism any here. In case at a later stage, it is found that I have indulged in plagiarism, then I am solely responsible for the same and the institution is at liberty to take any disciplinary action against me including cancellation of dissertation or any other penalties imposed by the university.

Date: **Signature of the Post-Graduate Student**

Place: Belagavi **REG. NO- IM0221005**

LIST OF ABBREVIATIONS USED IN THE STUDY

ABBREVIATIONS	FULL FORMS
C. albicans	*Candida albicans*
G. glabra	*Glycyrrhiza glabra*
DS	Denture stomatitis
SE	Standard error
SD	Standard deviation
MIZ	Minimum inhibition zone diameter
DIZ	Diameter of inhibition zone
mm	Millimeter
ml	Milliliter
µg	Microgram
gm	Grams
mm^3	Cubic millimeter
hrs.	Hours
^0C	Degree centigrade
%	Percentage
GLM	Generalized Linear Models
SPSS	Statistical Package for Social Science

ABSTRACT

STATEMENT OF PROBLEM

Denture-induced Candidiasis is mainly associated with infection caused by *Candida* species. The application of topical antifungal agents has become a challenge for geriatric denture wearers due to their reduced motor activity and loss of memory. Topical and systemic antifungal therapy requires patient compliance. Thus, the result or the outcome cannot be determined.

Continuous use of synthetic antifungal agents has resulted to have harmful effects on the patient's liver and kidney and has disadvantages like drug resistance. Excessive use of these synthetic drugs has increased the emergence of multidrug-resistant strains of microorganisms. Therefore, the use of natural and herbal drugs has come into picture that have fewer adverse effects. The root extract of Licorice *(Glycyrrhiza glabra)* has proven to be effective against *Candida albicans* hence it was incorporated into the soft liner.

PURPOSE

The aim of the study was to evaluate and compare the antifungal effect, water sorption and solubility of the denture soft liner incorporated with *Glycyrrhiza glabra.*

METHODS

A total of 228 samples were taken and they were divided into two groups: 84 samples to check for antifungal activity and 144 samples to check for water sorption and solubility. *Glycyrrhiza glabra* was incorporated into the soft liner at 5%, 10%, and 15% and checked for antifungal activity by measuring the zone of inhibition for

3, 7, and 14 days. The antifungal activity of the unmodified soft liner (control group) was also evaluated.

Water sorption and solubility was assessed at 1, 7, and 14 days for all the concentrations and the control according to the ISO specification #10139-2.

RESULT

The collected data was subjected to statistical analysis using dependent t-test, Generalized linear models (GLM) and mixed method models analysis. There was a statistically significant difference between the control and experimental group ($p < 0.05$) in antifungal property, water sorption and solubility. 15% *Glycyrrhiza glabra* showed maximum antifungal activity which gradually decreased over the period of 14 days with a zone of 20.79 mm on day 3, 15.0 mm on day 7 and 7.57 mm on day 14, Additionally, it had shown increasing water sorption and solubility as the concentration increased and days progressed.

CONCLUSION

Since the denture soft liner are intended to be used for shorter duration of time, incorporation of these antifungal agents is not contraindicated. Thus, incorporation of *Glycyrrhiza glabra* into the denture soft liner can prove to be beneficial to improve oral health status of the geriatric patients with cognitive disturbances, medically compromised conditions and reduced manual dexterity. But further research is required to assess the other physical properties and biocompatibility which could be affected with the addition of such agents.

KEYWORDS: Denture, Soft Liner, *Candida albicans*, *Glycyrrhiza glabra*, Licorice, Antifungal, Water Sorption, Solubility

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF GRAPHS

INTRODUCTION

Maintaining proper oral health and having a complete set of teeth are vital for overall well-being and quality of life. Edentulism is an enduring and irreversible ailment, often referred to as final indicator of oral-health diseases.[1] Despite advancements in preventive dental care, edentulism remain a substantial global health issue, particularly among the elderly population. Consequently, the need for full dentures is expected to persist as a significant demand and challenge in the foreseeable future.

The demand for prosthodontic treatments among the elderly and edentulous population is steadily increasing, especially in developed nations. Removable dentures have gained prominence in this context. According to a national dental survey in 2011, it was determined that 89% of adults aged 75 and older relied on removable dental prostheses for their oral care needs.[2]

Primary application of polymers (polymethyl methacrylate {PMMA}) in the field of dentistry was for the production of complete denture bases.[3] PMMA has become choice of material for fabrication of complete denture owing to its exceptional features. These attributes encompass its low density, aesthetic appeal, cost-effectiveness, ease of manipulation, and the capability to tailor its physical and mechanical properties to specific needs.[4] However, when exposed to the oral environment, the PMMA denture foundation is vulnerable to microbial colonization. This vulnerability is partially caused by the PMMA denture base's lack of ionic charge, which inhibits salivary defence molecules like histatins and defensins from adhering to the denture surface and encourages the growth of biofilms.

The primary microorganism frequently isolated from dentures is *Candida albicans*. Notably, microorganisms with higher hydrophobicity tend to adhere more readily. The formation of denture biofilm, combined with shifts in oral microflora, can result in local tissue irritation and denture stomatitis(DS).[5]

Denture stomatitis is a prevalent condition that commonly afflicts individuals who wear dentures. The oral mucosal areas covered by the denture exhibit inflammation and redness (erythema) in this situation. Numerous studies have indicated that a substantial proportion, potentially up to two-thirds or more, of removable complete denture wearers may experience denture stomatitis.[6] Various local factors contribute to the development of this condition, including denture porosity, surface-roughness, insufficient denture hygiene, and prolonged or night-time use of dentures.[5] While denture stomatitis has a multifaceted origin, the pathogenic response typically involves Candida infection, with *Candida albicans* being the predominant causative agent, seen as often as in 50-98% of the cases.[7]

Denture stomatitis can be treated with a variety of methods, including systemic and topical antifungal therapies, careful oral hygiene, cleaning and disinfecting dentures, replacing old dentures, treating anatomic irregularities, making sure occlusion is atraumatic, taking out dentures at night, and providing adequate nutrition.[7]

While systemic anti-fungal therapy is recommended for immunocompromised patients, it's important to note that these medications can carry potential risks, including hepatotoxic and nephrotoxic effects, as well as interactions with other drugs, which can exacerbate adverse systemic effects. Topical antifungal medications like nystatin and miconazole are frequently used to treat denture stomatitis.[8]

The challenges of conventional antifungal therapy are not solely linked to the inability to maintain therapeutic antifungal concentrations on denture surfaces. Other contributing factors to treatment failure include:

- Diminished antifungal effectiveness as a result of swallowing, tongue motions, and salivary flow.
- Patient noncompliance with antifungal therapy because of the stringent drug regimes, the expense of the related treatments, the taste of the topical medicines, and the ongoing usage of dentures.
- The continuous contact between contaminated internal denture surfaces and wounded mucosa, which damages supporting tissues and encourages mucosal re-infection, thereby extending the course of the pathology.[9]

Because of this, even after receiving standard therapy with topical and systemic antifungal medications, reinfection of the treated oral mucosa may occur. Additionally, the remarkably high rates of clinical relapse and recurrence within two weeks of treatment provide serious management issues for denture stomatitis.[7]

Tissue conditioning (TC) has traditionally found application in the treatment of denture stomatitis by relieving inflamed tissues in the region where dentures are worn, particularly in cases where dentures do not fit properly. Materials referred to as soft liners help condition the tissues. These substances can be categorized as either temporary or permanent, depending on whether they are made up of silicone or acrylic resin, and they can be processed through either chemical or heat polymerization methods.[11] The viscoelastic properties of this substance create a cushioning effect that enhances both the chewing experience and the even distribution

of occlusal forces on the tissues that support the denture.[10] Tissue conditioners offer various other prosthetic benefits, including their ability to secure overdenture bar attachments, support extraoral prostheses, distribute occlusal forces evenly, extend the lifespan of prosthetic devices, substitute the fitting surface of conventional rigid dentures, alleviate mucosal discomfort beneath hard dentures, enhance the rhythm of chewing movements, and compensate for the volumetric shrinkage of acrylic resin.[11]

In comparison to denture base materials made of acrylic resin, lining materials that are softer have demonstrated a greater vulnerability to microbial adherence. Their surface properties and the materials physical and chemical affinity, which allow them to interact with oral microbes, are credited with this heightened inclination.[12] The attachment of microorganisms to solid surfaces involves a two-step process. The first interactions result from surface free energies, while the subsequent interactions are facilitated by salivary or serum proteins, which offer binding sites for the adhesion of microorganisms.[13] The degradation of soft liners is primarily attributed to the leaching of plasticizers and the prolonged use of potent denture cleansers at high concentrations. Additional factors contributing to this issue include limited manual dexterity for prosthesis cleaning, memory loss concerns, and the naturally uneven surface of soft liners, which create conditions favourable for the chemical elements necessary for fungal colonization on dentures.[14]

The concept of introducing antifungal agents into soft liners, allowing for gradual release into the oral cavity, has been proposed as a strategy to mitigate biofilm buildup, hinder the colonization of *Candida albicans*, and contribute to the management of denture stomatitis. This approach eliminates the necessity for patients

to adhere to strict antifungal medication schedules, as it relies solely on the use of dentures. Moreover, the addition of drugs to denture liners breaks the cycle of reinfection through the prosthesis by preventing denture biofilms from contacting infected tissues. Since these drug-modified materials have a half-life of around 14 days, the amount of time needed to treat denture-induced stomatitis with them is similar to the amount of time needed to treat the condition with conventional topical antifungal medicines. As a result, denture stomatitis can be successfully treated in a rather short amount of time before switching to long-term liners or creating new dentures.[7]

The utilization of synthetic medications such as Nystatin, Fluconazole, Miconazole, and others in soft liners has been proposed to achieve an extended antifungal impact. Nevertheless, excessive or inappropriate use of synthetic antifungals is not without adverse effects and can lead to the development of fungal strains that are resistant to treatment.[15]

The growing interest in naturopathic therapies due to emergence of drug resistant strains has emphasised the importance of turning to natural remedies over conventional allopathic medicines. In many developing countries, medicinal plant extracts and essential oils such as Tea tree oil, Origanum oil, and Lemongrass essential oil have gained popularity as alternative solutions to health issues. These extracts have also shown superior safety and stability, qualities often absent in both organic and inorganic antimicrobial agents.[16]

Nevertheless, the inclusion of drugs at various concentrations can potentially compromise the physical, mechanical, or structural characteristics of flexible denture liners. Among the various mechanical attributes of these liners, sorption and solubility

stand out as particularly significant. Imbalance in these properties may result in elevated hardness and roughness leading to loss of superficial integrity and thus irritating the denture bearing areas.[17]

Thus, an in-vitro investigation was conducted to evaluate and compare the antifungal activity of licorice extract added to soft liner and its effect on water sorption and solubility of the soft liner.

NEED FOR THE STUDY

Soft-denture liners find application in both complete & partial removable dentures with the purpose of evenly dispersing functional loads across the tissues that support the denture. They serve as a cushioning mechanism, absorbing the impact generated during the chewing process and acting as a shock absorber. These materials are particularly advisable for scenarios involving uneven bone resorption, bony undercuts, thin atrophic mucosa, immediate prosthesis requirements, post-implant placement healing, as well as for patients experiencing bruxism and xerostomia.[18]

However, it is widely recognized that these substances are vulnerable to the colonization by bacterial and fungal species.[19] Surface roughness and material composition are acknowledged as the primary factors influencing the adhesion process.[20] The inherent imperfections in denture bases and the materials employed for denture relining create conditions where yeast cells can become trapped. All of these factors collectively contribute towards exacerbation of prosthetic stomatitis.

Denture stomatitis represents a pathological condition affecting the mucosa that supports dentures, primarily resulting from the irritation caused by improperly fitting dentures. It is characterized by widespread inflammation or reddening of the palate's mucosal surface beneath the denture, and it is more prevalent among complete denture wearers. The origin of denture stomatitis is often multifaceted; nevertheless, the primary etiological factor is the infection by Candida species, particularly *Candida albicans* (*C. albicans*). Alongside *C. albicans*, there are additional risk factors, including denture-related trauma, suboptimal oral and denture hygiene,

continuous or nighttime denture usage, xerostomia, and changes in salivary pH, all of which have been associated with the development of denture stomatitis.[15]

In the treatment of oral candidiasis, various antifungal medications, including nystatin, amphotericin, ketoconazole, miconazole, and others, have been integrated into tissue conditioners and have demonstrated promising results in restraining the growth of Candida. Nevertheless, the prolonged or improper use of these medications carries potential adverse effects, such as renal or hepatic toxicity, and may also lead to the emergence of drug-resistant pathogenic fungi.[15] In contrast, the use of natural and herbal remedies offers several advantages, including cost-effectiveness, easy accessibility, local sourcing, and minimal side effects when compared to synthetic antifungal agents. Recent trends indicate a growing preference for herbal medicine due to its natural origin and the relatively lower incidence of known side effects.[21]

Natural substances such as Tea tree oil (*Melaleuca alternifolia*), Origanum oil, and Lemongrass essential oil (*Cymbopogon citratus*) have been investigated for their potential as antifungal agents and have demonstrated positive results. Additionally, various inorganic antifungal agents, including silver zeolite, silver nanoparticles, and magnesium oxide, have been subjected to testing and evaluation.[15]

The herbaceous perennial shrub known as *Glycyrrhiza glabra*, or Licorice, is a member of the Leguminosae family and is native to Mediterranean regions and specific parts of Asia. The root of this plant serves as the most common source of Licorice, which finds applications in cosmetics, food, tobacco products, and both traditional and herbal medicine.[22]

Licorice has a long history of use in traditional medicine and is among the most frequently employed herbs. Active compounds derived from licorice exhibit various beneficial properties, including antitumor, antimicrobial, antiviral, anti-inflammatory, immunoregulatory, and more.[23]

Within the oral cavity, licorice is utilized in forms such as mouthwash, toothpaste, gel, and chewing gum to prevent and address oral infections, including dental caries, periodontal diseases, oral candidiasis, and recurrent aphthous ulcers. This is due to its substantial antimicrobial effectiveness against pathogens such as *S. mutans*, *C. albicans*, *A. actinomycetemcomitans*, and *P. gingivalis*.[22]

The antifungal characteristics of licorice can be attributed to two of its bioactive compounds, Licochalcone A and Glabridin, which act by inhibiting yeast-hyphae transition and biofilm formation—two crucial factors contributing to the virulence of Candida.[24]

Incorporating drugs into resilient liners can potentially have adverse effects on their physical, mechanical, and structural characteristics. Among the mechanical properties of resilient liners, special consideration is warranted for sorption and solubility, as these factors are closely tied to the most common issues encountered in the presence of oral fluids. While in active use, these liners come into contact with saliva inside the oral cavity, and when not in use, they are exposed to water or aqueous cleaning solutions. Sorption is the simultaneous process of diffusing liquids from the surrounding environment into the substance in order to fill the spaces between the polymer chains. Moreover, the solubility of plasticizers and alcohol in resilient liners might cause a decrease in their viscoelasticity and impact-absorbing capabilities, which compromises their ability to offer the required comfort.

Consequently, the use of interim resilient liners can eventually lead to irritation in the areas where the denture contacts the mouth.[25,26]

Therefore, this study is undertaken to evaluate the antifungal activity and impact on water-sorption & solubility of the soft-liner when incorporated with Licorice extract.

HYPOTHESIS

NULL HYPOTHESIS:

- There is no difference in the anti-fungal activity, water-sorption & solubility of the denture soft liner incorporated with Licorice (*Glycyrrhiza glabra*).

ALTERNATIVE HYPOTHESIS:

- There is a difference in the anti-fungal activity, water-sorption & solubility of the denture soft liner incorporated with Licorice (*Glycyrrhiza glabra*).

AIM & OBJECTIVES

AIM OF STUDY:

- To evaluate the anti-fungal activity against *Candida albicans*, water-sorption & solubility of a denture soft liner incorporated with Licorice (*Glycyrrhiza glabra*) at different concentrations.

OBJECTIVES OF THE STUDY:

- To evaluate the anti-fungal activity against *Candida albicans* of a denture soft liner incorporated with Licorice (*Glycyrrhiza glabra*) at different concentrations.
- To evaluate the water-sorption & solubility of denture soft liner incorporated with Licorice (*Glycyrrhiza glabra*).
- To evaluate and compare the antifungal activity against *Candida albicans*, water-sorption & solubility of a denture soft liner incorporated with Licorice (*Glycyrrhiza glabra*) at different concentrations.

