"COMPARATIVE EVALUATION OF ANTIFUNGAL ACTIVITY AGAINST *CANDIDA ALBICANS*, WATER SORPTION AND SOLUBILITY OF A SOFT LINER INCORPORATED WITH *THYMUS VULGARIS* (THYME) - MONTMORILLONITE -AN IN VITRO STUDY"

BY

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LIST OF ABBREVIATIONS USED IN THE STUDY

ABBREVIATIONS	FULL FORMS
C. albicans	*Candida albicans*
T. vulgaris	*Thymus vulgaris*
DS	Denture stomatitis
EO	Essential oil
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
ANOVA	Analysis Of Variance
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 natural extract of *Thymus vulgaris* has proven to be effective against *Candida albicans* hence, thyme essential oil was incorporated into the soft liner along with montmorillonite as a carrier.

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 *thyme-montmorillonite.*

METHODS

A total of 216 samples were taken and they were divided into two groups: 72 samples to check for antifungal activity and 144 samples to check for water sorption and solubility. *Thyme-montmorillonite* was incorporated into the soft liner at 3%, 5%, and 7% and checked for antifungal activity by measuring the zone of inhibition for 3, 7, and 12 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, One-way ANOVA and Two-way ANOVA. There was a statistically significant difference between the control and experimental group ($P < 0.05$) in antifungal property and water sorption and solubility. 7% thyme-montmorillonite showed maximum antifungal activity which gradually decreased over the period of 12 days with a zone of 20mm on day 3 and 14mm on day7 and 6mm on day 12, and it had showed increasing water sorption and solubility over the period of 14 days.

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 *thyme-montmorillonite* 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*, Thyme, Montmorillonite, Antifungal, Water Sorption, Solubility

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INTRODUCTION

For health and quality of life, an appropriate dentition is crucial. Despite improvements in preventative dentistry, edentulism continues to be a significant global public health issue.[1] It has been said that edentulism, a crippling and irreversible disorder, is the "ultimate marker of the burden of diseases for oral health"[1]. Even though the prevalence of total tooth loss has decreased over the past ten years, edentulism is still a serious condition, especially in elderly people.[1] As a result, the demand for full dentures will continue to be great and difficult for the foreseeable future.[2]

Prosthodontic treatment for the elderly and edentulous population is rapidly increasing in developed countries, particularly removable dentures.[3,4] In 2011, a national dental survey found that 89% of adults aged 75 and older had removable dental prostheses.[3] Polymers are the only material widely used in removable denture fabrication (methyl methacrylate).[4]

Complete dentures are generally made with acrylic resins because they have a number of desirable qualities, including good physiochemical characteristics that can endure biting force and, as a result, prevent breakage and deformation. However, this rigidity may make the patient uncomfortable, lead to mucosal lesions, or exacerbate already present diseases.[5] And, also due to it's high-water absorption, it is prone to denture plaque contamination. This factor appears to boost the capacity of *Candida albicans* to colonise the mucosal surface of the denture and the denture itself, where it acts as an opportunistic pathogen causing a range of clinical problems as angular cheilitis and denture stomatitis (DS).[6]

Denture stomatitis (DS) is a prevalent oral mucosal condition where trauma from ill-fitting dentures causes erythema or inflammation of the palatal surface, results in pain and discomfort to the acrylic denture wearers.[7] Approximately 72% of denture wearers are affected by this condition.[8] It is otherwise familiar as denture sore mouth, prosthetic stomatitis, inflammatory papillary hyperplasia, chronic denture palatitis, chronic atrophic candidiasis, stomatitis prosthetica, and denture related stomatitis.[9]

Despite having a complex etiology, the major cause of this condition is the colonization of acrylic bases and mucosa of denture wearers by species of *Candida* spp., particularly *Candida albicans*, which is present in 50 to 98% of cases.[10]

DS can be treated in a variety of ways, such as local antifungal and systemic therapy, oral hygiene maintenance, cleaning and disinfection of dentures, replacement of worn-out dentures, removal of anatomic irregularities, restoration of an atraumatic occlusion and removal of the denture at night.[10]

Whilst systemic antifungal therapy is advised for immunocompromised individuals, these medications have the potential to have negative systemic effects such as hepatotoxic and nephrotoxic effects, as well as interactions with other medications. [10] Nystatin and miconazole are two common topical antifungal medications used to treat DS. These antifungal medications can effectively treat the clinical symptoms and signs of DS caused by *Candida* spp., but they are unable to achieve a therapeutic antifungal concentration on the inner surfaces of dentures. [10] Failure to sustain therapeutic concentrations of antifungal medications on the surface of dentures, could be due to 1) Reduction in antifungal activity caused by salivary flow, swallowing, and tongue motions; 2) Patient noncompliance with antifungal therapy due to the high cost of drugs, the taste of topical agents, wearing dentures continuously, and rigorous medication schedule; 3) Continuous contact between infected internal denture surfaces and irritated mucosa, which encourages re-infection of the mucosa and damages supporting tissues, lengthening the clinical course of the disease.[11,12]

Consequently, following traditional therapy with topical and systemic antifungal medications, re-infection of the treated oral mucosa may happen. Clinical relapse and reappearance in the two weeks following therapy make treating denture stomatitis difficult. [10]

An alternative remedy in these circumstances is to apply soft liners beneath the tissue surface of dentures.[13]

Materials called soft denture liners create a cushioned layer between the oral mucosa and the hard denture foundation. By absorbing part of the masticatory pressures, the liners aid in more uniformly transferring the stresses to the underlying tissues. [13] In addition to being utilized on a long-term basis for full denture bases for patients who are unable to bear the stress brought on by dentures, soft liners are also used to heal abused tissues[13], relining of maxillofacial prosthesis, management of patients with xerostomia, ridge resorption and oral defects.[2] It's elasticity guarantees that it will return to its original shape while resilience controls how quickly the material recovers from deformation. So, they are also called resilient liners. [13]