REVIEW OF LITERATURE

1) Sivakumar et al. (2013) explores the challenges associated with microbial colonization of Polymethyl methacrylate (PMMA) acrylic resin, extensively used in denture fabrication. Its susceptibility to microorganisms, notably *Candida albicans*and *Candida glabrata*, colonize dentures, leading to local issues such as denture stomatitis and systemic issues such as malodor, aspiration pneumonia and infectious endocarditis. Author further mentions ways to mitigate this colonization by incorporation of substances such as antimicrobial polymers, biocide-releasing polymers, and polymeric surface coatings.[5]

2) Neppelenbroek et al. (2016) highlights denture-induced stomatitis as a prevalent oral condition caused by Candida spp. colonization, emphasizing the need for effective treatments. Current therapies include topical and systemic antifungals, oral hygiene measures, and denture maintenance. Incorporating antifungal agents into denture materials offers potential benefits in sustained drug release and improved treatment outcomes. However, maintaining a balance between drug efficacy and material properties poses a challenge. More study on this is crucial to evaluate the biocompatibility and effectiveness of drug-incorporated denture liners before widespread implementation.[7]

3) Neppelenbroek et al. (2008) investigated the management of denture stomatitis, focusing on the efficacy of microwave disinfection and antifungal therapy. Before treatment, all patients' dentures harbored Candida spp.,

highlighting the importance of eliminating these microorganisms to manage oral infections. Microwave disinfection proved effective in eliminating Candida mycelial forms and improving palatal mucosa inflammation severity, suggesting its potential as a therapeutic option. However, miconazole used alone showed limited efficacy, possibly due to factors like saliva dilution and Candida resistance in biofilm models. The study underscores the significance of denture hygiene practices and emphasizes the need for further research to optimize treatment strategies for denture stomatitis.[8]

4) Schneid et al. (1992) aimed to evaluate the effects of combining tissue conditioners with four different antifungal agents on the mechanical properties and drug release capabilities of the material, with the ultimate goal of addressing denture stomatitis effectively. The researchers selected LynaP Tissue Conditioner and Temporary Reliner as the material for investigation. They incorporated four representative antifungal agents into the tissue conditioner: chlorhexidine, clotrimazole, fluconazole, and nystatin, each at varying concentrations. The results demonstrated that the addition of antifungal agents did not substantially change the handling properties of the tissue conditioner, while effectively enhancing tensile strength and inhibiting Candida growth in vitro.[9]

5) Tari et al. (2007) looked into the relationship between the main fungus that causes denture stomatitis, Candida albicans, and the materials used for soft denture linings.[12]

6) Nevzatoğlu et al. (2007) investigated the surface properties of denture materials and their impact on *Candida albicans* adherence. They examined four denture base acrylic resins and five silicone-based resilient liner materials, simulating clinical conditions. Surface roughness measurements indicated significant differences between materials, with acrylic resins showing lower roughness compared to resilient liners. *Candida albicans* adherence varied based on material type and surface finish, underscoring the role of surface characteristics in microbial colonization on denture materials.[13]

7) Muttagi et al. (2017) explored the effects of incorporating seed oils from *L. usitatissimum, O. sanctum,* and *C. anthelminticum* into soft liners. They examined parameters such as surface roughness, antifungal properties, wettability, glucose sorption, and weight change. Their findings demonstrated that the inclusion of these seed oils led to significant reductions in *C. albicans* growth, lowered surface roughness, decreased glucose absorption, and improved wettability of the soft liners.[14]

8) Iqbal et al. (2016) explored the existing understanding of antifungal agents used in tissue conditioners for managing denture-induced stomatitis (DS). They found that these agents are efficient and generally do not adversely affect the physical or mechanical characteristics of the tissue conditioners. Their analysis suggests that incorporating antifungal agents into readily available tissue conditioners holds promise for effectively addressing DS.[15]

9) Lima et al. (2016) studied the impact of adding minimum inhibitory concentrations (MICs) of antifungals to interim denture resilient liners on water sorption and solubility. They found that incorporating nystatin (Ny) and ketoconazole (Ke) at MICs did not significantly change water sorption after 14 days in water. Additionally, the solubility of both materials remained unaffected by Ny incorporation for up to 14 days. However, chlorhexidine (Chx) incorporation increased water-sorption & solubility in both materials contrast to the control. Overall, the study suggests that incorporating Ny and Ke at MICs could effectively treat denture stomatitis without compromising the liners' physical properties.[17]

10) Graham et al. (1991) conducted an in vivo study using two commercial denture liners, finding that both liners supported the presence and growth of oral fungi, with no significant difference observed between them. This suggests that denture liners should be used cautiously in patients with oral fungal colonization and not relied upon as standalone treatments for denture stomatitis. Further research is recommended to explore combination therapies involving antifungal medications.[19]

11) Gopalkrishna et al. (2016) investigated the antifungal efficacy of *Centratherum anthelminticum* and *Ocimum sanctum* seed oils against Candida species, prevalent in oral infections. Both oils exhibited strong inhibition of Candida growth, including the resistant strain *C. krusei*, with *Centratherum anthelminticum* demonstrating higher activity. The study highlighted the synergistic effects of various oil components, such as phospholipids and

unsaponifiable matter, in enhancing their antifungal properties. Further research is necessary to identify and isolate specific active ingredients for potential clinical applications in managing oral candidiasis.[21]

12) Messier et al. (2012) highlighted licorice root's potential benefits in addressing oro-dental diseases, owing to its diverse bioactive compounds like saponins and flavonoids. Studies suggest licorice's anti-inflammatory and antibacterial properties could be effective in inhibiting oral pathogens and reducing inflammation in conditions like periodontal disease and dental caries.[22]

13) Messier et al. (2011) explored *Candida albicans* role in oral candidiasis and denture stomatitis, emphasizing its virulence factors and resistance to antifungal drugs. Licorice root compounds, including licochalcone A and glabridin, demonstrate antifungal activity against *C. albicans*, inhibit biofilm formation, and disrupt yeast-hyphal transition. Additionally, these compounds exhibit synergy with the antifungal drug nystatin, suggesting their potential as adjunctive therapies for *C. albicans* infections, while maintaining low cytotoxicity towards oral epithelial cells.[24]

14) Garg and colleagues (2016) discovered that the denture liner material exhibited the maximum water-sorption in distilled water, 5.25% sodium hypochlorite, and the lowest water sorption in Shellis' artificial saliva during 4^{th}, 7^{th}, and 11^{th} day intervals. By day 15, however, the data showed a reversal, with fake saliva showing the least water sorption and distilled water and 5.25% sodium hypochlorite exhibiting the greatest. Furthermore, soft liner's

solubility increased with artificial saliva, it decreased with 5.25% sodium hypochlorite, & was highest in distilled water during the 4^{th}, 7^{th}, 11^{th}, and 15-day intervals.[26]

15) Hofling et al. (2011) conducted a study comparing the antifungal agents fluconazole & amphotericin-B with proteinase-inhibitors like pepstatin-A, amprenavirr, & ritonavirr, to assess their effects on the production of proteinases by *C. albicans* clinical isolates. They also looked into the effectiveness of plant extracts against C. albicans and its proteinases, such as Casearía Rosmarinus, Mentha, Tabebuia, Arrabidaea, and Arctium. The findings demonstrated the particular action of these extracts and suggested that investigating chemicals originating from plants may result in the development of new antifungal medications.[27]

16) Bhat et al. (2013) discussed oral inflammation associated with Candida, attributing its occurrence to the transformation of typical oral organisms like Candida into pathogens under conducive conditions. Factors such as immunocompromised status, poorly fitting prostheses, systemic illnesses, and inadequate denture care by patients contribute to this imbalance, rendering the oral environment unsuitable for prosthesis wear. Globally, the prevalence of denture stomatitis due to Candida fungus ranges between 65 and 70% among denture users, with *C. albicans* traditionally identified as the primary causative species, though there has been a recent trend toward non-*Candida albicans* species.[30]

17) Kumar et al. (2018) examined the inhibition of *C. albicans* growth in resin-based denture soft lining materials treated with neem or garlic through an in vitro study. Using the streaking method at 2, 4, and 7 days, they found that both neem and garlic application on an acrylic soft liner inhibited *C. albicans*. Compared to the control group, neem and garlic showed promising outcomes in combating *C. albicans*. This study suggests that neem and garlic could serve as potential additions to tissue conditioners to reduce *C. albicans* adhesion, based on the findings of this in vitro investigation.[31]

18) Krishnamoorthy et al. (2019) carried out a study to evaluate the antifungal effectiveness and tensile strength of tissue conditioners containing Cocos nucifera oil. Their findings demonstrated that the addition of 10% w/w Cocos nucifera oil to Viscogel tissue conditioner resulted in decreased Candida colonization and enhanced tensile strength of the tissue conditioner.[32]

19) Abdallah et al. (2021) conducted a study on denture stomatitis (DS), a prevalent oral condition associated with Candida species infection and ill-fitting dentures. They investigated the effectiveness of curcumin, a natural antimicrobial agent, incorporated into denture liners. The study found that curcumin-modified denture liners demonstrated reduced surface roughness, improved bond strength, and concentration-dependent antifungal activity against Candida species, suggesting curcumin as a potential therapeutic option for DS.[34]

20) Sri et al. (2023) investigated the addition of Glycyrrhiza glabra (licorice) to soft denture liners to enhance their antimicrobial and anti-inflammatory properties. Their findings revealed evident antibacterial action, particularly at higher doses of G. glabra, against common oral infections such as Lactobacillus, Pseudomonas, Candida albicans, and Streptococcus mutans. Additionally, the study observed anti-inflammatory effects, indicating potential benefits for oral health. Notably, the modification did not compromise the the soft liners' mechanical and physical characteristics, such as their wettability, tensile bond strength, & surface roughness. These results suggest that G. glabra-incorporated soft-liners could offer improved therapeutic effects without compromising material integrity, making them a promising option for denture and maxillofacial prosthesis applications.[35]

21) Asl et al. (2008) extensively discussed the anti-inflammatory and antimicrobial properties of licorice. Glycyrrhizin, a major component of licorice root, has been shown to inhibit the replication of various viruses in vitro. Additionally, licorice contains flavonoids such as glabridin, glabrene, and licochalcone A, which exhibit anti-microbial activity against *H. pylori* & methicillin-resistant *S. aureus*. Their findings suggest that licorice possess promising anti-microbial properties.[36]

22) In an attempt to strengthen the antifungal qualities of a short-term denture soft liner against Candida albicans, Kumarich et al. (2020) looked into using Piper beetle extract.[37]

23) Bulad et al. (2004) investigated the retention, inhibition, and penetration of *Candida albicans* into various denture lining materials using in vitro methods. This involved testing different denture lining materials, including silicone elastomers and soft acrylic compounds, for their ability to inhibit *C. albicans* growth and retain the fungus on their surfaces. Results showed no significant inhibition of *C. albicans* growth by the materials, with varying degrees of adhesion and penetration observed, influenced by material composition and surface characteristics.[38]

24) Kawano et al. (1994) examined the solubility and sorption characteristics of 12 denture liners, comprising 9 copolymers, 2 silicones, and 1 polyphosphazene fluoroelastomer (Soft-Pals, Justisoft, Velvesoft, Molloplast-B, Flexor, Novus, Durosoft, Vermo-Soft, Vinsoft Super Soft, ProTech, and Prolastic), over periods ranging from 1 week to 1 year. The study revealed that after 7^{th} day, Molloplast-B, Flexor, Prolastic soft, & Durosoft denture liners exhibited a sorption value of 0.8mg/mm². Interestingly, after one year, the sorption value decreased for Prolastic and Molloplast-B soft liners compared to the initial measurement.[43]

25) Parr et al (2002) conducted an investigation to assess the alterations in material properties of two novel resilient denture lining materials employing different polymerization methods (auto-polymerization and conventional laboratory processing). The findings indicated that the laboratory-processed material exhibited elevated stiffness values, which further increased after one

week of water immersion. After six months and one year, the water sorption rates for both products were found to be similar. Interestingly, at 30 days, six months, and one year, significantly lower solubility was observed for auto-polymerized resins.[44]

26) Dong et al. (2015) explores the medicinal and industrial potential of licorice root, focusing on its bioactive compounds, such as triterpene glycyrrhetic acid (GA) and flavonoids (LF). The study investigates the adsorption and desorption behaviors of macroporous resins in extracting flavonoids from licorice leaves, finding that these resins exhibit comparable adsorption capacities and are influenced by factors like ethanol concentration and temperature.[47]

27) The solubility and water sorption of two soft liners, Viscogel (an acrylic-based soft liner) and Mollosil (a silicone-based soft liner), were evaluated and compared by Jawbal et al. (2017) in artificial saliva and distilled water at intervals of seven days, thirty days, and three months. They came to the conclusion that, in both artificial saliva and pure water, Viscogel showed higher rates of absorption and solubility than Mollosil at all times.[49]

28) Kazanji et al. (1988) investigated the impact of resilient denture liner materials' on their level of softness, focusing on clinical relevance. Findings suggest that thickness significantly impacts softness up to 1.8 mm, while

boxing-in shows minimal clinical significance on softness but potentially improves material bonding. Additionally, storage affects softness, with plasticizer leaching potentially altering material properties over time.[50]

MATERIALS AND METHODOLOGY

SOURCES FOR DATA ASSISMILATION:

This study was conducted in

1) KAHER's KLE VKIDS, Department of Prosthodontics and Crown & Bridge, Belagavi (for preparation of test specimens)

2) Department of Microbiology, Jawaharlal Nehru Medical College, Belagavi (formicrobiological procedures)

3) College of Pharmacy, Belagavi [for standardization of Licorice *(Glycyrrhiza glabra*) extract]

4) Dr. Prabhakar Kore's KLE VK IDS, Belagavi (for water- sorption & solubility)

INCLUSION CRITERIA

1) Specimens of identical dimensions.

2) Specimens free of voids.

EXCLUSION CRITERIA

1) Specimens of inaccurate dimensions.

2) Specimens with gross visual porosities.

ESTIMATION OF SAMPLE SIZE

The following formula was used to check the sample size:

$$\boldsymbol{n = \frac{\left(z_{1-a/2} + z_{1-\beta}\right)^2 \left(SD_1^2 + SD_2^2\right)}{(\bar{x}_1 - \bar{x}_2)^2}}$$

At 95% confidence level, $Z_{1-\alpha/2} = 1.96$

At 95% power $Z_{1-\beta} = 1.64$

n = Sample size

Antifungal group

Standard deviation in the 1st group, S1 = 0.83

Standard deviation in the 2nd group, S2 = 0.54

$\bar{x}_1$ = 19.8

$\bar{x}_2$ = 19.4

n = 80

Water sorption & Water solubility

Standard deviation in the 1st group, S1 = 12.1

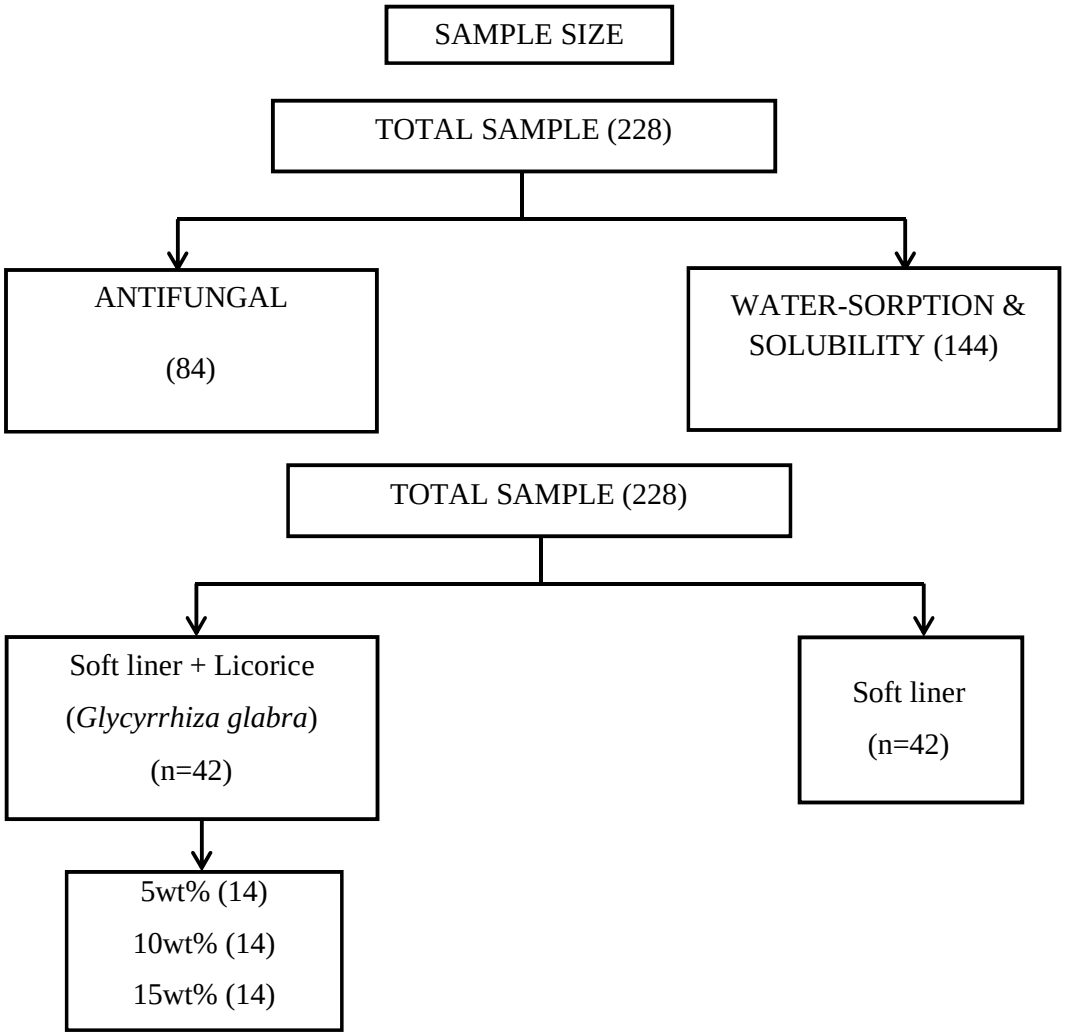
Standard deviation in the 2nd group, S2 = 21

$\bar{x}_1$ = 53.1

$\bar{x}_2$ = 60.6

n = 136

Sample size for Antifungal activity



Zone of inhibition was checked at the end of 3rd day, 7th day and 14th day.

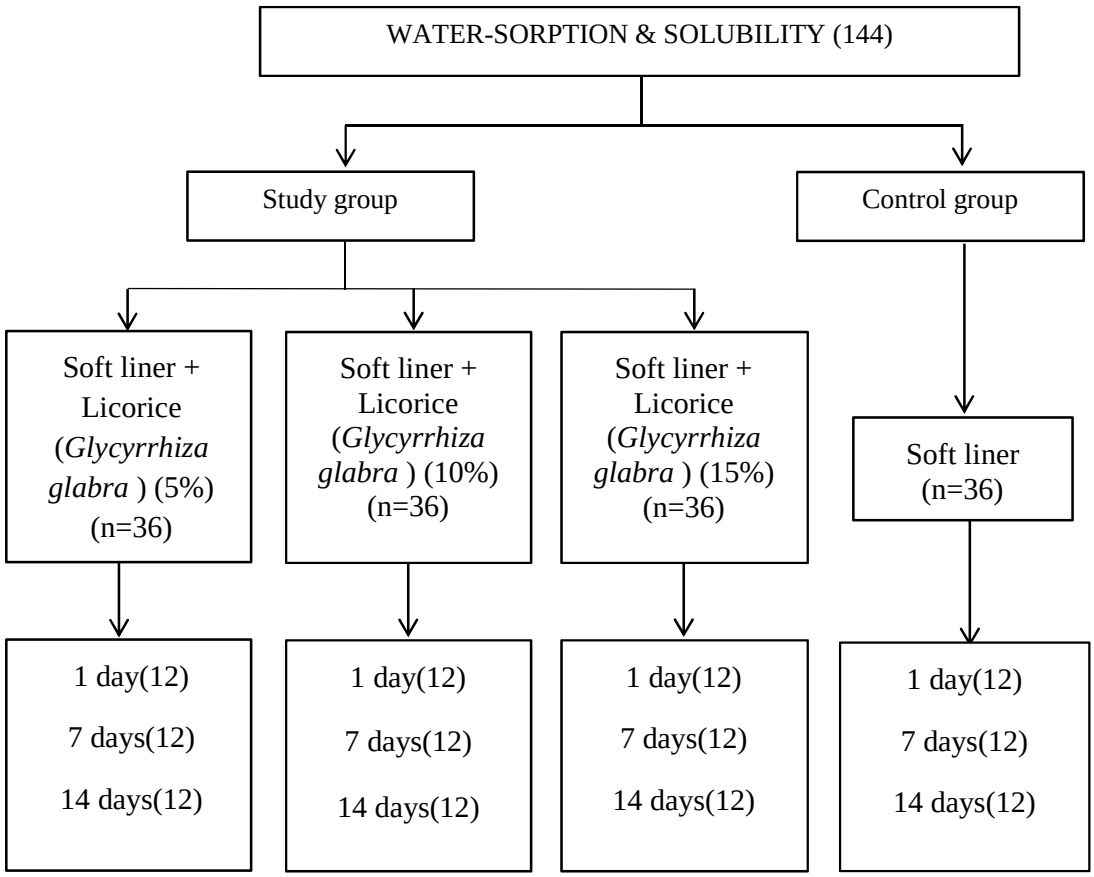
Total of 84 samples were taken to assess the antifungal efficacy. The samples were divided into 2 groups. They are:

Group 1: Control group(soft liner powder and liquid)

Group 2: *G. glabra* (licorice) incorporated in soft liner

Group 2 was further divided into 3 subgroups of concentration 5%(2a), 10%(2b) and 15%(2c) concentration with 14 samples each.