Based on their chemical makeup, soft denture liners are divided into four groups: silicone rubbers, vinyl resins, polyurethane and polyphosphazene type rubbers, and plasticized acrylic (chemical or heat-cured) resins.[14]

Nevertheless, investigations have shown that *C. albicans* may adhere to soft lining materials as well.[15] The surface characteristics of denture liners, low pH, the composition of saliva, and diets high in carbohydrates all hasten the colonisation and adherence of *C. albicans*, which leads to the development of DS. Because of plasticizer leaching and the persistent use of high-concentration denture cleansers, soft liners deteriorate. The inherently rough surface of soft liners, memory loss, and lack of dexterity to cleanse the prostheses all encourage the chemical elements necessary to act as receptors for the fungal colonization of dentures.[16]

The introduction of antifungal drugs into soft liners has been recommended as a strategy to halt this cycle of infection and reinfection.[10] Drugs incorporated into denture liners prevent denture biofilm from interacting with infected tissues. In this regard, the application of soft lining materials is strongly recommended since it improves the healing of injured tissues as well as the patient's comfort. Since short-term soft liners have a life cycle of about 14 days, the length of time needed to treat DS using these medication-modified materials is comparable to the time needed when using traditional topical antifungal agents.[10]

Numerous synthetic medications have been used, including nystatin, ketoconazole, fluconazole, miconazole, etc., but they have drawbacks and side effects such as the development of drug resistance and systemic complications.[17]

The recent resurgence of natural health has contributed to an increase in interest in naturopathic therapies. Medicinal plant extracts and essential oils like Tea tree oil, Origanum oil, Lemongrass essential oil, etc. have been used in developing countries as alternative treatments to health problems. The fundamental advantages of natural medicinal plant extracts as antibacterial agents are their enhanced safety and stability, which are lacking in both organic and inorganic antimicrobial agents.[18,19]

Introducing antifungal/antimicrobial agents to polymeric materials in commercially available concentrations can effectively inhibit the growth of *C. albicans*, but it may change their morphological structure and properties such as water sorption, solubility, tensile strength, and peel bond strength, elastic modulus and weight, hardness, roughness.[10]

As a result, an in-vitro study was carried out to compare and assess the antifungal activity of thyme oil combined with montmorillonite added to soft liners as well as its impact on water absorption and soft liners' solubility.

NEED FOR THE STUDY

Soft liners are used underneath ill-fitting dentures, to manage abused tissues, create functional impressions, condition tissue during implant healing, and reline maxillofacial prosthetics.[20] It serves to alleviate the pressure that the hard denture base exerts on the mucosa by acting as a cushion between the tissue supporting the denture and the base.[21] Soft liners or resilient denture liners are frequently employed to treat irritated supporting mucosa by stabilizing ill-fitting dentures and supplying tissue rest.[20]

However, it is well established that these materials are susceptible to bacterial and fungal species easy colonization and infection because the imperfections of the denture base and the materials used for denture relining allow yeast cells to become trapped, leading to denture stomatitis.[20]

The pathological condition of the mucosa called denture stomatitis is brought on by damage from improperly fitting dentures. The palatal mucosa underneath the denture will become generally inflamed or red.[17]

Although there are several potential causes of DS, Candida species, *particularly C. albicans*, are thought to be the primary culprit. In addition to *C. albicans*, denture stomatitis has also been linked to additional risk factors, including denture damage, poor oral and denture cleanliness, continuous and night time denture use, xerostomia, and altered salivary pH.[17]

Different products, such as synthetic antimicrobials like nystatin, fluconazole, miconazole, ketoconazole, etc., have been combined with soft liners to reduce the problem of colonisation, and have a slow continuous release and a sustained therapeutic effect but these medications have several drawbacks, including the increased emergence of multidrug resistant bacteria. As a result, herbal drugs with antifungal action are becoming increasingly popular due to their less well-known side effects.[17,22,23]

Natural agents like Origanum oil, Thai herbs, *C.nutans, C. sappan*, lemongrass oil, seed oils like *Ocimum sanctum, Linum usitatissimum*. Many inorganic antifungal agents were also used like silver zeolite, photocatalyst, silver nanoparticles, and magnesium oxide.[17]

The aromatic perennial plant *Thymus vulgaris* (thyme), which belongs to the *Lamiaceae* family, is native to the Mediterranean area and has been utilized by people all over the world as an aromatic, food-preserving, and therapeutic herb.[24]

T. vulgaris has been utilised in traditional medicine for many years. It could be used as mouth rinse, toothpaste, or aromatherapy to prevent and treat related oral infections due to its significant antibacterial action against *S.pyogenes, S.mutans, C.albicans, A.actinomycetemcomitans, and P.gingivalis*.[24]

Its antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, anticancer, anti-hypertensive, and anti-nematode properties are attributed to its two primary components, thymol and carvacrol.[25]

The disadvantage of directly incorporating essential oil into polymer materials is that, because of their volatile nature, they would quickly bloom to the surface and be lost by evaporation, resulting in a loss of antibacterial action. Controlled release of EO into the polymers would be required to offset this quick loss of action.[26]

Encapsulating therapeutics with a carrier system can result in improved drug stability and a regulated release profile. Furthermore, by adding a carrier system to hydrophobic medicines, a solubilizing effect can be achieved, increasing bioavailability.[27]

For this reason, montmorillonite, a porous clay mineral with a 2:1 layered structure and exchangeable cations between the layers, can be employed. The 2:1 layer consists of 2 tetrahedral silica sheets sandwiched between two octahedral alumina sheets. These cations are easily substituted by other organic or inorganic cations, which is related to montmorillonite's unusual hydrophilicity, swelling, adsorption, and fluidity features.[28]

Heating a drug over its melting point with montmorillonite has been tried and shown to be successful for adsorbing hydrophobic medicines. It has a wide surface area and is hydrophilic, which increases drug wettability and consequently the release rate of hydrophobic medicines.[27]

Introducing antimicrobial agents to these materials can effectively inhibit the growth of *C. albicans*, but it may change their morphological structure and physical properties such as water sorption, solubility, tensile strength, and peel bond strength, elastic modulus and weight, hardness, roughness.[10]

Water sorption and solubility are the common issues faced when soft liners are exposed to oral fluids. When not in use, the liners are either soaked in saliva inside the oral cavity or kept or exposed to water/aqueous cleansing solutions. Water and saliva are thus absorbed during immersion, and plasticizer and other water-soluble components are leached away resulting in a steady loss of resilience, increased hardness values, and reduced cushioning effect.[29]

Therefore, this study is taken to evaluate the antifungal, water sorption and solubility of the soft liner when incorporated with thyme-montmorillonite powder.

HYPOTHESIS

NULL HYPOTHESIS:

- There is no significant difference in the antifungal activity, water sorption, and solubility of the denture soft liner incorporated with thyme-montmorillonite.

ALTERNATIVE HYPOTHESIS:

- There is a significant difference in the antifungal activity, water sorption, and solubility of the denture soft liner incorporated with thyme-montmorillonite.

AIMS AND OBJECTIVES

AIM OF THE STUDY:

- To evaluate the antifungal activity against *Candida albicans*, water sorption, and solubility of a denture soft liner incorporated with Thyme–Montmorillonite at different concentrations.

OBJECTIVES:

- To evaluate and compare the antifungal activity against *Candida albicans* of a denture soft liner incorporated with Thyme-Montmorillonite powder at different concentrations.

- To evaluate and compare the water sorption and solubility of denture soft liner incorporated with Thyme-Montmorillonite.

REVIEW OF LITERATURE

1. **Graham et al (1991)** conducted an in vivo study to check the presence and growth of fungi on 2 resilient denture liners namely Veltec and Coe Comfort for 30 days. They concluded that both the liners supported the presence and growth of oral fungi without any difference between the two. He had concluded that since both the denture liners had fungal growth, they should be used along with antifungal agents.[20]

2. **Kawano et al (1994)** determined the solubility and sorption of 12 denture liners which included 9 copolymers, 2 silicones, and 1 polyphosphazene fluoroelastomer (Soft-Pals, Justisoft, Velvesoft, Molloplast-B, Flexor. Novus, Durosoft Verno-Soft, Vinsoft Super Soft, ProTech, and Prolastic) and was assessed for 1 week, 1, 3, and 6 months and 1 year. The study resulted in that sorption was 0.8mg/mm^3 for Molloplast-BFlexor, Prolastic soft, and Durosoft denture liners after 7 days and after a year the sorption value was less for Prolastic and Molloplast-B soft liners.[30]

3. **El-Hadary et al (2000)** In the study silicone-based soft liner (Luci-sof) was tested against a plasticized acrylic resin soft liner (Permasoft), using two processing techniques: laboratory-processed and auto-polymerized at chairside for the latter. The study assessed tensile bond strength, sorption, and solubility in mineral water for 1, 4, and 6 weeks. They concluded that the lower water sorption and solubility, and higher tensile bond strength of Luci-sof may improve clinical outcomes and success.[31]

4. **Kulak Ozkan et al (2002)** The goal of this study was to find out about elderly people's oral hygiene practices, denture cleanliness, yeast presence, and denture stomatitis. Swabs from the palate were obtained and mycologically examined to identify the yeast colonies. There was no statistical correlation between DS, denture brushing frequency, or denture cleaning procedures. However, there was a statistically significant relationship between DS, the presence of yeasts, and the cleanliness of dentures. [32]

5. **Parr et al (2002)** conducted a study to evaluate the material property changes of 2 new resilient denture lining materials with different polymerization modes (auto polymerization and conventional laboratory processing). The study concluded that the stiffness values of the laboratory-processed material were higher and after 1 week of water storage, the stiffness increased. Water sorption rates for the 2 products were comparable after 6 months and 1 year. At 30 days, 6 months, and 1 year significantly less solubility was noted for auto-polymerized resins.[33]

6. **Yilmaz et al (2004)** conducted a study to assess the effects of disinfectants like 3.5% Savflex, and 5% Deconex used for 10 minutes 5.25% sodium hypochlorite, and 2% sodium hypochlorite used for five minutes on the physical properties of temporary soft denture liner. The outcome of this study was that the disinfectants had significant effects on water sorption. Water solubility and hardness tests also had a remarkable effect on the liner with the use of disinfectants. Thus, they came to the conclusion that disinfection remarkably changes the properties of water sorption, solubility, and stiffness of the soft liners.[34]

7. **Yanikoglu et al (2004)** conducted a study in which they concluded that after 16 weeks of aging, the acrylic resin soft lining materials had better solubility (3.432% Visco-gel in artificial saliva) and absorption (3.349% Visco-gel in distilled water) than Molloplast-B. Acrylic soft lining materials demonstrated the highest toughness and color change.[35]

8. **Leo'n et al. (2005)** investigated and compared the water sorption, solubility, and tensile bond strength of denture lining materials copolymerized using different procedures following heat cycling. Light Liner was shown to have lower solubility levels, according to the study. Microwave radiation was used to polymerize Ever Soft, resulting in a high tensile strength. The adhesive/cohesive failure of materials polymerized using microwave radiation and visible light was noticeable. [36]

9. **Baena-Monror et al. (2005)** studied the presence of *C. albicans*, *Staphylococcus aureus*, and *Streptococcus mutan*s on the prosthesis and mucosal membrane in individuals with and without DS. The prevalence of *C. albicans, S. aureus, and S. mutans* was found to be 52.4%, 53.4%, and 68.6%, respectively. *C. albicans* was found in 86% of denture stomatitis patients, but *S. mutans* was found in just 16% of these individuals. They concluded that *C. albicans, S. aureus, and S. mutans* colonised the oral cavity of individuals using dental prosthesis on a regular basis, and that denture stomatitis was more common in these patients.[37]