Sample size for water-sorption & solubility



To check for water sorption and solubility, samples were distributed into 4 groups of 36 samples each.

Group 1: Control group(soft liner powder and liquid)

Group 2: *G. glabra* (licorice) incorporated in soft liner

Group 2a: 5% *G. glabra* (licorice) incorporated in soft liner

Group 2b: 10% *G. glabra* (licorice) incorporated in soft liner

Group 2c: 15% *G. glabra* (licorice) incorporated in soft liner

MATERIALS USED IN STUDY

MATERIALS	DESCRIPTION	MANUFACTRER
G. Glabra (Licorice)	Powder extract	Natural Remedies Private Ltd.
GC Soft liner	1609031	GC Corp. Tokyo, Japan
Sabouraud Dextrose agar	LOT D18JI4200-TR-13C	Hi-media, Mumbai
Candida albicans strain	90028	MTCC No. 2091
Petri plates	PW011	Hi-media
Absorbent papers	-	India mart
Silica gel	Silica gel packs	Silica gel products mfg.co, Gujarat

ARMAMENTARIUM:

- Bacteriological incubator – Biotechnics India (BTI-25)
- Digital analytic balance: UniBloc (AUW220D) and Kern and Sohn GmbH-(240-3N)
- Desiccator (ABG Initiative, BO79555LFD)
- Disk shaped metal molds of dimension 50mmx0.5mm
- Eppendorf microtiter pipette (1000 and 100 µl)
- Mixing jar and spatula – Prime Dental Products Pvt. Ltd.
- Sterile cork borer
- Dappen dish
- Metallic scale
- Glass beaker

DETAILS OF THE PROCEDURES CONDUCTED DURING THE RESEARCH

TO CHECK FOR THE ANTIFUNGAL PROPERTY IN DENTURE SOFT LINER:[51]

Candida albicans (ATCC 90028) strain was obtained from the Department of Microbiology, Jawaharlal Nehru Medical College, Belagavi. The obtained strain was subcultured on Sabouraud dextrose agar plates and incubated at 37°C. The *C. albicans* suspension after 24hours of incubation was then mixed with sterile saline to a density of 0.5 McFarland to standardize the concentration.

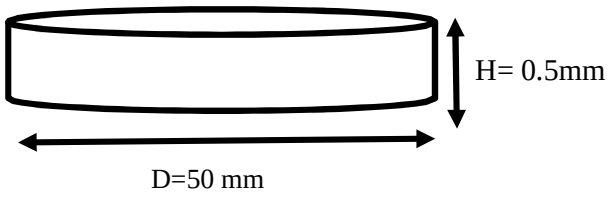
Well-diffusion method was used to evaluate the antifungal efficacy. Petri dishes of 90mm diameter were used and was filled with Sabouraud dextrose agar. *Candida albicans* inoculum was streaked on these culture plates using an inoculation loop. After the inoculum dries, wells of diameter 6mm and depth 5mm were made on the culture plates with a sterile cork borer. (A pilot study was conducted which concluded that, 5% of *G. glabra* in soft liner showed the minimum inhibition zone of 10mm against *Candida albicans*) Soft liner powder of different concentrations of 5%, 10%, 15%, and liquid were mixed to the ratio prescribed by the manufacturer for 30 seconds in a sterile jar using a sterile spatula. The punched wells were then packed with this mixture.14 samples were punched for each concentration (5%,10%,15%) and the same was done for the control.

Then the agar plates were placed in an incubator set at 37 degrees Celsius for 14 days. The diameter of the inhibition zone (DIZ) was measured at the end of the third, seventh and fourteenth day using a scale. The measured DIZ was subjected to statistical analysis.

TO CHECK FOR THE WATER-SORPTION & SOLUBILITY IN SOFT LINER:[17]

Master die fabrication

The specimens were made using a metal die that measured diameter of 50mm & thickness of 0.5mm. It was made in compliance with ADA spec. 12.



Sample fabrication

The soft-liner powder of control and of the different concentrations (5%, 10%, 15%) was mixed with the liquid as stated by the manufacturer. This mixture was then applied to the metal die. Thus 144 samples, 36 samples in each group (50mm in diameter and 0.5mm thick) were fabricated. After setting, the disks were submerged in artificial saliva and placed in an incubator at 37°C for 3 evaluation periods: 1 day, 7 days, and 14 days.

Water-sorption & Solubility assay:

Assays for solubility and sorption were conducted in compliance with ISO standard #10139-2. Specimens were placed in a silica gel-filled desiccator and kept in an incubator at 37°C to desiccate. Using a computerized analytical balance, each specimen was weighed every day until a consistent mass was achieved. When the difference between the average of each assessment interval was less than or equal to 0.0002g during a 24-hour period, the specimens' mass was deemed stable. Following the estimation of the initial mass (w1), the specimens were incubated for one day (24

hours), seven days, and fourteen days, with 36 samples each, in a sterile beaker containing 250 ml of artificial saliva at 37°C.

The artificial saliva was made-up of the following components: 0.1g NaCl, 0.1gKCI, 0.345g NaH2PO4, 0.198g $CaCl_2H_2O$, 0.25g urea. All the measured components were then mixed in 250ml of distilled water in a beaker and maintained at a pH of 7 using a pH meter. After that, absorbent papers were used to remove any remaining saliva from the specimens until there was no longer any obvious moisture present. After the specimens were taken out of the artificial saliva (w2), they were allowed to air dry for an additional fifteen seconds before being measured once more. It indicates the specimen's weight following the absorption of synthetic saliva. Lastly, all specimens underwent the previously outlined desorption process using silica gel once more, and they were weighed once more (w3). Water sorption values were determined using the following equations:

Water sorption (w2-w1)/V

Solubility values were determined using the following equations:

Solubility (w1-w3)/V

wl is the initial mass weighed after the first dry

w2 is the specimen mass after immersion in artificial saliva

w3 is the specimen mass after the second desorption process V is the volume of the specimen which is constant for all the specimens and is calculated using the formula V ar^3h, r is the radius, and h is the thickness of the specimen.

this, V = 981.75mm^3

The results obtained were then subjected to statistical analysis.

***Glycyrrhiza glabra* (Licorice) extract**

Extract *Glycyrrhiza glabra* (Licorice) is obtained from Natural Remedies, Bangalore.

natural REMEDIES
QUALITY CONTROL DEPARTMENT

Kosher certified

CERTIFICATE OF ANALYSIS

Product name	:	*Glycyrrhiza glabra* extract ≥ 12% Glycyrrhizin	**Batch No.**	:	FGG2102003
Botanical name	:	*Glycyrrhiza glabra*	**ULR No.**	:	TC400521000000126 5P
Product code	:	NRGGE12	**Lab Reference / Report No.**	:	FP2102034
Part used	:	Root	**Date of Report**	:	19.02.2021
Extract ratio	:	8 : 1	**Mfg. date**	:	February 2021
Solvent used	:	Methanol, water	**Retest date**	:	February 2023
Excipients	:	Nil	**Country of origin**	:	India
			Type of extract	:	Powdered extract
			Specification No.	:	NR/QCD/SSPC/GG12%/001

TEST RESULTS

SL. NO.	TESTS	SPECIFICATION	RESULT	TEST PROTOCOL
1.	Description	Light brown to brown, hygroscopic powder	Light Brown hygroscopic powder	Visual
2.	Identification	To pass the test	Passes the test	By TLC
3.	Loss on drying (%w/w)	< 5.0	3.7	As per USP <921> Method III
4.	Acid insoluble Ash (%w/w)	< 3.0	0.3	As per USP <561>
5.	Bulk density (g/cc)	0.20 – 0.80	0.47	As per USP <616> Method – I
6.	Tapped density	0.40 – 1.00	0.74	
7.	Material passing through 30# BS/35 ASTM (%w/w)	> 99.0	100	As per USP <786> Particle size distribution
8.	Heavy Metals			
	Lead	< 3.0 ppm	0.05	ICP –MS
	Arsenic	< 2.0 ppm	0.34	
	Cadmium	< 2.0 ppm	< 0.05	
	Mercury	< 0.1 ppm	< 0.05	
9.	Microbiology Test			
	Total aerobic microbial count	$<10^4$ cfu g^{-1}	*10*	As per USP <61> &<62>
	Total yeast and mould count	$<10^2$ org g^{-1}	*No growth*	
	Bile tolerant gram negative bacteria	$<10^2$ fs g^{-1}	*< 1*	
	E. coli	Absent/g	Absent	
	Salmonella species	Absent/10g	Absent	
	S. aureus	Absent/g	Absent	
10.	Aflatoxins (B_1+B_2+G_1+G_2)	< 5.0 ppb	Not Detected (LOD: 2ppb)	As per USP Test for Aflatoxins
11.	Residual solvent analysis Methanol (ppm)	< 3000	75	As per USP
12.	Pesticide residue analysis	To comply with USP <565>	Complies	As per AOAC/USP
13.	Phytochemical Analysis Glycyrrhizin (% w/w)	≥ 12.0	12.6	By HPLC

Remarks: The above referred batch conforms to the specification of *Glycyrrhiza glabra* extract (≥ 12%Glycyrrhizin) with respect to above mentioned tests.

Storage : The product should be stored in well closed containers, protected from light, moisture and heat, at a temperature between 18°C and 30°C.

AUTHORISED SIGNATORY
DATE:01.12.2021

Natural Remedies Private Limited
Regd. Office & R&D
CIN No: U24232KA1998PTC023573
5B, Veersandra Industrial Area, Hosur Road, Electronic City Phase 2, Bangalore 560100. Karnataka – INDIA
Tel: 91 8040209999/8/7,27382265, Fax: 91 80 40209817, Web: www.naturalremedy.com
E-Mail: qc@naturalremedy.com, info@naturalremedy.com

TO CHECK FOR THE ANTIFUNGAL PROPERTY

Candida albicans suspension will be obtained and antifungal susceptibility test will be done by well diffusion method.

⇩

Mueller-Hinton agar will be prepared and poured in 90mm diameter petri plates.

⇩

Culture plates will be streaked with inoculum and inoculum will be dried.

⇩

Wells will be punched (6mm diameter, 5mm depth) with sterile borer.

⇩

Mueller-Hinton agar will be prepared and poured in 90mm diameter petri plates.

⇩

Polymer of Group B will be mixed with monomer of soft liner according to manufacturer instruction and be placed into the punched wells at concentrations of 5%, 10%, 15%. Group A (GC Soft Liner) will be mixed same and act as a control.

⇩

Agar plates will be incubated at 37˚±1°C for fourteen days.

⇩

Diameter of inhibition zone will be measured with metallic scale after first, seventh and fourteenth day.

TO CHECK FOR THE WATER SORPTION AND SOLUBILTY[14]

Fabrication of the disk-shaped specimens of dimensions 50 mm×0.5 mm.

⇩

Specimens will be dried in a desiccator using silica gel at room temperature.

⇩

Disks will be weighed daily to an accuracy of 0.001g using a balancer.

⇩

Specimens will be weighed daily until a constant mass is obtained (0.002 g over 48 hours) w1.

⇩

Specimens will be subjected to desiccation again as previously done.

⇩

Specimens will be weighed after the specified time (w3).

⇩

Water-sorption & solubility will be calculated separately for water and artificial saliva using the formulae:
Water sorption = (w2-w1)/V Water solubility = (w1-w3)/V

Figure 1: Licorice (*Glycyrrhiza glabra*)