10. **Falah-Tafti et al (2010)** *C. albicans* were used to test the efficacy of two commonly used antifungal agents mixed with tissue conditioner. *C. albicans*

cannot adhere to tissue conditioner with 1% to 10% nystatin or 10% fluconazole. [38]

11. **Hofling et al (2011)** This study compared the antifungal fluconazole and amphotericin B, as well as proteinase inhibitors pepstatin A, amprenavir, and ritonavir, to the generation of proteinases by *C. albicans* from clinical isolates and the activity of plant extracts (*Casearia Rosmarinus*, *Mentha*, *Tabebuia, Arrabidaea, and Arctium*) against *C. albicans* and its generated proteinases. The findings revealed that these extracts have a specific activity and that the hunt for novel antifungal drugs might begin at the plant level. [39]

12. **Evren et al (2011)** This study focused on the elderly residents of three different retirement homes. DS was found in 44% of those with dentures. Differences in educational attainment, income, dental visits, denture status, brushing methods and frequency ($p = 0.001$) were found between residential homes. Poor denture hygiene habits were linked to denture-related stomatitis.[40]

13. **Bhat et al (2013)** *Candida* associated denture stomatitis (CADS) is caused by the conversion of the typical oral commensal *Candida* spp. into a pathogen under favorable circumstances. Immunocompromised status, ill-fitting prosthesis, other systemic illnesses, and the patient's inappropriate denture care are a few of the causes that change the oral balance into an unhealthy and inadequate foundation for the wearing of the prosthesis. The prevalence of denture stomatitis caused by the fungus *Candida* has been found to be between 65 and 70% of denture users globally. The most common species that causes infection has been *C. albicans*; however, there has lately been a shift to non-*Candida albicans* species.[41]

14. **Srivatstava et al (2013)** The antifungal activity, surface roughness, and tensile bond strength of tissue conditioner were evaluated after adding Origanum oil. 60% Origanum oil in tissue conditioner reduces *C. albicans* adherence without affecting its bond strength to heat polymerized acrylic resin.[42]

15. **Sunanda Sharma et al (2014)** The in vitro study compares the antifungal activity of *Melaleuca alterfolia* oil and fluconazole in tissue conditioner. Study concluded that 30% *M. alterfolia* oil was superior to 5% fluconazole in tissue conditioner as an antifungal agent. Both showed antifungal activity against *C. albicans* for 24 hours.[43]

16. **Amornvit et al (2014)** conducted an in-vitro study to check the inhibitory efficacy of lemon grass when integrated with COE-COMFORT tissue conditioner opposing *C. albicans*. They concluded that oil of lemon grass oil has evidently exhibited an impressive anti-Candida effect.[44]

17. **Pachava et al (2015)** The study assessed whether tea tree oil in soft denture liner would inhibit *C. albicans* growth and show activity up to 60 days. These results suggest that tea tree oil could be used as an effective antifungal to treat DS and other oral infections.[45]

18. **Iqbal et al (2016)** This review examined the current knowledge on antifungal agents in tissue conditioners for treating denture-induced stomatitis. Antifungal agents in tissue conditioners are effective and have minimum or no effect on the physical and mechanical properties of the tissue conditioners. The conclusion was that adding antifungal agents into commercially available tissue conditioners can help treat DS.[17]

19. **Krishnamurthy et al (2016)** examined differences in surface roughness, retention, colonization, and penetration of *C. albicans*, and antifungal effect among 4 different denture liners (Molloplast B, Ufi Gel Hard C Permaflex, GC Soft Liner). The study concluded that rougher surfaces retain more cells than smooth surfaces, denture lining materials allow *Candida* penetration through their structure; and denture lining materials offer no anti-fungal activities.[46]

20. **Garg et al (2016)** The water sorption of the GC RELINE denture liner material was greatest in distilled water, followed by 5.25% sodium hypochlorite, and least in Shellis artificial saliva at 4-, 7-, and 11-day intervals. However, on the 15th day, the findings showed that 5.25% sodium hypochlorite had the highest water sorption, followed by distilled water, and artificial saliva had the lowest. At 4-, 7-, 11-, and 15-day intervals, the solubility of the GC RELINE soft liner was greatest in artificial saliva, followed by distilled water, and lowest in 5.25% sodium hypochlorite.[29]

21. **Lima et al (2016)** conducted a study to check the solubility and sorption of the antifungals in denture soft liners. The result of the investigation was that at the 14-day interval increase in water sorption was only seen in the Chlorhexidine group when compared with the control group. For all the intervals, the solubility increased with the incorporation of chlorhexidine and ketoconazole when compared with the control group. They concluded that the inclusion of nystatin and ketoconazole in both Trusoft and Softtone and the inclusion of Chlorhexidine in true soft had no influence on water sorption after two weeks. The solubility of these two materials wasn't affected by the inclusion of nystatin for up to 14 days.[47]

22. **Fani M et al (2017)** tested *Thymus vulgaris* oil for antibacterial activity against 30 clinical isolates of *S. pyogenes, S. mutans, C. albicans, P. gingivalis, and A. actinomycetemcomitans*. Thyme oil at dosages ranging from 16 to 256 µg/ml inhibited all clinical isolates with inhibition zones ranging from 7.5 to 42 mm when assessed using the agar disc diffusion technique. *C. albicans'* minimum inhibitory concentration was determined to be 16 µg/ml.[24]

23. **Seshagiri Muttagi et al (2017)** investigated surface roughness, antifungal properties, wettability, glucose sorption, and weight change. They concluded that adding *L. usitatissimum, O. sanctum, and C. anthelminticum* seed oils to soft liners remarkably reduced *C. albicans* progression, decreased surface roughness, and glucose absorption, and showed better wettability.[16]

24. **Jabbal et al (2017)** conducted a study to assess and compare the solubility and water sorption of two soft liners namely Viscogel (acrylic-based soft liner) and Mollosil (silicone based soft liner) at different time intervals of 7 days, 30 days and 3 months in both artificial saliva and distilled water. They concluded that at all the intervals high rates of absorption and solubility were seen in Viscogel than Mollosil both in artificial saliva and distilled water.[48]