Figure 2: Soft liner

Figure 3: Soft liner with different concentration

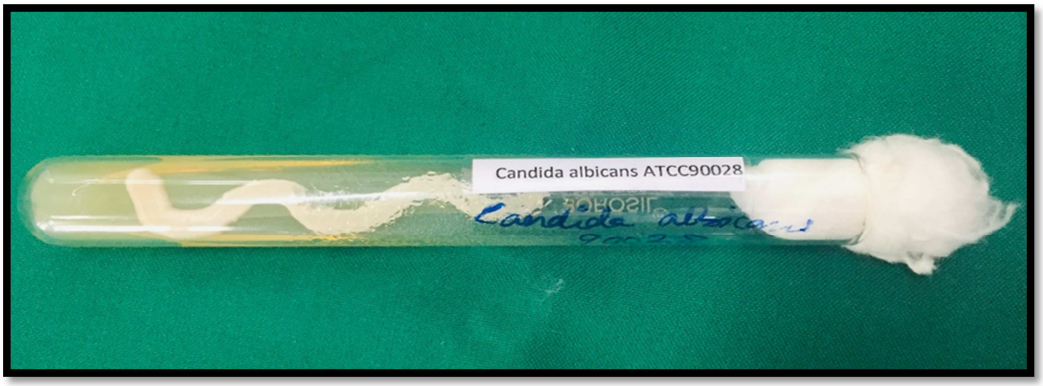


Figure 4: Candida albicans strain

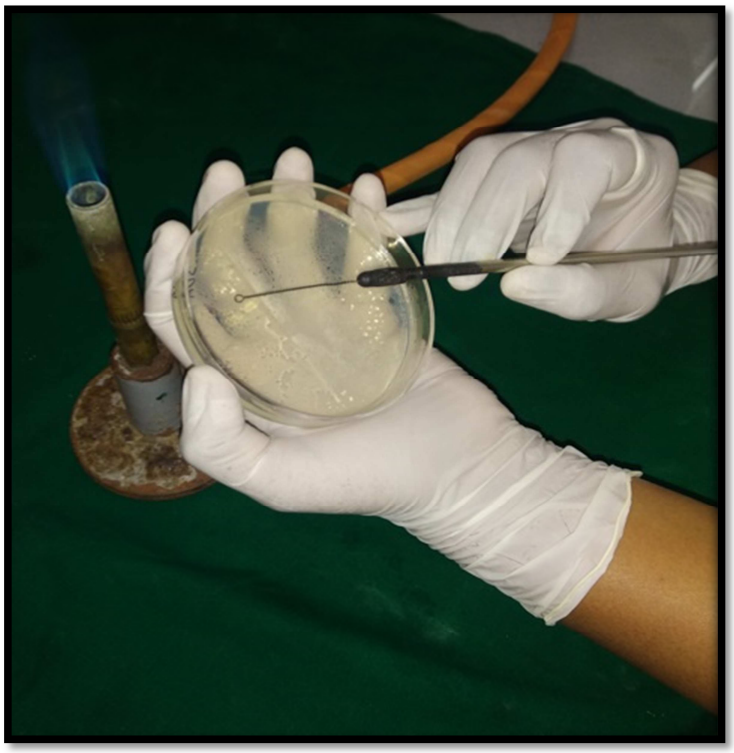


Figure 5: Streaking of Candida albicans inoculum on the Saboraud dextrose agar

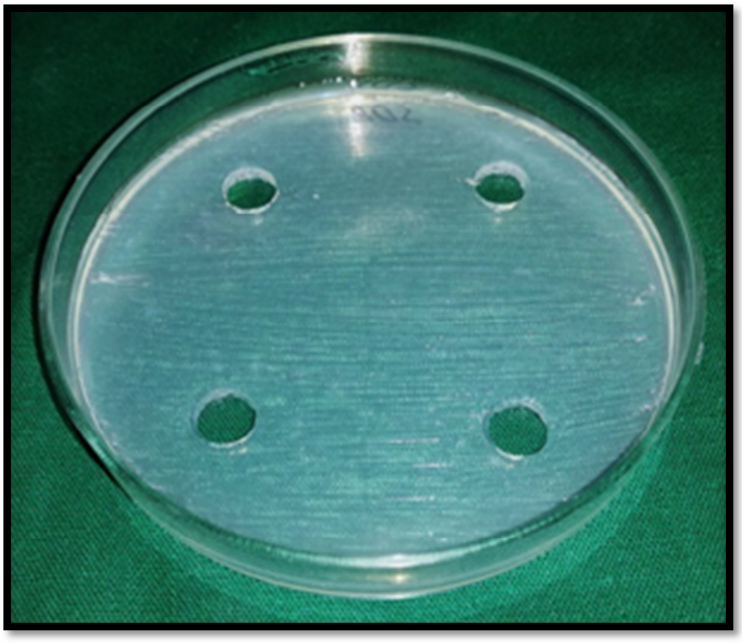


Figure 6: Wells of 6mm diameter and 5mm depth punched

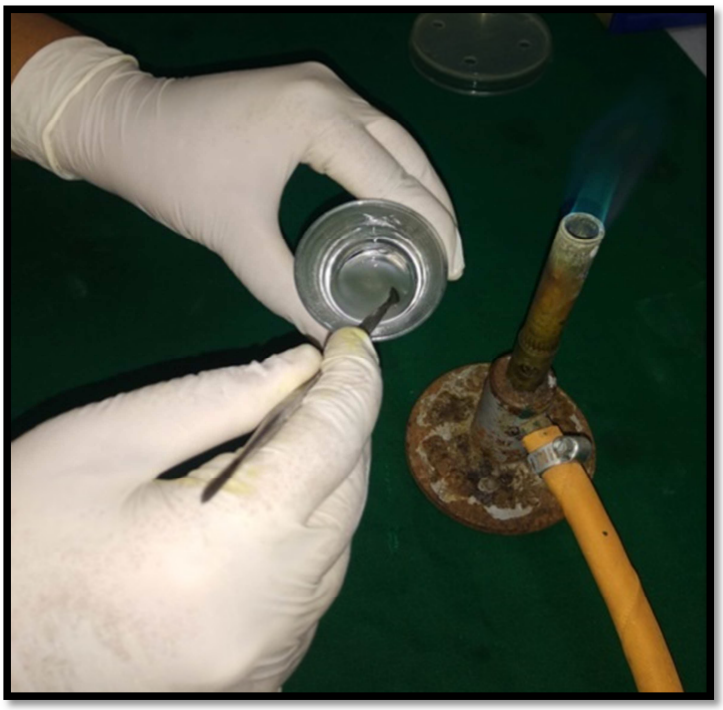


Figure 7: Soft liner mixed in a jar

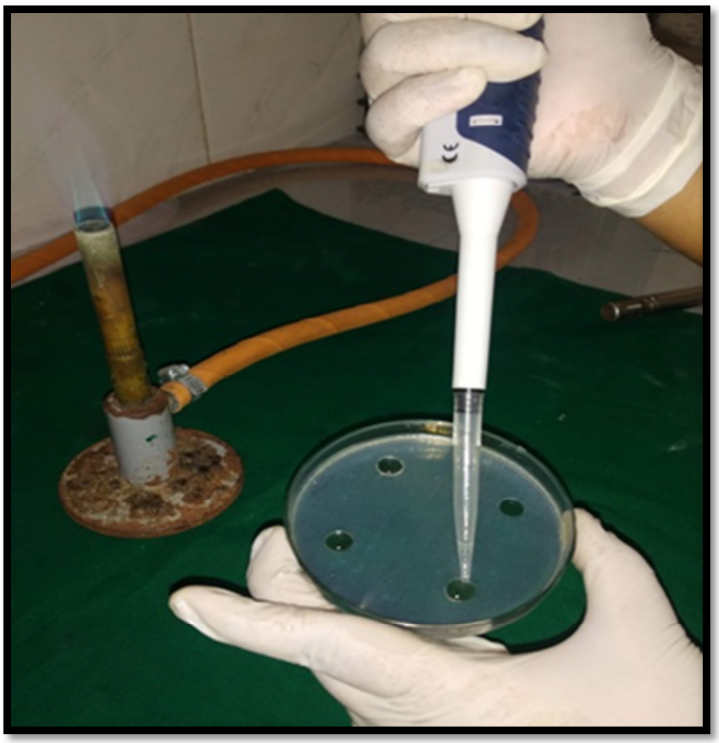


Figure 8: Punched wells filled with soft liner

Figure 9: Incubator

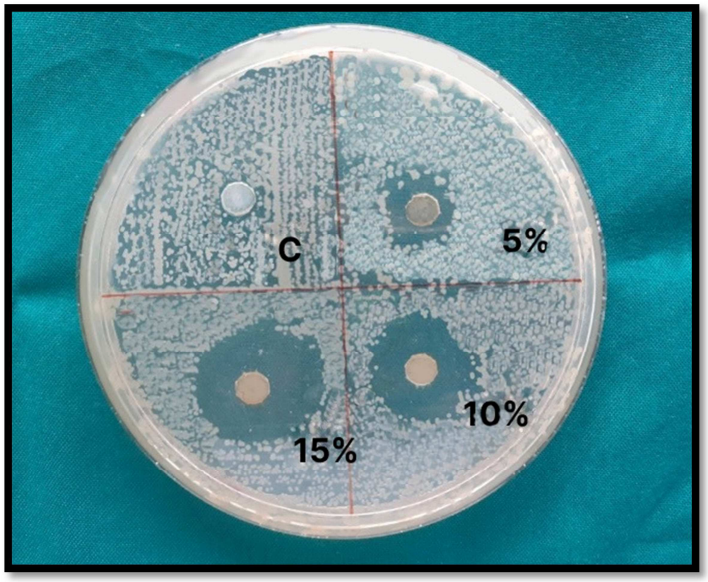


Figure 10: Soft liner with control, 5%, 10%, and 15% *G. glabra* on day 3

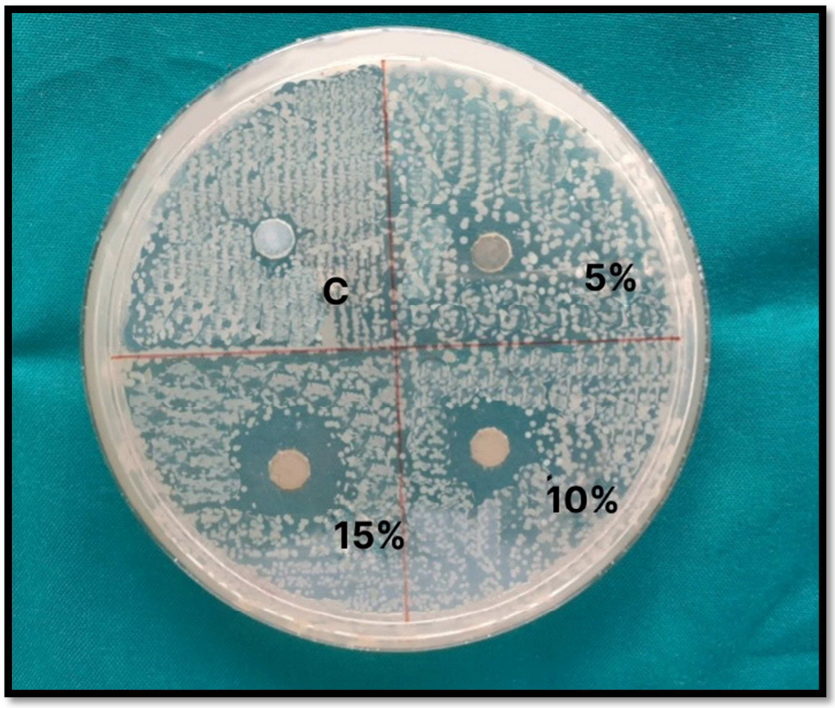


Figure 11: Soft liner with control, 5%, 10%, and 15% *G. glabra* on day 7

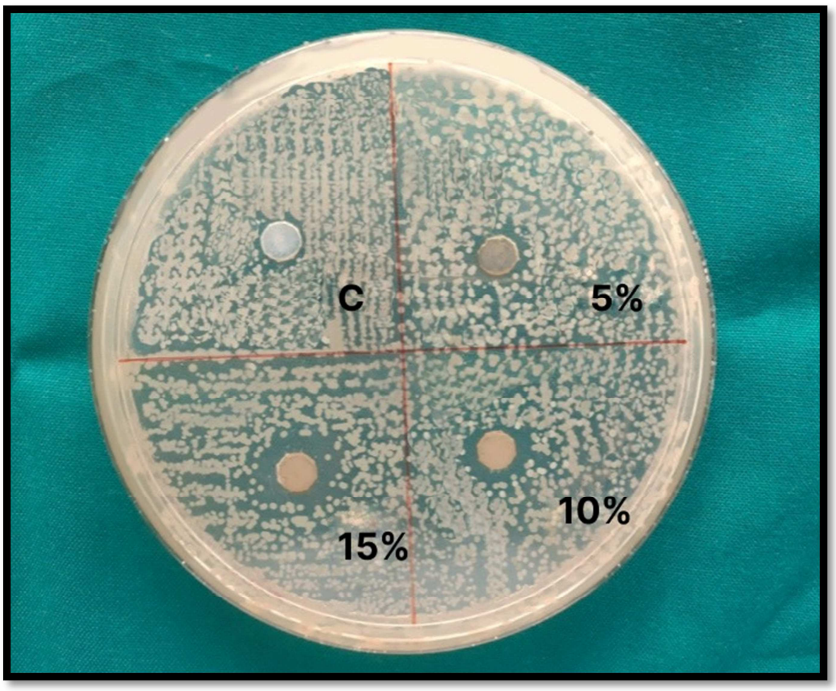


Figure 12: Soft liner with control, 5%, 10%, and 15% *G. glabra* on day 14

Figure 13: Specimens of controlgroup for water-sorption & solubility day 1

Figure 14: Specimens of controlgroup for water-sorption & solubility for day 7

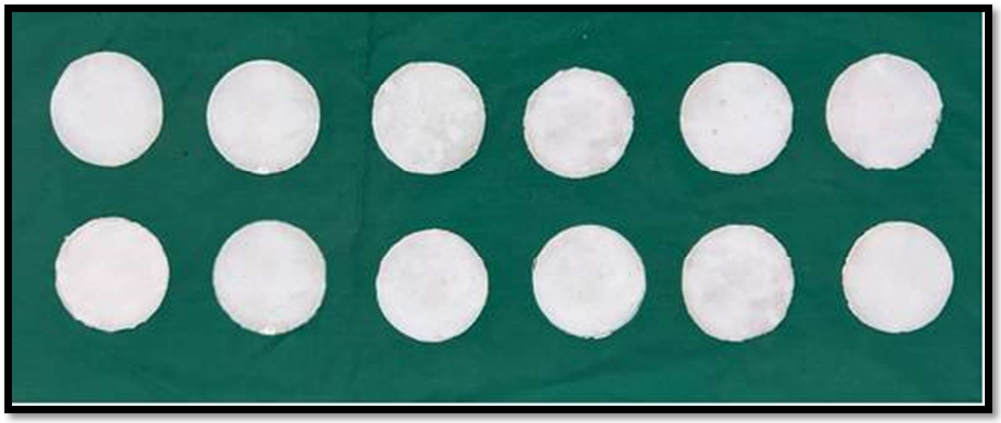


Figure 15: Specimens of controlgroup for water-sorption & solubility for day 14

Figure 16: Specimens of 5% *G. glabra* group for water sorption and solubility for day 1

Figure 17: Specimens of 5% *G. glabra* group for water sorption and solubility for day 7

Figure 18: Specimens of 5% *G. glabra* group for water sorption and solubility for day 14

Figure 19: Specimens of 10% *G. glabra* group for water sorption and solubility for day 1

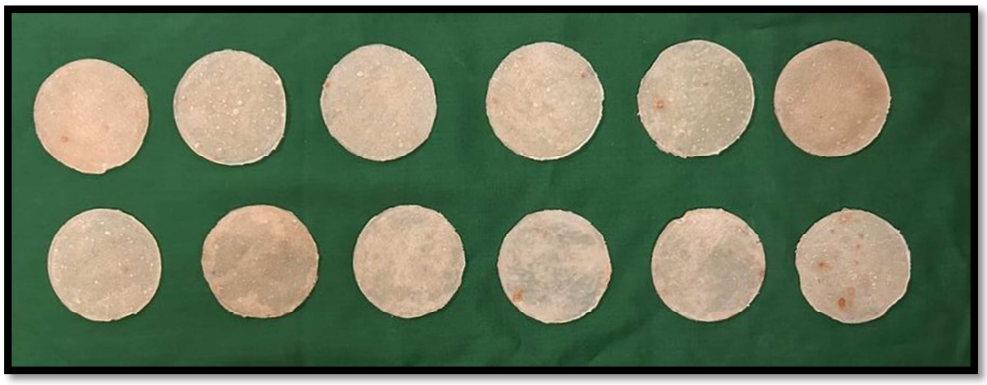


Figure 20: Specimens of 10% *G. glabra* group for water sorption and solubility for day 7

Figure 21: Specimens of 10% *G. glabra* group for water sorption and solubility for day 14

Figure 22: Specimens of 15% *G. glabra* group for water sorption and solubility for day 1

Figure 23: Specimens of 15% *G. glabra* group for water sorption and solubility for day 7

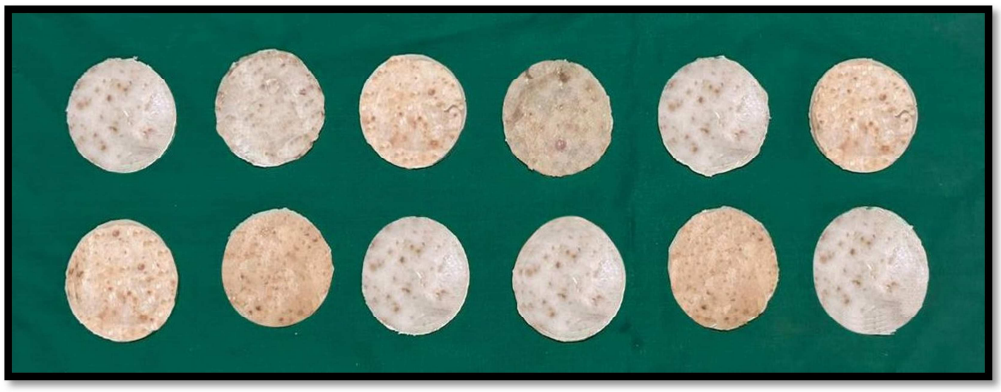


Figure 24: Specimens of 15% *G. glabra* group for water sorption and solubility for day 14

Figure 25: Weighing of the specimens in digital analytic balance

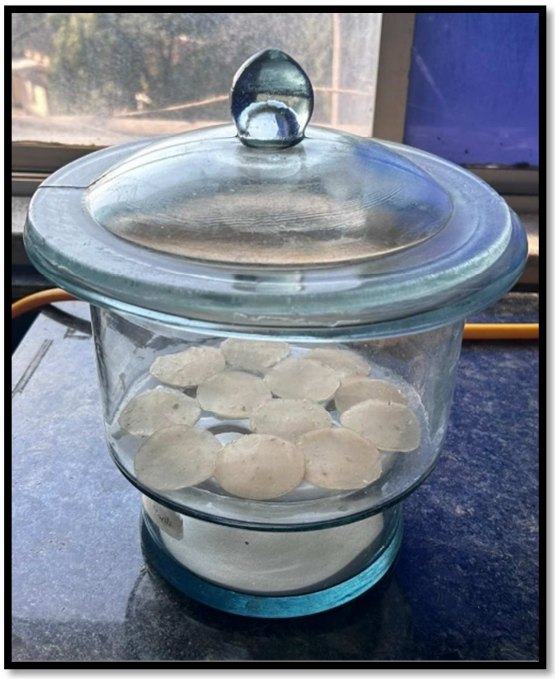


Figure 26: Specimens placed in the desiccator

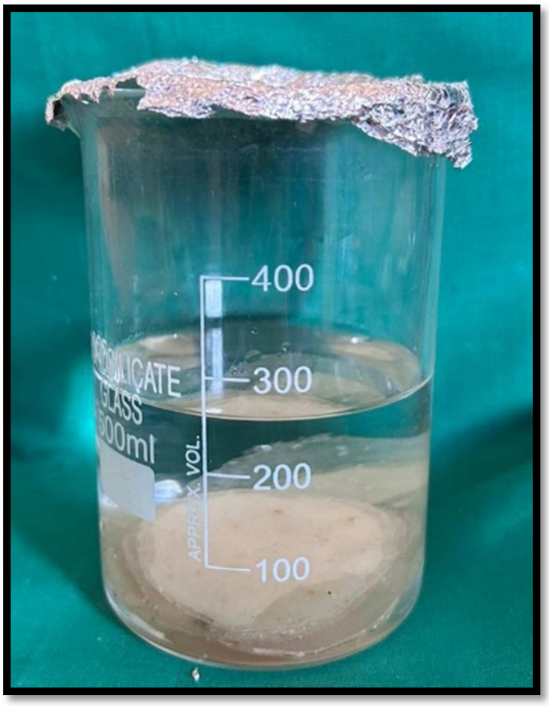


Figure 27: Specimens in artificial saliva

RESULTS

The present study was conducted to evaluate the antifungal activity, water sorption, and solubility of soft liner incorporated with *Glycyrrhiza glabra*.

Statistical analysis

The acquired data was input into a Microsoft Excel worksheet. SPSS version 25.0 statistical software was used to analyze the data. The fundamental characteristics of the data were described using descriptive statistics.

Generalized linear models (GLM) analysis was performed on Anti-Fungal activity (as the data assumptions were violated). The data was expressed in terms of mean and standard deviation.

As the data assumptions of normality was met by the Water Sorption and Water Solubility, Mixed-model analysis was carried out.

Dependent t-test was used for comparison of 3 groups with MIZ in millimetres (mm) at different time intervals. Comparison of the 3 experiment groups with the mean antifungal score at different time points (days 3, 7, and 14) was done by mixed method models.

Comparison of 4 groups and 3 different time points (days 1, 7, and 14) with mean water sorption and solubility was done by mixed method models.

Comparison of Anti-Fungal activity between 5% *Glycyrrhiza glabra*, 10% *Glycyrrhiza glabra* and 15% *Glycyrrhiza glabra*

DESCRIPTIVE STATISTICS

Table 1: Mean and standard deviation of anti-fungal groups.

Experiment Group	Mean ± SD
Day 3	14.67 ± 4.822
Day 7	9.29 ± 4.341
Day 14	3.74 ± 3.246
5% *Glycyrrhiza glabra*	4.9 ± 4.05
10% *Glycyrrhiza glabra*	8.33 ± 4.23
15% *Glycyrrhiza glabra*	14.45 ± 5.61
	9.23 ± 6.11

Table 2: Mean and standard deviation of anti-fungal activity in group over time.

Anti-fungal activity			
Days	Interventions		
	5% *Glycyrrhiza glabra* (Mean ± SD)	10% *Glycyrrhiza glabra* (Mean ± SD)	15% *Glycyrrhiza glabra* (Mean ± SD)
Day 3	9.64 ± 1.082	13.57 ± 1.016	20.79 ± 1.528
Day 7	5.07 ± 0.829	7.79 ± 0.893	15 ± 1.109
Day 14	0 ± 0	3.64 ± 1.082	7.57 ± 1.089

The control group exhibited no antifungal activity; therefore, it was excluded from the analysis.

The mean values of the diameter zone with respect to time within and between the groups are presented in the table. The GLM model analysis revealed a evident mean difference in the effectiveness among all three groups. A evident decrease was observed in all three intervention groups after 7 and 14 days compared to Day 3. Furthermore, the diameter zones showed a evident group influence with time.

Table 3: Mean Comparison of Antifungal activity on Day 3, Day 7 and Day14.

Days	Day 3	Day 7	Day 14
	(Mean ± SD) 14.67 ± 4.822	(Mean ± SD) 9.29 ± 4.341	(Mean ± SD) 3.74 ± 3.246
Day 3	---		
Day 7	-5.38 p<0.001*	----	
Day 14	-10.93 p<0.001*	5.55 p<0.001*	---

A statistically evident value is $p<0.05$*.

The GLM model indicated a evident difference in means across all three time periods. There was a notable decrease in the mean antifungal activity values across the three time periods (Day 3, Day 7, Day 14), independent of groups, with $p<0.001$. The antifungal activity decreased over time, with Day 3 showing higher activity compared to 7^{th} & 14^{th} day.

Table 4: Mean Comparison of Antifungal activity among three different groups.

Groups	5% *Glycyrrhiza glabra*	10% *Glycyrrhiza glabra*	15% *Glycyrrhiza glabra*
	(Mean ± SD) 4.9 ± 4.05	(Mean ± SD) 8.33 ± 4.23	(Mean ± SD) 14.45 ± 5.61
5% *Glycyrrhiza glabra*	----		
10% *Glycyrrhiza glabra*	3.43 p<0.001*	----	
15% *Glycyrrhiza glabra*	9.55 p<0.001*	6.12 p<0.001*	----

A statistically evident value is p<0.05*.

Pairwise Comparison of Mean Antifungal Activity Among Three Groups. The GLM model revealed a evident difference in mean antifungal activity among the three groups (5%, 10%, and 15%), independent of time points. The antifungal activity values increased in ascending order as follows: 5% < 10% < 15%.