25. **Das et al (2018)** conducted a study to estimate and compare the solubility and water sorption of an acrylic resin soft liner with a silicone liner. The study resulted that after six weeks of immersion, Eversoft had higher solubility and absorption than Molloplast B. They also concluded that due to a decrease in solubility and water sorption Molloplast B dispensed better clinical success.[14]

26. **Kumar et al (2018)** investigated the in vitro growth inhibition of *C. albicans* in soft-liner materials derived from resin-based denture soft lining materials treated with neem or garlic using the streaking method after 2, 4, and 7 days. *C. albicans* was inhibited by neem and garlic applied to an acrylic soft liner. When compared to the control group, both neem and garlic had favourable outcomes against *C. albicans.* Within the constraints of this in vitro investigation, it was discovered that neem and garlic can be employed as a tissue conditioner addition to minimise *C. albicans* adhesion.[15]

27. **Maciel et al (2018)** conducted a study to assess the solubility and water sorption of seven tissue conditioning materials like Coe Comfort, Softtone, Rite-line, Dura conditioner, Hydrocast, Dentusoft, and Viscogel and two interim resilient liners Trusoft and CoeSoft throughout their lifespans. They concluded that Coecomfort, Rite-Line, Dura Conditioner, Hydrocast, Dentusoft, Coe Soft and Truesoft were better denture liners when used for seven days. Among these materials, Coe comfort, Coe soft and trusoft were observed to be the best acceptable denture lining materials when used for 14 days.[49]

28. **Joseph AM et al (2019)** investigated the impact of organically modified montmorillonite inclusion in 1%, 3%, and 5% by weight on the flexural strength, linear polymerization shrinkage, and surface hardness, of heat cure denture base material. It was determined that adding 1 wt.% nanoclay improved surface hardness and minimized linear polymerization shrinkage of the resin. However, there was no substantial improvement in the flexural strength of the resultant nanocomposite.[50]

29. **Anagha et al (2019)** The silicone-based soft liner Mollosil demonstrated the highest percentage of sorption and solubility in artificial saliva than in distilled water, while an acrylic-based Viscogel demonstrated the highest percentage of sorption in artificial saliva and the lowest percentage of sorption in distilled water, with the exception of Molloplast-B, which demonstrated the highest percentage of sorption and solubility in distilled water when compared to all of these soft lining materials.[51]

30. **Krishnamoorthy et al. (2019)** conducted a study to assess the antifungal efficacy and tensile strength of tissue conditioners having *Cocos nucifera* oil. They found that incorporating 10% w/w *Cocos nucifera* into Viscogel tissue conditioner reduced *Candida* colonisation and increased tissue conditioner tensile strength.[52]

31. **Jafri et al (2020)** *T. vulgaris* and thymol were tested for effectiveness against medication-resistant isolates of *Candida* species. The study's findings highlight the promising role of T. *vulgaris* and thymol as alternative agents in the treatment of biofilm-associated with *C. albicans* and *C. tropicalis* infections, as well as their synergistic interaction with antifungal drugs (fluconazole and amphotericin B) that could be used to combat infection caused by drug-resistant *Candida* species.[53]

32. **Ramos et al (2020)** created Poly Lactic Acid (PLA) films by incorporating thymol and commercial montmorillonite at various concentrations. Several analytical approaches were utilized to assess the effects of adding nano clay and thymol on the characteristics of PLA nano-biocomposites. The addition of 8 wt.% thymol and 2.5 wt.% montmorillonite enhanced the antibacterial effect

against *E. coli* and *S. aureus*. The inclusion of thymol and montmorillonite lowered the modulus of elasticity, according to the tensile tests.[54]

33. **Naoe et al (2020)** investigated the antimicrobial activity, mechanical characteristics, and biocompatibility of a new antimicrobial tissue conditioner comprising Cetylpyridinium chloride (CPC) in combination with montmorillonite (CPC-Mont). The addition of CPC-Mont to the tissue conditioner dramatically reduced the amounts of both adherent and free-floating C. albicans, and this effect lasted for a week. Furthermore, the mechanical characteristics and biocompatibility of the tissue conditioners were equivalent to currently available tissue conditioners.[55]

34. **Hejazi et al (2021)** Study's findings suggest that the *C. copticum L*. EO-loaded tissue conditioner can treat both injured mucosa and denture stomatitis. The *Carum copticum L.* essential oil-loaded tissue conditioner demonstrated appropriate physical, biological, and release qualities as a new therapy for denture stomatitis.[56]

35. **AZ Godil et al (2021)** conducted an in-vitro study to investigate the activity of incorporated antifungal agents like Fluconazole and *Ocimum sanctum* oil (Tulsi) in the denture soft liners to reduce the risks of *C. albicans.* They concluded that this approach allows the prolonged drug release in the oral cavity which simultaneously treats the injured denture-bearing tissues and the infection, biofilms of *Candida* without compromising on their physical properties.[57]

36. **Khaled Hosny et al (2021)** conducted an in vitro study to produce an oregano essential oil-based nanoemulsion (OEO-SNEDD) that would have antibacterial

and antifungal effects against oral microbiota. This study concluded that nano emulsion essential oil can provide good protection against oral microbial infections like *C. albicans* and *S. mutans*.[58]

37. **Lee et al (2021)** conducted an in-vitro study to investigate the antifungal efficacy and surface characteristic of soft denture liner linked with herbal *Cnidium officinale* extracts and concluded that material having the extract showed antimicrobial efficacy against *C. albican*s and exhibited no significant difference between the surface characterization of the experimental and control group.[59]

38. **Asahara et al (2022)** The regulated release of CPC from a tissue conditioner comprising CPC-Mont, antibacterial activity *(S. aureus, C. albicans, and S. mutans)*, and oral mucosa irritation were investigated in this work. The CPC release test was carried out in three test solutions: distilled water, 0.2 M NaCl, and 0.2 M HCl daily for 28 days. The quantity of CPC released each day, as well as the total amount released over 28 days (6.12 mg), was less than the daily safe maximum of sore throat medications (8 mg). Furthermore, TC with CPC-Mont might maintain antibacterial action against adhering bacteria for 14 days and has no possibility for oral mucosa irritation.[60]

MATERIALS AND METHODOLOGY

SOURCE OF DATA:

This study was conducted in

1. KLE Academy of Higher Education and Research
2. Department of Prosthodontics and Crown & Bridge, KLE Vishwanath Katti Institute of Dental Sciences, Belagavi (for preparation of test specimens)
3. Department of Microbiology, Jawaharlal Nehru Medical College, Belagavi (for microbiological procedures)
4. College of Pharmacy, Belagavi (for preparation of Thyme-Montmorillonite)
5. Dr. Prabhakar Kore’s Basic Science Research Centre, Belagavi (for water sorption and 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 abnormalities.

SAMPLE SIZE ESTIMATION

Sample size was obtained by the formula:

$$n = \frac{2S^2(Z_\alpha + Z_\beta)^2}{d^2}$$

Where,

$S1 = 0.1021$, $S2 = 0.1619$, $S = 0.132$

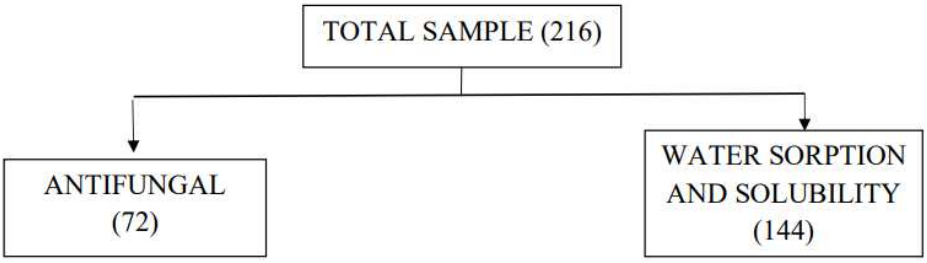
$Z_\alpha = 2.58$ at 1% α-error

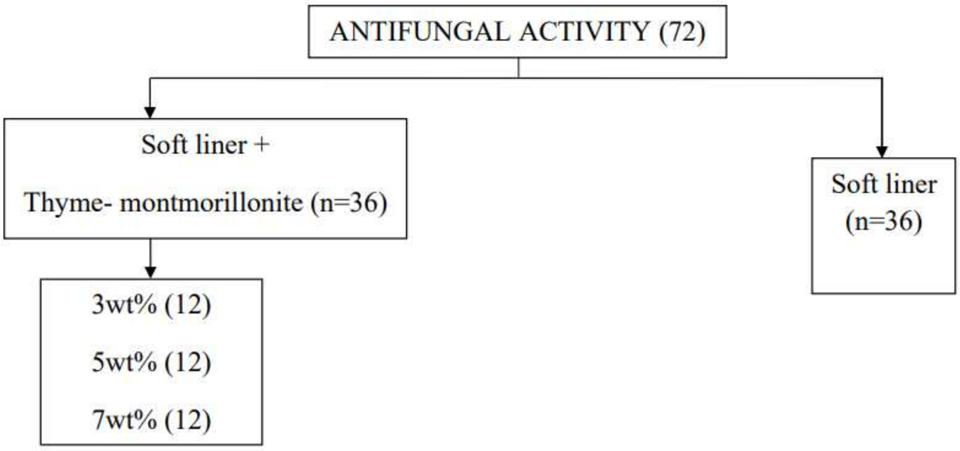
$Z_{\beta} =$ 1.68 at 5% β-error

$d = 0.1354$

$n = 36$

Total sample size= 216





Zone of inhibition will be checked at the end of 3rd day, 7th day and 12th day.

Table 1: Sample size for Antifungal activity

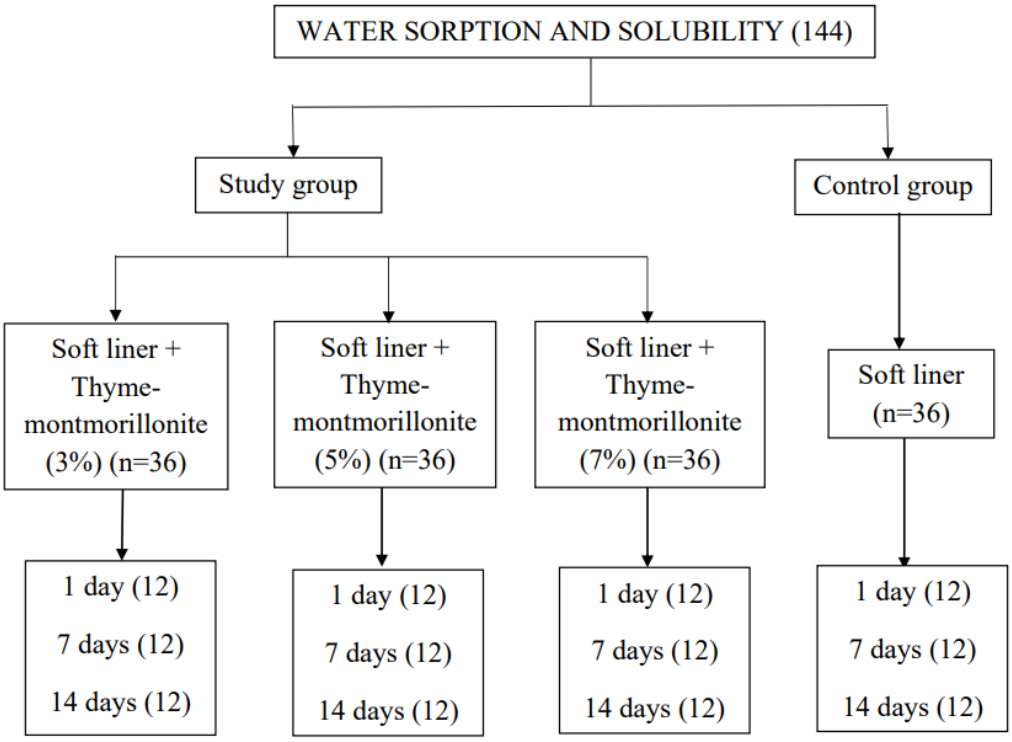
Total of 72 samples were taken to assess the antifungal efficacy. These samples were divided into 2 groups. They are-

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

Group 2: Thyme-montmorillonite incorporated in soft liner

Group 2 was further divided into 3 subgroups of concentration 3%(2a), 5%(2b) and 7%(2c) concentration with 12 samples each.