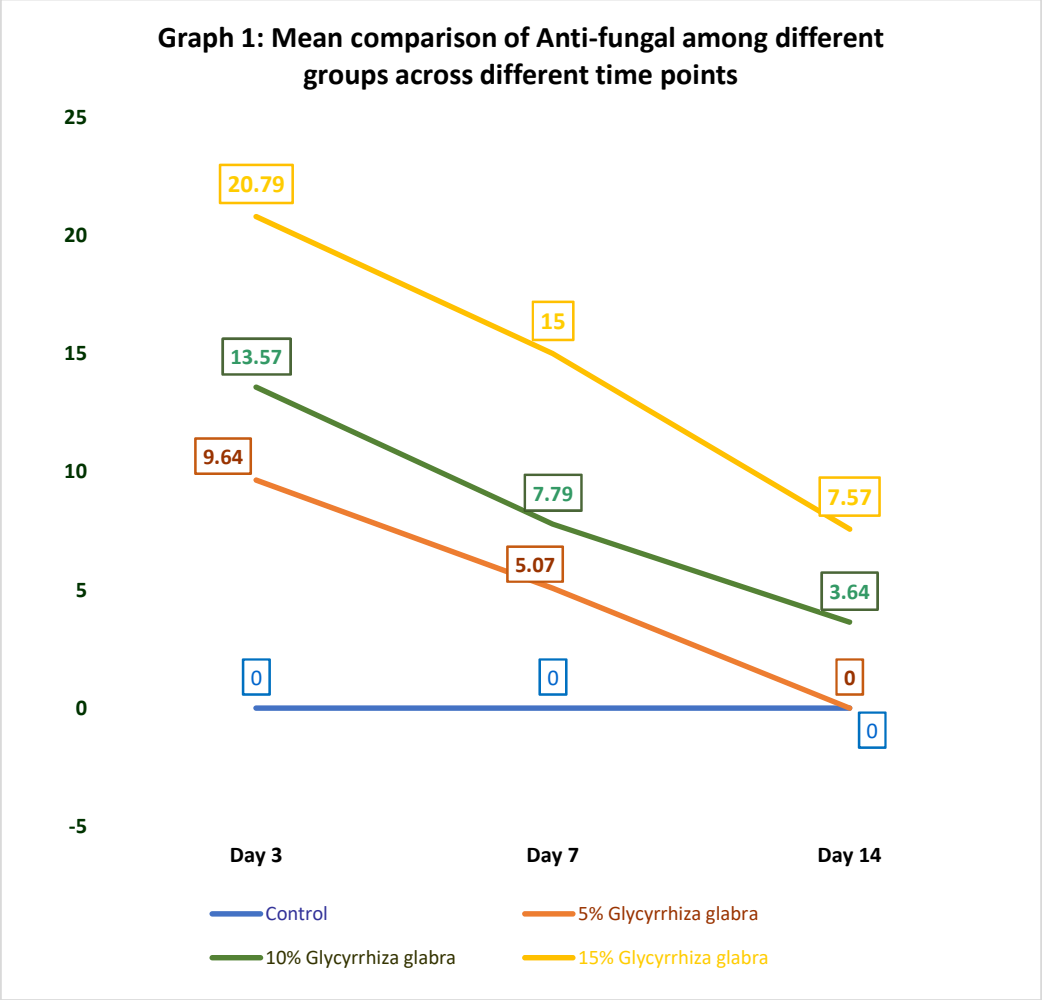

Graph 1: Mean comparison of Anti-fungal among different groups across different time points
25
20
15
10
5
0
-5
20.79
15
7.57
13.57
7.79
3.64
9.64
5.07
0
0
0
0
Day 3
Day 7
Day 14
Control
5% Glycyrrhiza glabra
10% Glycyrrhiza glabra
15% Glycyrrhiza glabra

Table 5: Comparison of Mean Antifungal Scores Across Three Experimental Groups (5%, 10%, and 15%) at Various Time Points.

Mean	Day 3	Day 7	Day 14
10% - 5%	3.929 p<0.001*	2.714 p<0.001*	3.643 p<0.001*
15% - 5 %	11.143 p<0.001*	9.929 p<0.001*	7.571 p<0.001*
15% - 10 %	7.214 p<0.001*	7.214 p<0.001*	3.929 p<0.001*

Pairwise Comparison of Antifungal Scores Among the Three Groups at Different Time Points indicates a evident mean difference among each pair of groups at every time point, with $p<0.05$.

Table 6: Comparing mean differences in time points among groups

Experiment Group	Day (3 -7)	Day (14-3)	Day (7-14)
5% *Glycyrrhiza glabra*	4.57 ± 0.938	9.64 ± 1.08	5.07± 0.83
10% *Glycyrrhiza glabra*	5.79 ± 0.98	9.93 ± 0.73	4.14 ± 1.03
15% *Glycyrrhiza glabra*	5.79 ± 0.89	13.21 ± 1.53	7.43 ± 1.02
Mean			
10% - 5%	1.214 p<0.001 *	0.286 p= 1.0	-0.929 p=0.004*
15% - 5 %	1.214 p<0.001*	3.571 p < 0.001*	2.357 p<0.001*
15% - 10 %	0.00 p= 1	3.286 p<0.001*	3.286 p<0.001*

The analysis of mean differences in time points among groups revealed statistically evident differences between the 3rd, 7th, and 14th days.

Table 7: Comparison of different treatment time points with mean Antifungal scores in three experiment groups (5%, 10%, 15 %) by dependent t-test.

Experiment Group	Days	(Mean ± SD)	(Mean ± SD) Diff	% of change	t-value	p-value
5% *Glycyrrhiza glabra*	DAY 3 DAY 7	9.64 ± 1.082 5.07 ± 0.829	4.571 ± 0.938	-47.41	18.243	<0.001*
	DAY 3 DAY 14	9.64 ± 1.082 0 ± 0	9.643 ± 1.082	-100	33.35	<0.001*
	DAY 7	5.07 ± 0.829				
	DAY 14	0 ± 0	5.071 ± 0.829	-100	22.89	<0.001*
10% *Glycyrrhiza glabra*	DAY 3	13.57 ± 1.016				
	DAY 7	7.79 ± 0.893	5.78 ± 0.975	42.59	22.204	<0.001*
	DAY 3	13.57 ± 1.016				
	DAY 14	3.64 ± 1.082	9.929 ± 0.73	73.16	50.88	<0.001*
	DAY 7	7.79 ± 0.893				
	DAY 14	3.64 ± 1.082	4.14 ± 1.027	53.14	15.092	<0.001*
15% *Glycyrrhiza glabra*	DAY 3 DAY 7	20.79 ± 1.528 15 ± 1.109	5.786 ± 0.893	-27.83	24.25	<0.001*
	DAY 3 DAY 14	20.79 ± 1.528 7.57 ± 1.089	13.214 ± 1.528	-63.5	32.35	<0.001*
	DAY 7 DAY 14	15 ± 1.109 7.57 ± 1.089	7.429 ± 1.016	-49.52	27.341	<0.001*

p<0.05 * is statistically evident.

A comparison of Days 3, 7, and 14 with respect to MIZ in all three groups (5%, 10%, and 15%) was conducted using dependent t-tests. Statistically evident differences were observed between all days across all groups, with p<0.001***.

Table 8: Pair-wise comparison of four groups and three time points with mean Anti-Fungal activity.

Anti-Fungal activity	5% Gg with Day 3	5% Gg with Day 7	5% Gg with Day 14	10% Gg with Day 3	10% Gg with Day 7	10% Gg with Day 14	15% Gg with Day 3	15% Gg with Day 7	15% Gg with Day 14
5% Gg with Day 3	----								
5% Gg with Day 7	4.57 p<0.001*	----							
5% Gg with Day 14	9.64 p<0.001*	5.07 p<0.001*	----						
10% Gg with Day 3	3.93 p<0.001*	8.5 p<0.001*	13.57 p<0.001*	----					
10% Gg with Day 7	1.86 p<0.001*	2.71 p<0.001*	7.79 p<0.001*	5.79 p<0.001*	----				
10% Gg with Day 14	6.0 p<0.001*	1.43 p<0.001*	3.64 p<0.001*	9.93 p<0.001*	4.14 p<0.001*	----			
15% Gg with Day 3	11.14 p<0.001*	15.71 p<0.001*	20.79 p<0.001*	7.21 p<0.001*	13 p<0.001*	17.14 p<0.001*	----		
15% Gg with Day 7	5.36 p<0.001*	9.9 p<0.001*	15 p<0.001*	1.43 p<0.001*	7.21 p<0.001*	11.36 p<0.001*	5.79 p<0.001*	----	
15% Gg with Day 14	2.07 p<0.001*	2.5 p<0.001*	7.57 p<0.001*	6.00 p<0.001*	0.21 p = 0.55	3.93 p<0.001*	13.21 p<0.001*	7.43 p<0.001*	----

$p<0.05$ * is statistically evident.

When conducting a pair-wise comparison of four groups across three time points with mean anti-fungal activity, all groups showed statistically evident differences at all time points.

Comparison of Water Sorption between Control, 5% *Glycyrrhiza glabra*, 10% *Glycyrrhiza glabra* and 15% *Glycyrrhiza glabra.*

DESCRIPTIVE STATISTICS

Table 9: Mean and standard deviation of water-sorption groups.

Water Sorption	(Mean ± SD)
Day 3	119.16 ± 48.17
Day 7	155.34 ± 56.74
Day 14	191.70 ± 59.63
Control	74.19 ± 20.00
5% *Glycyrrhiza glabra*	149.53± 39.05
10% *Glycyrrhiza glabra*	176.18 ± 32.73
15% *Glycyrrhiza glabra*	221.70 ± 32.31

Table 10: Mean and Standard Deviation of Water Sorption for Groups Over Time.

WATER SORPTION				
Timeline		Interventions		
	Control (Mean ± SD)	5% *Glycyrrhiza glabra* (Mean ± SD)	10% *Glycyrrhiza glabra* (Mean ± SD)	15% *Glycyrrhiza glabra* (Mean ± SD)
Day 3	55.17 ± 5.49	100.31 ± 4.95	137.16 ± 5.44	184.023 ± 5.79
Day 7	67.91 ± 7.98	155.99 ± 9.46	176.55 ± 5.85	220.91 ± 7.07
Day 14	99.51 ± 6.56	192.31 ± 7.33	214.84 ± 7.29	260.17 ± 8.73

The mean values of water sorption with respect to time within and between the groups are displayed in the table. The mixed ANOVA model analysis revealed a evident mean difference in the effectiveness among all four groups. There was a notable increase in water sorption observed in all three intervention groups after 7 and 12 days compared to Day 3. Additionally, there was a evident effect of group and time on water sorption.

Graph 2: Mean comparison of Water-Sorption among different groups across different time points

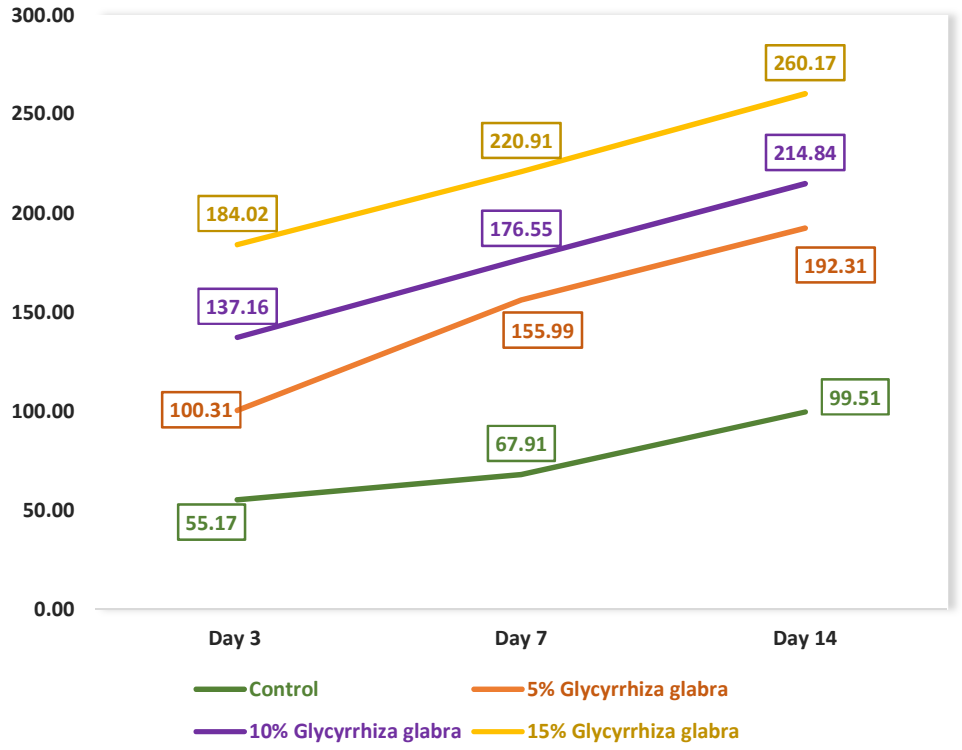


Table 11: Mean comparison of Water sorption among four different groups.

Water-Sorption	Control	5% *Glycyrrhiza glabra*	10% *Glycyrrhiza glabra*	15% *Glycyrrhiza glabra*
	74.19 ± 20.00	149.53± 39.05	176.18 ± 32.73	221.70 ± 32.31
Control	----			
5% *Glycyrrhiza glabra*	75.34 p< 0.001*	-----		
10% *Glycyrrhiza glabra*	101.90 p<0.001*	26.65 p<0.001*	---	
15% *Glycyrrhiza glabra*	147.50 p<0.001*	72.16 p<0.001*	45.52 p<0.001*	----

The Mixed ANOVA model indicated a evident difference in mean water sorption among all four groups (Control, 5%, 10%, and 15%), independent of time points. The water sorption values increased in ascending order: Control < 5% < 10% < 15%.

Table 12: Mean comparison of water sorption on Days 1, day 7 and Day 14.

Water-Sorption Days	Day 1	Day 7	Day 14
	(Mean ± SD) 119.16 ± 48.17	(Mean ± SD) 155.34 ± 56.74	(Mean ± SD) 191.70 ± 59.63
Day 1	----	---	----
Day 7	36.17 p<0.001*	----	---
Day 14	72.54 p<0.001*	36.37 p<0.001*	----

The Mixed ANOVA model revealed a evident difference in means across all three time periods. Additionally, there was a evident increase in the mean water sorption values across these three time periods (Day 1, Day 7, Day 14), independent of groups, with $p<0.001$. Furthermore, the analysis demonstrated that water sorption increased over time, with the mean values showing a progression from Day 1 to Day 14 as follows: Day 1 < Day 7 < Day 14.

Table 13: Comparison of Four groups (Control, 5%, 10% and 15%) with mean Water-Sorption scores at different time points.

Time	Day 1	Day 7	Day 14
Water - Sorption	Difference		
5% - control	45.141 p<0.001*	88.08 p<0.001*	92.79 p<0.001*
10 % - control	81.992 p<0.001*	108.645 p<0.001*	115.33 p<0.001*
15% - control	128.86 p<0.001*	153.00 p<0.001*	160.66 p<0.001*
10% - 5%	36.85 p<0.001*	20.56 p<0.001*	22.534 p<0.001*
15% - 5 %	83.716 P<0.001*	64.92 p<0.001*	67.86 p<0.001*
15% - 10 %	46.865 p<0.001*	44.36 p<0.001*	45.33 p<0.001*

A statistically evident value is p<0.05*.

A pairwise comparison of all four groups with Water-Sorption scores at different time points revealed evident mean difference among each pair of every group at time point with p<0.05.

Table 14: Comparing mean differences between time points among groups.

Water -sorption	Day (7-1)	Day (14-7)	Day (14-1)
Control	12.73 ± 7.78	31.60 ± 4.4	44 .34 ± 6.67
5%	55.68 ± 8.71	36.33 ± 5.93	92 ± 6.90
10%	39.39 ± 7.65	38.29 ± 7.27	77.68 ± 8.03
15%	36.88 ± 8.46	39.26 ± 9.72	76.14 ± 9.24
5% - control	42.947 p<0.001*	4.711 p = 0.116	47.65 p<0.001*
10 % - control	26.653 p<0.001*	6.68 p = 0.047	33.342 p<0.001*
15% - control	24.15 p<0.001*	7. 657 p= 0.168	31.87 p <0.001*
10% - 5%	- 16.29 p<0.001*	1.977 p =1.00	- 14.32 p<0.001*
15% - 5 %	-18.8 p=0.002*	2.947 p = 1.00	-15.852 p=0.01
15% - 10 %	-2.504 p=1.00	0.969 p =1.00	-1.54 p= 1.00

A statistically evident value is p<0.05*.

The table presents mean water sorption values and differences between time points for four groups (Control, 5%, 10%, and 15%). Statistically evident differences were found between groups at various time points, with notable increases observed in water sorption over time for each group.

Table 15: Comparison of different treatment time points with mean Water-Sorption in four groups (Control, 5%, 10%, 15 %) by dependent t-test.

	Days	(Mean ± SD)	(Mean ± SD) Diff	% of change	t-value	p-value
Control	DAY 1 DAY 7	55.17 ± 5.49 67.91 ± 7.98	12.74 ± 7.78	23.09	5.671	<0.001*
	DAY 1 DAY 14	55.17 ± 5.49 99.51 ± 6.56	44.34 ± 6.67	80.36	23.01	<0.001*
	DAY 7 DAY 14	67.91 ± 7.98 99.51 ± 6.56	31.60 ± 4.44	46.5	24.68	<0.001*
5% *Glycyrrhiza glabra*	DAY 1 DAY 7	100.31 ± 4.95 155.99 ± 9.46	55.68 ± 8.71	55.5	22.12	<0.001*
	DAY 1 DAY 14	100.31 ± 4.95 192.31 ± 7.33	92 ± 6.90	91.7	46.15	<0.001*
	DAY 7 DAY 14	155.99 ± 9.46 192.31 ± 7.33	36.31 ± 5.93	23.27	21.2	<0.001*
10% *Glycyrrhiza glabra*	DAY 1 DAY 7	137.16 ± 5.44 176.55 ± 5.85	39.39 ± 7.65	28.71	17.83	<0.001*
	DAY 1 DAY 14	137.16 ± 5.44 214.84 ± 7.29	77.68 ± 8.03	56.63	33.49	<0.001*
	DAY 7 DAY 14	176.55 ± 5.85 214.84 ± 7.29	38.29 ± 7.27	21.68	18.22	<0.001*
15% *Glycyrrhiza glabra*	DAY 1 DAY 7	184.023 ± 5.79 220.91 ± 7.07	36.88 ± 8.46	20.04	15.08	<0.001*
	DAY 1 DAY 14	184.023 ± 5.79 260.17 ± 8.73	76.14 ± 9.24	41.38	28.51	<0.001*
	DAY 7 DAY 14	220.91 ± 7.07 260.17 ± 8.73	39.26 ± 9.72	17.77	13.98	<0.001*

Comparison of different days on Days 1 ,7 and 14 with respect to Water-Sorption in all 4 groups (Control, 5%, 10% and 15%) was done by dependent t-test. A statistically evident difference (p<0.001***) was observed among all groups throughout all days.c

Table 16: Pair-wise comparison of four groups and three time points with mean water sorption.

Water Sorption	Cont rol Day 1	Control Day 7	Control Day 14	5% Gg Day 1	5% Gg Day 7	5% Gg Day 14	10% Gg Day 1	10% Gg Day 7	10% Gg Day 14	15% Gg Day 1	15% Gg Day 7	15% Gg Da 14
Control Day 1	---											
Control Day 7	12.73 p<0.001*	----										
ControlDay 14	44.34 p<0.001*	31.6 p<0.001*	----									
5% Gg Day 1	45.14 p<0.001*	32.40 p<0.001*	0.7992 p=0.725	----								
5% Gg Day 7	100.82 p<0.001*	88.08 p<0.001*	56.48 p<0.001*	55.68 p<0.001*	----							
5% Gg Day 14	137 p<0.001*	124 p<0.001*	92.7 p<0.001*	92 p<0.001*	36 p<0.001*	----						
10% Gg Day 1	82 p<0.001*	69.5 p<0.001*	37.65 p<0.001*	36.85 p<0.001*	-18.83 p<0.001*	-55.14 p<0.001*	----					
10% Gg Day 7	121.38 p<0.001*	108.64 p<0.001*	77.04 p<0.001*	76.24 p<0.001*	20.55 p<0.001*	-15.75 p<0.001*	39.39 p<0.001*	----				

10% Gg Day 14	159.67 p<0.001*	146.93 p<0.001*	115.33 p<0.001*	114.53 p<0.001*	58.84 p<0.001*	22.53 p<0.001*	77.68 p<0.001*	38.29 p<0.001*	----			
15% Gg Day 1	128.85 p<0.001*	116.11 p<0.001*	84.51 p<0.001*	83.71 p<0.001*	28.02 p<0.001*	-8.28 p<0.001*	46.86 p<0.001*	7.47 p<0.001*	-30.81 p<0.001*	----		
15% Gg Day 7	165.74 p<0.001*	153.0 p<0.001*	121.4 p<0.001*	120.60 p<0.001*	64.91 p<0.001*	28.6 p<0.001*	83.75 p<0.001*	44.36 p<0.001*	6.07 p<0.001*	-36.88 p<0.001*	----	
15% Gg Day 14	205.00 p<0.001*	192.26 p<0.001*	160.66 p<0.001*	159.86 p<0.001*	104.17 p<0.001*	67.86 p<0.001*	123 p<0.001*	83.62 p<0.001*	45.33 p<0.001*	76.14 p<0.001*	39.26 p<0.001*	----

A statistically evident value is p<0.05*.