Table 2: Sample size for water sorption and solubility



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

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

Group 2: Thyme-montmorillonite incorporated in soft liner

Group 2a: 3% Thyme-montmorillonite incorporated in soft liner

Group 2b: 5% Thyme-montmorillonite incorporated in soft liner

Group 2c: 7% Thyme-montmorillonite incorporated in soft liner

Water sorption and solubility was checked on 1, 7, and 14 days for all the groups.

MATERIALS USED IN THE STUDY

Table 3: Material used in the study.

MATERIALS	DESCRIPTION	MANUFACTURER
Thyme essential oil	Essential oil	Commercially available
Montmorillonite	Clay mineral	Sigma-Aldrich
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	-	Indiamart
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 WERE CONDUCTED DURING THE RESEARCH

PREPARATION OF THYME-MONTMORILLONITE MIXTURE:

5g of Montmorillonite powder was dried in a china dish at 90°C for 1 hour to remove moisture. In a china dish, 5grams of Thyme essential oil was heated to 100°C.

Thyme oil vapours were condensed into a montmorillonite bed stored in a funnel tube over the china dish. The heating process was repeated until the vapours were completely absorbed into the montmorillonite bed.

Montmorillonite was then extracted and weighed from the funnel tube. Adsorbed montmorillonite was stored in an air-tight container.[61]

TO CHECK FOR THE ANTIFUNGAL PROPERTY IN DENTURE SOFT LINER:[16]

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 37C. 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, 3% of thyme montmorillonite in soft liner showed the minimum inhibition zone of 10mm against *Candida albicans*) Soft liner powder of different concentrations of 3%, 5%, 7%, 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.

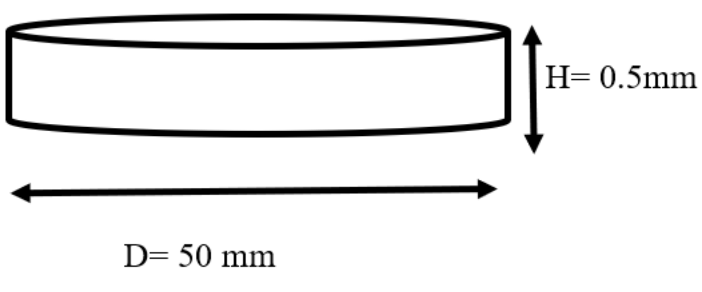
12 samples were punched for each concentration (3%, 5%, 7%) and the same was done for the control.

The agar plates were then put in an incubator at 37^0C for 12 days. The diameter of the inhibition zone (DIZ) was measured at the end of the third, seventh and twelfth day using a scale. The measured diameter of the inhibition zone was subjected to statistical analysis.

TO CHECK FOR THE WATER SORPTION AND SOLUBILITY IN DENTURE SOFT LINER:[29]

Master die fabrication

A metal die of 50mm diameter and 0.5mm thickness was fabricated in accordance with ADA specification no. 12 and was used for the fabrication of the specimens.



Sample fabrication

The soft-liner powder of control and of the different concentrations (3%, 5%, 7%) 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 37^0C for 3 evaluation periods: 1 day, 7 days, and 14 days.

Water sorption and Solubility assay:[29,47]

Sorption and solubility assays were done according to ISO specification #10139-2. Specimens were set in a desiccator containing silica gel and stored in an incubator at 37^0C for desiccation. All the specimens were weighed daily in a digital analytic balance until a persistent mass was obtained. The mass of the specimens was considered steady when the dissimilarity between the average of each assessment period was below or equal to 0.0002g over 24 hours. After the initial mass (w1) was estimated, the specimens were placed in 250ml of artificial saliva in a sterile beaker at 37^0C in an incubator for 1 day (24 hours), 7 days, and 14 days of 36 samples each.

The artificial saliva was made-up of the following components: 0.1g NaCl, 0.1gKCl, 0.345g NaH_2PO_4, 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. Afterward, the excess saliva was eliminated from the specimens with absorbent papers until the specimen was free of visible moisture. Then, for another 15 seconds, the specimens were enabled to air dry and measured again one minute after removal from the artificial saliva (w2). It denotes the weight of the specimen after absorption of artificial saliva. Finally, all specimens were again subjected to desorption using silica gel as previously described and were weighed again (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

w1 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 $V= \pi r^2 h$, 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.

PREPARATION OF THYME-MONTMORILLONITE MIXTURE

Montmorillonite powder was dried in china dish at 90℃ for1 hour.

⇩

Thyme oil was heated in a china dish at 100℃.

⇩

Vapours of thyme oil get condensed into montmorillonite bed kept over the china dish in a funnel tube.

⇩

Heating was continued till vapours were completely adsorbed into montmorillonite bed.

⇩

Montmorillonite was removed from the funnel tube and weighed.

⇩

Adsorbed montmorillonite was stored in an airtight container.

TO CHECK FOR THE ANTIFUNGAL PROPERTY[16]

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

⇩

Sabouraud dextrose agar was prepared and was poured in 90mm diameter petri plates.

⇩

Culture petri plates were then streaked with inoculum and kept for drying.