When pair-wise comparison of four groups and three time points with mean water sorption, all the groups had shown statistically evident differences at all the time.

Comparison of Water solubility between Control, 5% *Glycyrrhiza glabra*, 10% *Glycyrrhiza glabra* and 15% *Glycyrrhiza glabra*

DESCRIPTIVE STATISTICS

Table 17: Mean and standard deviation of water-solubility groups.

Water Solubility	(Mean ± SD)
Day 1	25.18 ± 5.077
Day 7	28.62 ± 4.94
Day 14	31.61 ± 4.91
Control	21.96 ± 3.14
5% *Glycyrrhiza glabra*	26.76 ± 2.90
10% *Glycyrrhiza glabra*	30.20 ± 2.63
15% *Glycyrrhiza glabra*	34.96 ± 3.21

Table 18: Mean and Standard Deviation of Water Solubility for Groups Over Time.

WATER - SOLUBILITY				
Days		Interventions		
	Control (Mean ± SD)	5% *Glycyrrhiza glabra* (Mean ± SD)	10% *Glycyrrhiza glabra* (Mean ± SD)	15% *Glycyrrhiza glabra* (Mean ± SD)
Day 1	18.38 ± 1.28	23.53 ± 1.21	27.27 ± 1.00	31.54 ± 1.95
Day 7	22.44 ± 1.63	26.67 ± 1.04	30.10 ± 0.84	35.31 ± 1.70
Day 14	25.08 ± 1.26	30.09 ± 0.89	33.25 ± 0.91	38.05 ± 1.68

The mean values water solubility with respect to time within and between the groups are shown in the table. Mixed Anova model analysis showed that evident mean difference in effectiveness of all the 4 groups. There was a evident increase in water solubility in all 3 intervention groups after 7 and 14 days as compared to Day 1.

There was no evident effect of groups time on Water solubility with $p = 0.202 > 0.05$.

Graph 3: Mean comparison of Water-Solubility among different groups across different time points

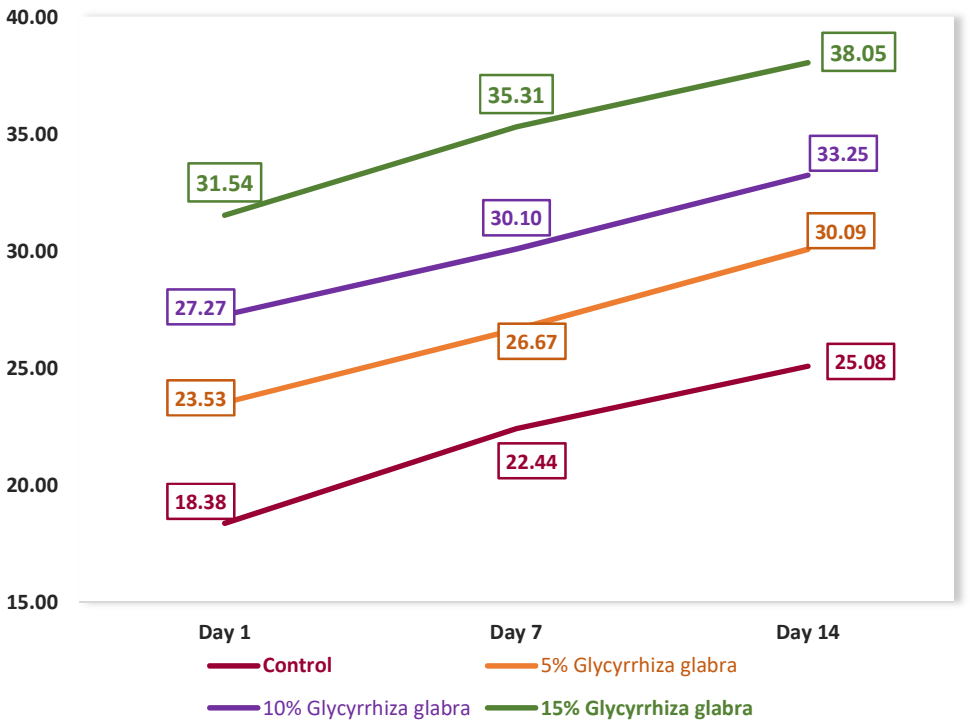


Table 19: Mean Comparison of Water- Solubility on Day 1, Day 7 and Day14.

Water-Solubility	Day 1	Day 7	Day 14
	25.18 ± 5.077	28.62 ± 4.94	31.61 ± 4.91
Day 1	----	---	----
Day 7	3.448	----	---
	p<0.001*		
Day 14	6.436	2.989	----
	p<0.001*	p<0.001*	

A statistically evident value is p<0.05*.

The mixed model analysis showed a evident difference in means in all three time periods. There was evident increase in Water- Solubility mean value across three time periods (Day1, Day 7, Day 14) independent of groups with p<0.001. There was increase in Water-Solubility with passage of time as follows Day1 < Day 7 <Day 14.

Table 20: Pair wise comparison of four groups with mean Water Solubility.

Water-Solubility	Control	5% *Glycyrrhiza glabra*	10% *Glycyrrhiza glabra*	15% *Glycyrrhiza glabra*
	21.96 ± 3.14	26.76 ± 2.90	30.20 ± 2.63	34.96 ± 3.21
Control	-----			
5% *Glycyrrhiza glabra*	4.796 p<0.001*	----		
10% *Glycyrrhiza glabra*	8.236 p<0.001*	3.441 p<0.001*	----	
15% *Glycyrrhiza glabra*	13.00 p<0.001*	8.205 p<0.001*	4.765 p<0.001*	----

The mixed model analysis showed a evident difference in mean Water-Solubility among all the groups 4 groups (Control, 5%, 10% and 15%) independent of time points.

The Water-Solubility values increased as follows in ascending order Control < 5% < 10% < 15%.

Table 21: Comparison of Four groups (Control, 5%, 10% and 15%) with mean Water-Solubility scores at different time points.

Solubility time Mean	Day 1	Day 7	Day 14
5% - control	5.153 p<0.001 *	4.232 p<0.001*	5.00 p<0.001*
10 % - control	8.885 p<0.001*	7.660 p<0.001*	8.16 p<0.001*
15% - control	13.15 p<0.001*	12.87 p<0.001*	12.97 p<0.001*
10% - 5%	3.73 p<0.001*	3.428 p<0.001*	3.16 p<0.001*
15% - 5 %	8.002 p<0.001*	8.645 p<0.001*	7.96 p<0.001*
15% - 10 %	4.27 p<0.001*	5.217 p<0.001*	12.97 p<0.001*

Comparison of different days on Days 1 ,7 and 14 with respect to Water-Solubility in all 4 groups (Control, 5%, 10% and 15%) was done by dependent t-test. Statistically, evident difference was seen between all the days among all the groups with p<0.001***.

Table 22: Comparing mean differences between time points among groups

Water solubility			
	Day (7-1)	Day (14- 7)	Day (14 -1)
Control	4.05 ± 2.17	2.64 ± 1.08	6.70 ± 1.70
5% *Glycyrrhiza glabra*	3.13 ± 1.53	3.41 ± 0.99	6.55 ± 1.65
10% *Glycyrrhiza glabra*	2.82 ± 0.89	3.15 ± 0.71	5.97 ± 1.09
15% *Glycyrrhiza glabra*	3.77 ± 0.72	2.74 ± 0.68	6.51 ± 1.19
Mean			
5% - control	-0.92 p=0.764	-0.151 p=1.00	0.796 p=0.236
10 % - control	-1.225 p=0.266	- 0.722 p=1.00	0.503 p= 1.00
15% - control	-0.277 p=1.00	-0.185 p=1.00	0.092 p=1.00
10% - 5%	-0.305 p=1.00	-0.571 p=1.00	-0.266 p=1.00
15% - 5 %	0.642 p=1.00	-0.034 p=1.00	-0.677 p=0.411
15% - 10 %	0.948 p =0.7	0.537 p=1.00	-0.411 p =1.00

A statistically evident value is $p<0.05$*.

The mean differences among groups across different time periods were assessed, with statistical significance considered at $p<0.05$. However, none of the observed differences were statistically evident.

Table 23: Comparison of different treatment time points with mean Water-Solubility in four groups (Control, 5%, 10%, 15 %) by dependent t-test.

Water - Solubility	Days	(Mean ± SD)	(Mean ± SD) Diff	% of change	t-value	p-value
Control	Day 1 Day 7	18.38 ± 1.28 22.44 ± 1.63	4.05 ± 2.17	22.03	6.469	<0.001*
	Day 1 Day 14	18.38 ± 1.28 25.08 ± 1.26	6.70 ± 1.70	36.45	13.61	<0.001*
	Day 7 Day 14	22.44 ± 1.63 25.08 ± 1.26	2.64 ± 1.08	11.76	8.43	<0.001*
5% *Glycyrrhiza glabra*	Day 1 Day 7	23.53 ± 1.21 26.67 ± 1.04	3.13 ± 1.53	13.3	7.06	<0.001*
	Day 1 Day 14	23.53 ± 1.21 30.09 ± 0.89	6.55 ± 1.65	27.87	11.88	<0.001*
	Day 7 Day 14	26.67 ± 1.04 30.09 ± 0.89	3.41 ± 0.99	12.78	13.71	<0.001*
10% *Glycyrrhiza glabra*	Day 1 Day 7	27.27 ± 1.00 30.10 ± 0.84	2.82 ± 0.89	10.34	10.945	<0.001*
	Day 1 Day 14	27.27 ± 1.00 33.25 ± 0.91	5.97 ± 1.09	21.9	15.26	<0.001*
	Day 7 Day 14	30.10 ± 0.84 33.25 ± 0.91	3.15 ± 0.71	10.46	18.99	<0.001*
15% *Glycyrrhiza glabra*	Day 1 Day 7	31.54 ± 1.95 35.31 ± 1.70	3.77 ± 0.72	11.95	17.92	<0.001*
	Day 1 Day 14	31.54 ± 1.95 38.05 ± 1.68	6.51 ± 1.19	20.64	13.93	<0.001*
	Day 7 Day 14	35.31 ± 1.70 38.05 ± 1.68	2.74 ± 0.68	7.76	18.84	<0.001*

Comparison of different days on Days ,7 and 14 with respect to Water-Sorption in all 4 groups (Control, 5%, 10% and 15%) was done by dependent t-test. Statistically, evident difference was seen between all the days among every group with p<0.001***.

Table 24: Pair-wise comparison of 4 groups & 3 time points with mean water solubility.

Solubility	ControlDay 1	Control Day 7	Control Day 14	5% Gg Day 1	5% Gg Day 7	5% Gg Day 14	10%Gg Day 1	10%Gg Day 7	10% Gg Day 14	15% GgDay 1	15% Gg Day 7	15% GgDa 14
Control Day1	---											
Control Day 7	4.0533 p<0.001*	----										
Control Day 14	6.70 p<0.001*	2.64 p<0.001*	----									
5% Gg Day 1	5.15 p<0.001*	1.09 p=0.051 ns	- 1.58 p=0.001*	----								
5% Gg Day 7	8.28 p<0.001*	4.23 p<0.001*	1.58 p<0.001*	3.13 p<0.001*	----							
5% Gg Day14	11.07 p<0.001*	7.64 p<0.001*	5.00 p<0.001*	6.55 p<0.001*	3.41 p<0.001*	----						
10% Gg Day 1	8.88 p<0.001*	4.83 p<0.001*	2.18 p<0.001*	3.73 p<0.001*	0.599 p0=0.133	-2.817 p<0.001*	----					
10% Gg Day 7	11.71 p<0.001*	7.66 p<0.001*	5.01 p<0.001*	6.56 p<0.001*	3.42 p=0.001*	0.0108 p=0.974	2.82 p<0.001*	----				
10% Gg Day 14	14.84 p<0.001*	10.81 p<0.001*	8.16 p<0.001*	9.71 p<0.001*	6.57 p<0.001*	3.16 p<0.001*	5.97 p<0.001*	3.15 p<0.001*	----			
15% Gg Day 1	13.15 p<0.001*	9.10 p<0.001*	6.45 p<0.001*	8.00 p<0.001*	4.86 p<0.001*	1.45 p=0.014	4.27 p<0.001*	1.44 p=0.014	-1.709 p=0.004	----		
15% Gg Day 7	16.93 p<0.001*	12.8 p<0.001*	10.23 p<0.001*	11.77 p<0.001*	8.64 p<0.001*	5.22 p<0.001*	8.04 p<0.001*	5.22 p<0.001*	2.06 p<0.001*	3.77 p<0.001*	----	
15% Gg Day 14	19.60 p<0.001*	15.61 p<0.001*	12.97 p<0.001*	14.51 p<0.001*	11.38 p<0.001*	7.96 p<0.001*	10.7 p<0.001*	7.95 p<0.001*	4.80 p<0.001*	6.51 p<0.001*	2.74 p<0.00 1*	----

A statistically evident value is p<0.05*.

During the pair-wise comparison of four groups across three time points with mean water solubility, all groups exhibited statistically evident differences at every time point, except for the comparisons between 5% at Day 1 with Control at Day 7 and between 10% at Day 1 with 5% at Day 7.

LIMITATIONS OF THE STUDY

- Since this study was conducted in a in-vitro setting, it is important to note that the findings may vary when applied to clinical settings.

- The evaluation of the inhibition zone diameter of *G. glabra* was limited to a single strain of *Candida albicans.* Creation of prosthetic biofilms is complicated, involving bacteria as well as fungi. Through co-aggregation, bacteria can promote fungal cell adherence to internal prosthesis surfaces.

- This study focused solely on one type of acrylic interim resilient liner. Different types of liners may produce varying results.

- The incorporation of *G. glabra* led to discoloration of the soft liner, potentially resulting in a slightly aesthetically undesirable outcome. Nevertheless, the discoloration was minimal and could potentially be masked with the acrylic resin denture surface.

DISCUSSION

The human mouth is recognized as a distinctive habitat providing diverse ecological niches for microbial colonization.[27] The oral cavity comprises two distinct surfaces where bacteria can habituate i.e. Teeth representing the rough surface and oral mucosa representing the soft tissues. This creates an atmosphere which is nourishing to the microorganisms.[28]

Recently, remarkable increase in oral and systemic fungal infections has been noticed. Candidiasis has become a particularly concerning infection, posing a threat primarily to patients with compromised immune systems, older individuals, & those under medications.[21] The predominant issue experienced by patient who wears denture is denture stomatitis which is marked by inflammation and redness of the oral mucosa, as well as pathological alterations related to the denture use. *C. albicans* is commonly identified as primary agent in causation of DS, which affects approximately"30–70% of denture wearers.[29] Other C. albicans strains that are commonly found in the oral cavity include *C. krusei, C. glabrata, C. tropicalis, and C. parapsilosis.*[30]

The rising occurrence of fungal infections has resulted in a growing demand for antifungal medications. While topical antifungal agents like nystatin and miconazole are commonly employed to treat denture stomatitis, they often fail to achieve therapeutic levels on the denture surface. Systemic antifungal treatment may carry risks of hepatotoxicity and nephrotoxicity.[7] Due to the escalating resistance and potential toxicity associated with conventional anticandidal drugs, there is a preference for plant-based traditional antifungal agents that offer fewer or no side effects.[14]

Addressing the drawbacks of synthetic antifungal medications, there is a need for alternative therapies that are more effective in treating fungal infections. Some research has investigated the antifungal properties of herbal extracts obtained from garlic, neem, curcumin, aloe vera, and ocimum seeds as potential solutions to these challenges. Due to their natural composition, these extracts exhibit potent antifungal activity, high antimicrobial effectiveness, and reduced toxicity.[21,31,34]

Recently, tissue conditioners have been employed as a method of delivering drugs to elderly patients with denture stomatitis. Various in vitro and in vivo studies have demonstrated positive antifungal effects when organic substitutes such as tea-tree oil, lemon-grass oil, Thai-herbs, and origanum-oil are added to soft liners.[32] Furthermore, soft liners enhance denture retention and stability by promoting even distribution of masticatory pressure to the underlying tissues. This diminishes denture-induced soft tissue trauma and thus serves as an effective drug delivery mechanism.

The typical treatment duration with conventional topical antifungal medications is around 14 days. Given that the lifespan of a short-term soft liner is also approximately 14 days, utilizing these materials modified with drugs for treating denture-induced stomatitis aligns with the timeframe needed for conventional topical antifungal agents.[7,33] Hence, a 14-day duration was selected for this study.

Glycyrrhiza glabra (Licorice), is a plant renowned for its significant medicinal value. Asl and Hosseinzadeh reported that licorice roots possess numerous beneficial pharmacological properties, including anti-inflammatory, antiviral, antioxidative, antimicrobial, anticancer,

immunomodulatory, hepatoprotective, and cardioprotective effects. Medicinally, it is employed in treating various conditions such as arthritis, Addison's disease, peptic ulcer, asthma, bronchitis, and others.[36]

The soft-lining material is prone to habituation by *Candida albicans.*[37,38] *Candida albicans* induces significant host damage through its virulence factors, including adhesion, proteinase secretion, yeast-hyphal transition, and phenotypic switching.[39,40] The transition from blastospores to hyphal cells is particularly crucial in various processes, including biofilm formation.[22] Bioactive compounds found in licorice, such as Licochalcone A, glabridin, and liquiritigenin, have demonstrated effectiveness in suppressing fungal growth, preventing biofilm formation, and inhibiting yeast-hyphal transition.[41]

This study involved incorporating licorice root extract into soft liner which was then evaluated for their antifungal effectiveness against *Candida albicans* using the well diffusion method. The extract was obtained through Natural Remedies Private Limited, Bangalore, Karnataka. While introducing antifungal agents to such materials can effectively restrain *C. albicans* growth, it could change their morphological makeup and characteristics.[7]

Hence, the study also examined alterations in the water-sorption & solubility of these soft liners.

As per the findings of this study, the mean value for the MIZ calculated in millimetres for the 5% *G. glabra* group on days 3,7 and 14 were 9.64mm, 5.07mm, and 0mm respectively. The mean value for the 10% *G. glabra* group on days 3,7 and 14 were 13.57mm, 7.79mm, and 3.64mm respectively. The 15% *G. glabra* group on days 3,7 and 14 were

20.79mm, 15mm, and 7.57mm respectively. The control group had no antifungal effect.

The addition of *G. glabra* to the soft-liner yielded anti-fungal effect to the reference *C. albicans* strain. The antifungal effect is mostly attributed to inhibition of two key virulence property that is biofilm formation and yeast-hyphae transition of *C. albicans* by bioactive compounds such as Licochalcone A and glabridin.[42]

Based on Graph 1, there was a noticeable increase in the zone of inhibition (mm) with the rise in *G. glabra* concentration on the same day. As the concentration of *G. glabra* combined with soft liner increased, the zone of inhibition also increased. Consequently, the most significant antifungal effect was observed with the 15% *G. glabra* group, followed by 10% and 5%. These findings regarding the antifungal properties closely mirrored previous studies [35, 51, 52]. For reference, as per a research by Sri et al.[35,] the antimicrobial effects of *G. glabra* extract incorporated into soft liners were assessed at concentrations of 5% and 10%. This investigation revealed notable efficacy against *Candida albicans*, particularly at higher concentrations. Similarly, another study explored the incorporation of grape seed extract (GSE) into acrylic soft liners at concentrations of 5% and 10%, showing significant antifungal activity against *Candida albicans* with higher concentrations exhibiting enhanced effectiveness[51]. Additionally, a study examined the antifungal properties of denture-lining material containing *Cnidium officinale* (CO) at varying concentrations (200-600 µg/ml) against *Candida albicans*, exhibiting promising antifungal efficacy of the denture-lining material, with effectiveness increasing proportionally with higher concentrations of CO[52]. This could be attributed to the greater release of antifungal compounds from the soft liners as the concentration increased.

According to Table 2, the zone of inhibition (mm) decreased as the time interval (days 3, 7, and 14) progressed within the same group. Previous studies investigated similar trends [14,9]. For instance, Muttagi et al.[14] incorporated *Centratherum anthelminticum, Linum usitatissimum, and Ocimum sanctum* into a soft liner, assessing their antifungal activity over a week. Similarly, Schneid et al.[9] examined the integration of various antifungal agents into an interim resilient liner, studying their anticandidal effects over a period of 14 days. In both investigations, the zone of inhibition decreased over time, while the minimum inhibitory zone (MIZ) expanded with higher concentrations. This trend was attributed to drug leaching or diffusion, with speculation that ethanol content in the liner could also influence antifungal activity. Consequently, there appears to be a decline in the antifungal efficacy of *G. glabra* over time, likely stemming from the depletion or leaching of bioactive compounds from the mixture. Thus, it can be concluded that the antifungal activity is influenced by both concentration and time. Although *G. glabra* is generally considered non-toxic, excessive amounts may lead to irritation.

Although the traditional medicinal applications of *G. glabra* have been widely recognized, its potential as an antifungal agent in dental applications have not been thoroughly investigated in the past. This study demonstrated the antifungal efficacy of *G. glabra* when incorporated into soft liners, offering a potential solution to the limitations associated with conventional soft liners.

Qualities of resilient liners may be jeopardized by the addition of pharmaceuticals in quantities that are commercially available. Solubility and sorption are two of the many mechanical characteristics of resilient liners that need special consideration because they are associated with the

problems that these materials encounter most frequentlySorption is the process of taking in material by diffusion and absorbing it from the surrounding atmosphere to fill the gaps between the polymer chains. Solubility in resilient materials refers to the release of plasticizer and other soluble compounds, such as ethanol, into the surrounding media at the same time. Temporary strong liners balance the release of components and fluid absorption, which results in unpleasant odors, bacterial adhesion and colonization, color changes, and sometimes even displacement of the denture base resin.[17,43] Therefore, water sorption and solubility are important properties to assess lifespan of a particular liner.[44]

Several researchers have demonstrated that water sorption in poly (methyl methacrylate) (PMMA) follows Fickian diffusion kinetics.[45] Even though PMMA only absorbs little water, this can have a big effect on the polymer's dimensional and mechanical properties. Water infiltration is mostly caused by diffusion, even though the contrariety of PMMA molecules aid in absorption.[26] The polymer network is easily penetrated by water molecules, which permits the flow of additives and uncured or loose monomers.[45] Water molecules enter the polymer and take up spaces between polymer chains, which has two important effects: it causes the polymerized mass to slightly expand and interferes with the entanglement of the polymer chains, so serving as plasticizers and changing the physical characteristics of the final polymer. This modification results in notable changes in the physical and dimensional properties, reduces stresses experienced during polymerization, and increases the mobility of the polymer chain. Prolonged water sorption can lead to increasing distortion, stress at the liner/denture base contact, swelling, and decreased bonding. Consequently, soft- liners gradually transition towards increased rigidity as these processes unfold.[26]

One important criterion for determining whether soft-liner materials are appropriate is their solubility. Dibutyl phthalate and other plasticizers increase the pliability of soft liners, but they also come with drawbacks. When soft liners are exposed to aqueous environments, plasticizers can be released because they are not bonded inside the resin mass. Soft liner materials experience two different reactions during clinical usage, when the denture comes into contact with saliva, and storage, when they are soaked in water or disinfecting solutions: diffusion of plasticizers & various soluble components of water or saliva. It is believed that impurities in the acrylic resin soft-liners, ethanol loss, and plasticizer leaching are the root causes of solubility issues. The material's mechanical and physical qualities may suffer as a result. As a result, using liners that are less prone to leaching phenomena is preferred.[26]

This study followed the guidelines outlined in the ISO specification #10139-2. It aimed to assess the impact of various concentrations of *G. glabra* on the solubility and water-sorption of a denture liner material which is acrylic based over intervals of 1, 7, and 14 days. Since dentures with soft liners come into contact with saliva in clinical settings, artificial saliva was used instead of distilled water to mimic oral conditions.

The mean water sorption values for the control group on days 1, 7, and 14 are 55.17µg/mm³, 67.91 µg/mm³, and 99.51 µg/mm³. The water sorption for 5% on days 1, 7, and 14 is 100.31 µg/mm³ 155.99 µg/mm³ and 192.31 µg/mm³. For 10% on days 1, 7, and 14 is 137.16 µg/mm³, 176.55 µg/mm³, and 214.84 µg/mm³. For 15% on days 1. 7, and 14 is 184.02 µg/mm³, 220.91 µg/mm³, and 260.17 µg/mm³.

As indicated in Graph 2, the average water sorption values across all groups (control, 5%, 10%, 15%) increased consistently across the time

points (1, 7, 14 days). Among these, the 15% concentration exhibited the highest water absorption, while the control group showed the lowest.

Table 10 illustrates that the mean water sorption values rose across three different time intervals (days 1, 7, and 14) regardless of the group (control, 5%, 10%, and 15%). The highest absorption occurred on day 14, whereas the lowest was observed on day 1.

As depicted in Table 13, there was a notable increase in the average water sorption values for all groups (control, 5%, 10%, and 15%) across all days (1, 7, and 14), with evident statistical significance.

The mean solubility values for the control group on days 1, 7, and 14 are 18.38 µg/mm³, 22.44 µg/mm³, and 25.08 µg/ mm³. Thevalues for 5% on days 1, 7, and 14 are 23.53 µg/mm³, 26.67 µg/mm³, and 30.09 µg/mm³. For 10%, the mean solubility values were 27.27 µg/mm³, 30.10 µg/mm³, and 33.25 µg/mm³ on days 1,7 and 14 respectively. For 15% on day 1 was 31.54 µg/mm³, on day 7 was 35.31 µg/ mm³, and on day 14 were 38.05 µg/mm³.

Graph 3 indicates that the average water sorption values for all groups increased consistently regardless of the time point. The control group exhibited the lowest solubility value, whereas the 15% group displayed the highest.

In accordance with Table 18, there was a rise in the mean solubility values across three distinct periods i.e. 1st, 7th, and 14th day irrespective of the groups (control, 5%, 10%, and 15%). The highest solubility was observed on day 14, while the lowest was recorded on day 1.

Table 21 illustrates that the mean solubility values increased for all groups (Control, 5%, 10%, 15%) across all time points (day 1, 7, and 14), with evident statistical significance.

It was observed that incorporating antifungal agents into soft liners resulted in increased water-sorption & solubility as the conc. and duration of exposure increased during a 14-day immersion. These findings align with previous research studies [17, 49, 50, 53, 54, 55]. The duration-dependent gradient closely aligned with the study done by Lima et al.[17] who observed increased water-sorption & solubility in soft-liners when enriched with antimicrobial agents like chlorhexidine, ketoconazole, and nystatin over a period of 14 days. Notably, the presence of nystatin did not significantly alter these properties, unlike chlorhexidine and ketoconazole. This observation suggests that certain drugs, due to their molecular characteristics, may induce changes in the polymeric structure, thereby enhancing these properties. This escalation in values for the denture soft liner could be attributed to the potential alteration of the diffusion coefficient by the preparation of *G. glabra*. Additionally, increased leaching of the drug from the denture soft liner, coupled with replacement by smaller molecules such as water and components of artificial saliva, may also contribute to this phenomenon[46].

Furthermore, Jabbal et al.[49] found that acrylic-based soft liners exhibited greater sorption and solubility compared to silicone-based counterparts. He attributed this difference to the increased crosslinking present in the acrylic soft liner, resulting in greater sorption.

Yanikoglu et al.[55] reported increased solubility in artificial saliva in contrast to distilled water & denture cleansing solution for most soft denture liners. Similarly, Kazanji et al.[50] also noted higher solubilities in

saliva against distilled water for various soft-lining materials. This phenomenon is attributed to the composition of synthetic saliva, which comprises various salts and additives that may interact with the soft lining material. The incorporation of *G. glabra* might interfere with the polymeric structure, leading to heightened flow and diffusion through the channels and pores formed within the polymeric matrix leading to increased solubility.

A concentration-dependent increase in water sorption and solubility was observed, with higher concentrations of *G. glabra* exhibiting greater water sorption and solubility. This concentration-related dependency mirrors the findings of Sadeq et al.[53], who incorporated Ag-Zn zeolite into acrylic soft liners, resulting in significantly higher water sorption and solubility. This was attributed to interactions between the liner and the hygroscopic nature of zeolite. Likewise, Chladek et al.[54] observed that water sorption and solubility escalated with aging time and silver nanoparticle concentration, consistent with our results indicating a progressive increase in these properties over time and with higher concentrations of *G. glabra* extract.

Bioactive substances extracted from *Glycyrrhiza glabra* roots possess notable medicinal properties. Typically, the extraction and isolation of these compounds employ macroporous resins like polystyrene and methacrylate.[47] These compounds primarily are complexes of triterpenes, saponins, and flavonoids, which exhibit water solubility.[48] These inherent properties could also potentially impact the solubility and water absorption observed in the study.

Overall, these studies aligned with the findings of the current research, demonstrating a gradual rise in water-sorption & solubility as concentration increased and the days progressed.

Thus, the null hypothesis was rejected as there was a significant difference in anti-fungal activity, water sorption, and solubility of soft-liners added with *G. glabra.*

SCOPE OF THE STUDY

The study aimed to assess and compare the antifungal effectiveness, water absorption & solubility of denture soft-liners containing *G. glabra*.

Future investigations should focus on elucidating the precise mechanism of action underlying *G. glabra* antifungal properties.

In addition, more investigation is necessary to assess *G. glabra's* stability and durability.

Additionally, it is necessary to explore the impact of incorporating these antifungal agents on the physical and surface characteristics of acrylic-based denture soft liners.

Understanding the reasons behind the observed increase in water absorption and solubility following the addition of *G. glabra* to the soft liner requires further investigation.

Furthermore, comprehensive studies are needed to assess various properties of acrylic-based denture soft liners post-incorporation of these antifungal agents, including bond strength, hardness, and color stability.

Considering the limitations of this in-vitro study, future research should incorporate in-vivo parameters under variable clinical conditions. Factors such as saliva presence, temperature fluctuations, pH variations, and chewing cycles have been shown to influence denture liner properties and the efficacy of antifungal drugs in other studies.

CONCLUSION

Keeping in mind the limitations of this in-vitro study, following conclusions were drawn:

- The soft liner containing *G. glabra* exhibited antifungal effectiveness, with higher concentrations (15%) demonstrating greater efficacy compared to lower concentrations (5%, 10%) throughout the 14-day study period. While antifungal activity was evident at 5%, 10%, and 15% concentrations, its effectiveness diminished over time across all concentrations.
- Water sorption notably increased following the inclusion of *G. glabra* at all time intervals across all groups. The 15% concentration displayed the highest water sorption, with levels escalating over the duration of the study.
- The presence of *G. glabra* led to increased solubility across all time intervals and groups. Solubility was most pronounced at the 15% concentration, showing a progressive rise over time.
- Further research is essential to evaluate the biocompatibility of *G. glabra* and its impact on other properties before its clinical application.

SUMMARY

This in-vitro study aimed to evaluate and compare the solubility, water sorption capacity, and antifungal activity of a denture soft-liner infused with Glycyrrhiza glabra (licorice) root extract.

The extract was obtained through Natural Remedies Private Limited, Bangalore, Karnataka. A total of 228 samples were included in this study. 84 samples were assessed for antifungal activity using the well diffusion method. At 3, 7, and 14 days, 14 samples of *G. glabra* at three different concentrations (5%, 10% and 15%) were mixed into the soft liner were evaluated for the zone of inhibition.

To evaluate the water absorption and solubility of the soft-liner, another 144 samples with dimensions of 50mm x 0.5mm were fabricated i.e. 36 control samples (soft liner and liquid alone), and 36 samples for each concentration of *G. glabra*. Each group was further subdivided according to the time of interval of evaluation, which was after 1 day, 7 days, and 14 days. Thus, each subgroup comprised 12 samples. Solubility and water sorption assays were carried out in accordance with ISO specification #10139-2.

The resultant data was charted and subjected to statistical analysis using SPSS software version 25.0. For antifungal activity, comparison of 3 experiment groups (5%, 10%, and 15%) with mean antifungal scores at different treatment time points was done by Generalized linear models (GLM) analysis. The dependent t-test was used to compare the MIZ values in 2 research groups at 3^{rd}, 7^{th}, and 14^{th} day.

For water-sorption & solubility: a dependent t-test was used for comparison of 1^{st}, 7^{th}, & 14^{th} day time points with both mean water sorption and solubility ($\mu g/mm^3$) scores in the 4 study groups. Pair wise comparison of four groups and different time intervals with mean water sorption and solubility ($\mu g/mm^3$) was done by mixed method models analysis.

According to the results obtained, *G. glabra* showed antifungal activity against *Candida albicans.* 15% concentration of *G. glabra* showed the highest activity against *C. albicans* but its activity reduced gradually over the period of 14 days.

There was also a statistically significant increase in denture soft liner solubility and water sorption with the addition of *G. glabra*. The maximum values in both attributes were found at a 15% concentration of *G. glabra*, and the values increased throughout 14 days.

Since the denture soft liner is intended to be used for a shorter duration of time, incorporation of these antifungal agents is not contraindicated. Thus, the infusion of *G. glabra* into the soft-liner can prove to be beneficial to improve the oral health status of geriatric patients with cognitive disturbances, medically compromised conditions and reduced manual dexterity. But further research is required to assess the other physical properties and biocompatibility which could be affected with the addition of such agents.

BIBLIOGRAPHY

1. Emami E, de Souza RF, Kabawat M, Feine JS. The impact of edentulism on oral and general health. International journal of dentistry. 2013 May 8;2013.
2. Douglass CW, Shih A, Ostry L. Will there be a need for complete dentures in the United States in 2020?. The Journal of prosthetic dentistry. 2002 Jan 1;87(1):5-8.
3. Frazer RQ, Byron RT, Osborne PB, West KP. PMMA: an essential material in medicine and dentistry. Journal of long-term effects of medical implants. 2005;15(6).
4. Zafar MS. Prosthodontic applications of polymethyl methacrylate (PMMA): An update. Polymers. 2020 Oct 8;12(10):2299.
5. Sivakumar I, Arunachalam KS, Sajjan S, Ramaraju AV, Rao B, Kamaraj B. Incorporation of antimicrobial macromolecules in acrylic denture base resins: a research composition and update. Journal of Prosthodontics. 2014 Jun;23(4):284-90.
6. Gendreau L, Loewy ZG. Epidemiology and etiology of denture stomatitis. Journal of Prosthodontics: Implant, Esthetic and Reconstructive Dentistry. 2011 Jun;20(4):251-60.
7. Neppelenbroek KH. Sustained drug-delivery system: a promising therapy for denture stomatitis?. Journal of Applied Oral Science. 2016 Sep;24:420-2.
8. Neppelenbroek KH, Pavarina AC, Palomari Spolidorio DM, Sgavioli Massucato EM, Spolidório LC, Vergani CE. Effectiveness of microwave disinfection of complete dentures on the treatment of Candida-related denture stomatitis. Journal of Oral Rehabilitation. 2008 Nov;35(11):836-46.

9. Schneid TR. An in vitro analysis of a sustained release system for the treatment of denture stomatitis. Special Care in Dentistry. 1992 Nov;12(6):245-50.
10. Marín Zuluaga DJ, Gómez Velandia OC, Rueda Clauijo DM. Denture-related stomatitis managed with tissue conditioner and hard autopolymerising reline material. Gerodontology. 2011 Dec;28(4):258-63.
11. Prasad A, Prasad BR, Shetty V, Shastry CS. Tissue conditioners: a review. Journal of Health and Allied Sciences NU. 2014 Jun;4(02):152-7.
12. Tari B, Nalbant D, Dogruman Al FU, Kustimur S. Surface roughness and adherence of Candida albicans on soft lining materials as influenced by accelerated aging. The journal of contemporary dental practice. 2007;8(5).
13. Nevzatoğlu EU, Özcan M, Kulak-Ozkan Y, Kadir T. Adherence of Candida albicans to denture base acrylics and silicone-based resilient liner materials with different surface finishes. Clinical oral investigations. 2007 Sep;11:231-6.
14. Muttagi S, Subramanya JK. Effect of incorporating seed oils on the antifungal property, surface roughness, wettability, weight change, and glucose sorption of a soft liner. The Journal of prosthetic dentistry. 2017 Jan 1;117(1):178-85.
15. Iqbal Z, Zafar MS. Role of antifungal medicaments added to tissue conditioners: A systematic review. Journal of prosthodontic research. 2016 Oct 1;60(4):231-9.
16. Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, Warmington JR, Wyllie SG. The mode of antimicrobial action of the essential oil of Melaleuca alternifolia (tea tree oil). Journal of applied microbiology. 2000 Jan 1;88(1):170-5.
17. Lima JF, Maciel JG, Arrais CA, Porto VC, Urban VM, Neppelenbroek KH. Effect of incorporating antifungals on the water sorption and solubility of

interim resilient liners for denture base relining. The Journal of Prosthetic Dentistry. 2016 May 1;115(5):611-6.

18. Hashem MI. Advances in soft denture liners: An update. J Contemp Dent Pract. 2015 Apr 1;16(4):314-8.
19. Graham BS, Jones DW, Burke J, Thompson JP. In vivo fungal presence and growth on two resilient denture liners. The Journal of prosthetic dentistry. 1991 Apr 1;65(4):528-32.
20. Nevzatoğlu EU, Özcan M, Kulak-Ozkan Y, Kadir T. Adherence of Candida albicans to denture base acrylics and silicone-based resilient liner materials with different surface finishes. Clinical oral investigations. 2007 Sep;11:231-6.
21. Gopalkrishna AH, Seshagiri M, Muddaiah S, Shashidara R. In vitro antifungal activity of different components of Centratherum anthelminticum and Ocimum sanctum seed oils and their synergism against oral pathogenic fungi. Journal of Dental Research, Dental Clinics, Dental Prospects. 2016;10(2):92.
22. Messier C, Epifano F, Genovese S, Grenier D. Licorice and its potential beneficial effects in common oro-dental diseases. Oral diseases. 2012 Jan;18(1):32-9.
23. Yang R, Wang LQ, Yuan BC, Liu Y. The pharmacological activities of licorice. Planta medica. 2015 Dec;81(18):1654-69.
24. Messier C, Grenier D. Effect of licorice compounds licochalcone A, glabridin and glycyrrhizic acid on growth and virulence properties of Candida albicans. Mycoses. 2011 Nov;54(6):e801-6.
25. Lima JF, Maciel JG, Arrais CA, Porto VC, Urban VM, Neppelenbroek KH. Effect of incorporating antifungals on the water sorption and solubility of

interim resilient liners for denture base relining. The Journal of Prosthetic Dentistry. 2016 May 1;115(5):611-6.

26. Garg A, Shenoy KK. A comparative evaluation of effect on water sorption and solubility of a temporary soft denture liner material when stored either in distilled water, 5.25% sodium hypochlorite or artificial saliva: An in vitro study. The Journal of the Indian Prosthodontic Society. 2016 Jan;16(1):53.
27. Höfling JF, Mardegan RC, Anibal PC, Furletti VF, Foglio MA. Evaluation of antifungal activity of medicinal plant extracts against oral Candida albicans and proteinases. Mycopathologia. 2011 Aug;172:117-24.
28. Deo PN, Deshmukh R. Oral microbiome: Unveiling the fundamentals. Journal of oral and maxillofacial pathology: JOMFP. 2019 Jan;23(1):122.
29. O'Donnell LE, Robertson D, Nile CJ, Cross LJ, Riggio M, Sherriff A, Bradshaw D, Lambert M, Malcolm J, Buijs MJ, Zaura E. The oral microbiome of denture wearers is influenced by levels of natural dentition. PloS one. 2015 Sep 14;10(9):e0137717.
30. Bhat V, Sharma SM, Shetty V, Shastry CS, Rao V, Shenoy SM, Saha S, Balaji S. Prevalence of Candida associated denture stomatitis (CADS) and speciation of Candida among complete denture wearers of south west coastal region of Karnataka. Journal of Health and Allied Sciences NU. 2013 Sep;3(03):059-63.
31. Kumar SM, Kumar VA, Natarajan P, Sreenivasan G. Antifungal efficacy and the mechanical properties of soft liners against Candida albicans after the incorporation of garlic and neem: An in vitro study. Journal of International Society of Preventive & Community Dentistry. 2018 May;8(3):212.
32. Krishnamoorthy G, Narayana AI, Peralam PY, Balkrishanan D. To study the effect of Cocos nucifera oil when incorporated into tissue conditioner on its

tensile strength and antifungal activity: An in vitro study. The Journal of the Indian Prosthodontic Society. 2019 Jul;19(3):225.

33. Lima JF, Maciel JG, Arrais CA, Porto VC, Urban VM, Neppelenbroek KH. Effect of incorporating antifungals on the water sorption and solubility of interim resilient liners for denture base relining. The Journal of Prosthetic Dentistry. 2016 May 1;115(5):611-6.
34. Abdallah RM, Aref NS. Curcumin containing soft liner as an alternative treatment modality for oral candidiasis. World Journal of Dentistry. 2021 Nov 24;12(6):435-40.
35. Sri H, Maiti S, Shanmugam R. Comparative Evaluation of Glycyrrhiza glabra Incorporated Soft-Liner Over Conventional Soft-Liner Based on Antimicrobial Activity, Anti-Inflammatory Efficacy, Surface Roughness, Wettability, and Tensile Bond Strength: An: In Vitro: Study. Journal of International Oral Health. 2023 May 1;15(3):290-7.
36. Asl MN, Hosseinzadeh H. Review of pharmacological effects of Glycyrrhiza sp. and its bioactive compounds. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2008 Jun;22(6):709-24.
37. Kumpanich J, Eiampongpaiboon T, Kanchanavasita W, Chitmongkolsuk S, Puripattanavong J. Effect of Piper beetle extract on anti-candidal activity, gelation time, and surface hardness of a short-term soft lining material. Dental Materials Journal. 2020 Nov 27;39(6):1016-21.
38. Bulad K, Taylor RL, Verran J, McCord JF. Colonization and penetration of denture soft lining materials by Candida albicans. Dental materials. 2004 Feb 1;20(2):167-75.

39. Lopes JP, Lionakis MS. Pathogenesis and virulence of Candida albicans. Virulence. 2022 Dec 31;13(1):89-121.
40. Talapko J, Juzbaşic M, Matijevic T, Pustijanac E, Bekic S, Kotris I, Śkrlec I. Candida albicans—the virulence factors and clinical manifestations of infection. J Fungi (Basel) 7: 79.
41. Sidhu P, Shankargouda S, Rath A, Ramamurthy PH, Fernandes B, Singh AK. Therapeutic benefits of liquorice in dentistry. Journal of Ayurveda and integrative medicine. 2020 Jan 1;11(1):82-8.
42. Messier C, Grenier D. Effect of licorice compounds licochalcone A, glabridin and glycyrrhizic acid on growth and virulence properties of Candida albicans. Mycoses. 2011 Nov;54(6):e801-6.
43. Kawano F, Dootz ER, Koran Iii A, Craig RG. Sorption and solubility of 12 soft denture liners. The Journal of prosthetic dentistry. 1994 Oct 1;72(4):393-8.
44. Parr GR, Rueggeberg FA. In vitro hardness, water sorption, and resin solubility of laboratory-processed and autopolymerized long-term resilient denture liners over one year of water storage. The Journal of prosthetic dentistry. 2002 Aug 1;88(2):139-44.
45. Bettencourt AF, Neves CB, de Almeida MS, Pinheiro LM, e Oliveira SA, Lopes LP, Castro MF. Biodegradation of acrylic based resins: A review. Dental materials. 2010 May 1;26(5):e171-80.
46. Brook IM, Douglas CW, Van Noort R. Controlling drug release from acrylic polymers: in vitro studies with potential oral inserts. Biomaterials. 1986 Jul 1;7(4):292-6.
47. Dong Y, Zhao M, Sun-Waterhouse D, Zhuang M, Chen H, Feng M, Lin L. Absorption and desorption behaviour of the flavonoids from Glycyrrhiza glabra

L. leaf on macroporous adsorption resins. Food chemistry. 2015 Feb 1;168:538-45.

48. Sharma V, Katiyar A, Agrawal RC. Glycyrrhiza glabra: chemistry and pharmacological activity. Sweeteners. 2018:87.
49. Jabbal RS, Datta K. Comparative evaluation of water sorption and solubility of two autopolymerizing soft denture liners in distilled water and artificial saliva. Indian Journal of Dental Sciences. 2016 Oct 1;8(4):208-14.
50. Kazanji MN, Watkinson AC. Influence of thickness, boxing, and storage on the softness of resilient denture lining materials. The Journal of prosthetic dentistry. 1988 Jun 1;59(6):677-80.
51. Aref NS. An In vitro assessment of surface roughness, tensile bond strength and antifungal activity of grape seed extract-modified soft liner. J Contemp Dent Pract. 2020 Apr 1;21(4):353-8.
52. Lee MJ, Shim YS, An SY, Kang MK. Surface characterization, biocompatibility and antifungal efficacy of a denture-lining material containing cnidium officinale extracts. Molecules. 2021 Mar 7;26(5):1440.
53. Sadeq HA, Hummudi AP. Water sorption and solubility of acrylic soft liner after incorporation of silver-zinc Zeolite.
54. Chladek G, Kasperski J, Barszczewska-Rybarek I, Żmudzki J. Sorption, solubility, bond strength and hardness of denture soft lining incorporated with silver nanoparticles. International journal of molecular sciences. 2012 Dec 27;14(1):563-74.
55. Yanikoglu ND, Duymus ZY. Comparative study of water sorption and solubility of soft lining materials in the different solutions. Dental materials journal. 2004;23(2):233-9.

ANNEXURE I

ETHICAL CLEARANCE

Research and Ethics Committee
KLE VK INSTITUTE OF DENTAL SCIENCES
A Constituent Unit of KLE Academy of Higher Education & Research
Accredited 'A' Grade by NAAC | Placed in Category 'A' by MHRD (GoI)
Nehru Nagar, Belagavi - 590 010, Karnataka State
☎: 0831-2470362
FAX: 0831-2470640
Web: http://www.kledental-bgm.edu.in
E-mail:principal@kledental-bgm.edu.in

Sl. No. : 1588

CERTIFICATE

ECR/NEW/INST/2021/2435
Research & Ethics Committee

This is to Certify that the synopsis titled

Evaluation of antifungal activity Candida albicans water sorptions and solubility of a soft liner incorporated with Root-extract of Licorice (Glycyrrhiza glabra) - an in vitro study

Submitted by

Dr. **REG. NO- IM0221005** _____ *P. G. Student / Staff, Guided by* _____ *from Department of* Prosthodontics Crown and Bridge *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 3/4/24

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

MEMBER SECRETARY
Research & Ethical Committee
KLEVK Institute of Dental Sciences
BELAGAVI.

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLE VK Institute of Dental Sciences
Belgaum

ANNEXURE II

AUTHENTICATION FORM

natural
QUALITY CONTROL DEPARTMENT

Kosher certified

CERTIFICATE OF ANALYSIS

Product name	:	*Glycyrrhiza glabra* extract ≥ 12% Glycyrrhizin	**Batch No.**	:	FGG2102003
			ULR No.	:	TC400521000000126SP
Botanical name	:	*Glycyrrhiza glabra*	**Lab Reference / Report No.**	:	FP2102034
Product code	:	NRGGE12	**Date of Report**	:	19.02.2021
Part used	:	Root	**Mfg. date**	:	February 2021
Extract ratio	:	8 : 1	**Retest date**	:	February 2023
Solvent used	:	Methanol, water	**Country of origin**	:	India
Excipients	:	Nil	**Type of extract**	:	Powdered extract
			Specification No.	:	NR/QCD/SSPC/GG12%/001

TEST RESULTS

SL. NO.	TESTS	SPECIFICATION	RESULT	TEST PROTOCOL
1.	Description	Light brown to brown, hygroscopic powder	Light Brown hygroscopic powder	Visual
2.	Identification	To pass the test	Passes the test	By TLC
3.	Loss on drying (%w/w)	< 5.0	3.7	As per USP <921> Method III
4.	Acid insoluble Ash (%w/w)	< 3.0	0.3	As per USP <561>
5.	Bulk density (g/cc)	0.20 – 0.80	0.47	As per USP <616> Method – I
6.	Tapped density	0.40 – 1.00	0.74	
7.	Material passing through 30# BS/35 ASTM (%w/w)	> 99.0	100	As per USP <786> Particle size distribution
8.	Heavy Metals			
	Lead	< 3.0 ppm	0.05	ICP –MS
	Arsenic	< 2.0 ppm	0.34	
	Cadmium	< 2.0 ppm	< 0.05	
	Mercury	< 0.1 ppm	< 0.05	
9.	Microbiology Test			
	Total aerobic microbial count	$<10^4$ cfu g^{-1}	*10*	As per USP <61> &<62>
	Total yeast and mould count	$<10^2$ org g^{-1}	*No growth*	
	Bile tolerant gram negative bacteria	$<10^2$ fs g^{-1}	*< 1*	
	E. coli	Absent/g	Absent	
	Salmonella species	Absent/10g	Absent	
	S. aureus	Absent/g	Absent	
10.	Aflatoxins (B_1+B_2+G_1+G_2)	< 5.0 ppb	Not Detected (LOD: 2ppb)	As per USP Test for Aflatoxins
11.	Residual solvent analysis Methanol (ppm)	< 3000	75	As per USP
12.	Pesticide residue analysis	To comply with USP <565>	Complies	As per AOAC/USP
13.	Phytochemical Analysis Glycyrrhizin (% w/w)	≥ 12.0	12.6	By HPLC

Remarks: The above referred batch conforms to the specification of *Glycyrrhiza glabra* extract (≥ 12%Glycyrrhizin) with respect to above mentioned tests.

Storage : The product should be stored in well closed containers, protected from light, moisture and heat, at a temperature between 18°C and 30°C.

AUTHORISED SIGNATORY
DATE:01.12.2021

Natural Remedies Private Limited
Regd. Office & R&D
CIN No: U24232KA1998PTC023573
58, Veersandra Industrial Area, Hosur Road, Electronic City Phase 2, Bangalore 560100. Karnataka – INDIA
Tel: 91 8040209999/8/7,27382265, Fax: 91 80 40209817, Web: www.naturalremedy.com
E-Mail: qc@naturalremedy.com, info@naturalremedy.com

ANNEXURE III

Table 1: Diameter of Inhibition zone (mm) (antifungal activity) of control after 3,7 and 14 days.

SAMPLE NO.	DAY 3	DAY 7	DAY 14
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

ANNEXURE IV

Table 2: Diameter of Inhibition zone (mm) (antifungal activity) of *Glycyrrhiza glabra* at 5% concentration after 3,7 and 14 days.

SAMPLE NO.	DAY 3	DAY 7	DAY 14
1	11	6	0
2	8	5	0
3	9	6	0
4	10	5	0
5	9	4	0
6	11	5	0
7	9	4	0
8	9	6	0
9	8	4	0
10	10	5	0
11	9	4	0
12	11	6	0
13	10	5	0
14	11	6	0

ANNEXURE V

Table 3: Diameter of Inhibition zone (mm) (antifungal activity) of *Glycyrrhiza glabra* at 10% concentration after 3,7 and 14 days.

SAMPLE NO.	DAY 3	DAY 7	DAY 14
1	14	8	3
2	13	9	3
3	14	8	4
4	12	8	2
5	15	9	5
6	13	7	2
7	15	8	4
8	14	9	5
9	12	7	3
10	15	8	5
11	13	7	4
12	13	6	3
13	14	8	5
14	13	7	3

ANNEXURE VI

Table 4: Diameter of Inhibition zone (mm) (antifungal activity) of *Glycyrrhiza glabra* at 15% concentration after 3,7 and 14 days.

SAMPLE NO.	DAY 3	DAY 7	DAY 14
1	20	14	8
2	22	16	9
3	20	15	8
4	21	16	9
5	19	13	6
6	22	16	7
7	18	14	8
8	21	15	7
9	23	17	9
10	21	14	8
11	22	15	6
12	20	15	7
13	23	16	8
14	19	14	6

ANNEXURE VII

WATER SORPTION

Table 5: Water sorption values of control on days 1,7 and 14.

SAMPLE NO.	DAY 1	DAY 7	DAY 14
1	52.53	81.56	105.42
2	47.91	63.25	96.79
3	65.20	80.61	113.45
4	54.20	68.94	95.78
5	50.40	56.42	87.50
6	55.20	60.42	96.82
7	46.90	64.53	101.20
8	53.30	74.28	106.40
9	57.45	59.54	97.80
10	62.25	67.61	99.43
11	57.76	65.87	95.70
12	58.90	71.84	97.81

ANNEXURE VIII

Table 6: Water sorption values of 5% *Glycyrrhiza glabra* on days 1,7 and 14.

SAMPLE NO.	DAY 1	DAY 7	DAY 14
1	93.81	140.56	179.45
2	102.80	150.42	191.62
3	100.14	158.67	194.82
4	98.45	148.43	188.74
5	106.63	170.20	193.85
6	102.45	165.50	201.56
7	97.56	149.65	186.65
8	102.89	142.67	184.72
9	110.45	163.60	199.43
10	97.31	157.83	197.21
11	96.85	163.21	203.24
12	94.35	161.19	186.4

ANNEXURE IX

Table 7: Water sorption values of 10% *Glycyrrhiza glabra* on days 1,7 and 14.

SAMPLE NO.	DAY 1	DAY 7	DAY 14
1	132.84	165.56	201.20
2	136.56	183.21	214.43
3	146.76	178.74	216.4
4	132.67	176.32	206.78
5	133.54	176.41	216.41
6	139.98	184.64	224.76
7	135.76	183.21	206.81
8	147.32	168.94	210.76
9	132.45	170.42	219.56
10	137.65	176.43	221.85
11	139.56	177.53	223.61
12	130.81	177.2	215.53

ANNEXURE X

Table 8: Water sorption values of 15% *Glycyrrhiza glabra* on days 1,7 and 14.

SAMPLE NO.	DAY 1	DAY 7	DAY 14
1	176.83	211.23	257.43
2	184.21	223.65	261.42
3	178.71	214.34	241.56
4	186.41	212.88	277.87
5	185.54	233.70	265.45
6	189.21	230.41	262.43
7	185.40	226.56	268.42
8	176.41	224.42	255.87
9	180.41	220.65	260.42
10	182.42	220.43	259.87
11	197.32	215.89	253.54
12	185.41	216.78	257.78

ANNEXURE XI

WATER SOLUBILITY

Table 9: Solubility values of control on days 1,7 and 14.

SAMPLE NO.	DAY 1	DAY 7	DAY 14
1	19.20	23.65	26.54
2	17.63	24.65	24.53
3	18.23	20.12	22.78
4	20.65	23.65	25.43
5	17.04	20.76	23.67
6	17.82	21.76	24.48
7	16.34	24.75	26.73
8	18.45	23.79	26.32
9	18.95	20.41	23.85
10	17.43	22.55	25.97
11	20.42	21.80	25.90
12	18.43	21.34	24.80

ANNEXURE XII

Table 10: Solubility values of 5% *Glycyrrhiza glabra* on days 1,7 and 14.

SAMPLE NO.	DAY 1	DAY 7	DAY 14
1	21.65	25.67	29.65
2	24.89	26.34	30.12
3	25.76	28.54	30.26
4	22.74	27.07	31.45
5	24.32	25.82	29.98
6	22.65	27.63	30.41
7	22.87	28.21	31.45
8	22.65	26.56	30.54
9	24.56	25.78	28.45
10	22.43	26.87	29.17
11	24.04	26.32	29.13
12	23.86	25.21	30.41

ANNEXURE XIII

Table 11: Solubility values of 10% *Glycyrrhiza glabra* on days 1,7 and 14.

SAMPLE NO.	DAY 1	DAY 7	DAY 14
1	27.87	30.65	33.56
2	26.50	28.78	32.13
3	27.67	30.65	33.75
4	27.89	30.76	33.21
5	25.76	29.65	32.40
6	27.78	29.65	32.65
7	28.63	30.43	34.83
8	26.65	31.21	33.78
9	25.78	29.43	33.52
10	27.45	30.76	34.56
11	28.67	30.54	32.45
12	26.56	28.64	32.12

ANNEXURE XIV

Table 12: Solubility values of 15% *Glycyrrhiza glabra* on days 1,7 and 14.

SAMPLE NO.	DAY 1	DAY 7	DAY 14
1	34.43	37.65	40.87
2	28.76	32.45	34.56
3	30.81	35.57	38.67
4	30.54	35.67	38.45
5	28.21	32.78	36.70
6	31.23	34.78	37.52
7	32.81	35.65	37.54
8	34.52	38.53	40.63
9	32.56	35.62	38.43
10	30.81	34.61	37.42
11	31.21	34.89	38.54
12	32.56	35.56	37.31