⇩

Wells were punched (6mm diameter, 5mm depth) with a sterile cork borer.

⇩

The punched wells are filled with soft liner with varying concentrations of thyme-montmorillonite powder (3wt%, 5wt%, 7wt%) and the control.

⇩

Agar plates were then incubated at 37˚±1°C for 12 days.

⇩

DIZ was measured with a metallic scale after the third, seventh and twelfth day of incubation.

TO CHECK FOR THE WATER SORPTION AND SOLUBILTY

Disk shaped specimens of dimensions 50mmx0.5mm were made of soft liner of different concentrations and control

⇩

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

⇩

Disks were weighed to an accuracy of 0.001g using a digital analytic balance.

⇩

Specimens were weighed daily until a constant mass is obtained (0.002 g over 24 hours) w1.

⇩

Specimens were immersed in 250ml artificial saliva and placed in an incubator at 37^0C for 1 day, 7 days, and 14 days.

⇩

Excess water was removed with absorbent papers and the specimens were measured after the specified time accordingly (w2).

⇩

Specimens were treated to desiccation again as previously done.

⇩

Specimens were weighed again after the evaluation time (w3).

⇩

Water sorption and solubility were then calculated separately for water and artificial saliva using the formulae:

Water sorption = (w2-w1)/V

Water solubility = (w1-w3)/V

Figure 1: Thyme essential oil

Figure 2: Montmorillonite

Figure 3: Soft liner

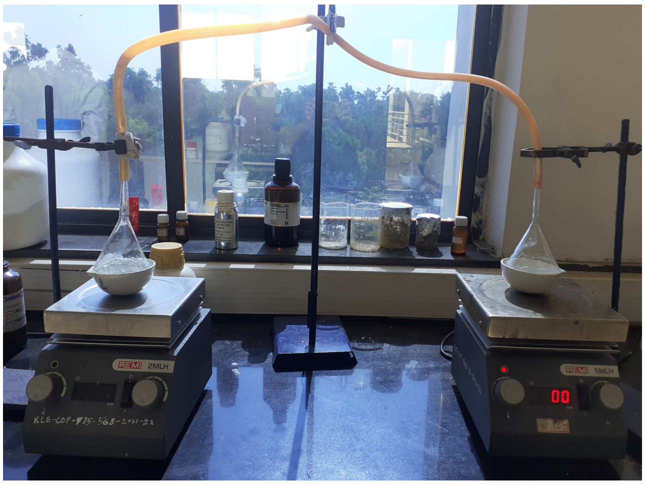


Figure 4: Preparation of thyme- montmorillonite

Figure 5: Thyme- montmorillonite

Figure 6: Soft liner with different concentration

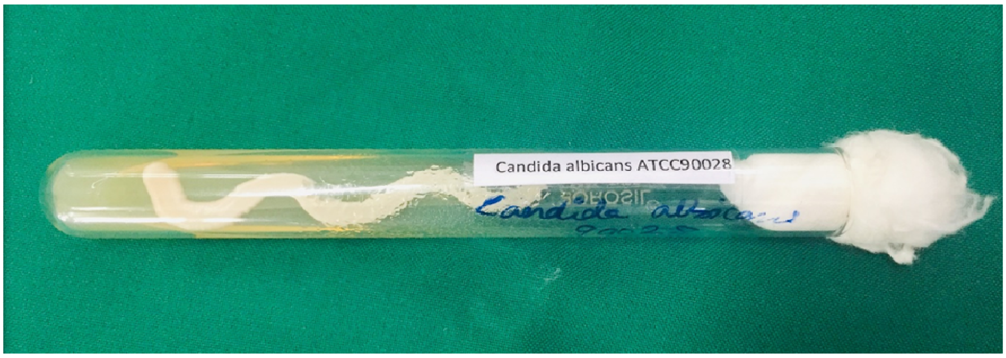


Figure 7: Candida albicans strain

Figure 8: Streaking of Candida albicans inoculum on the Saboraud dextrose agar

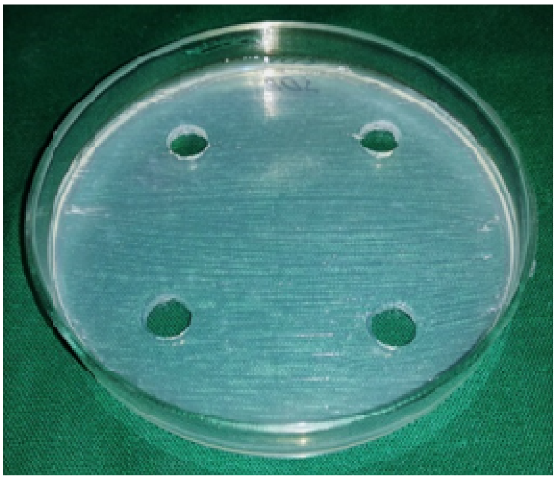


Figure 9: Wells of 6mm diameter and 5mm depth punched

Figure 10: Soft liner mixed in a jar

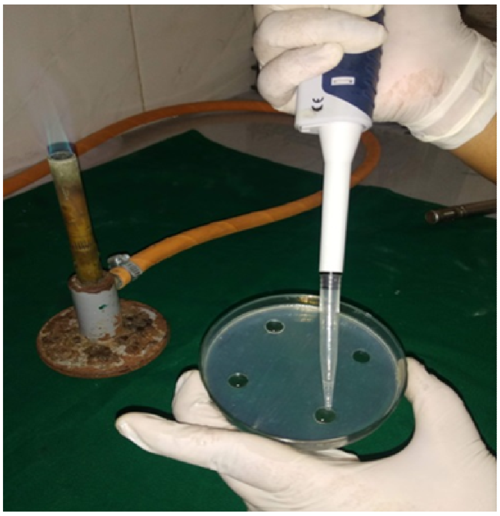


Figure 11: Punched wells filled with soft liner

Figure 12: Incubator

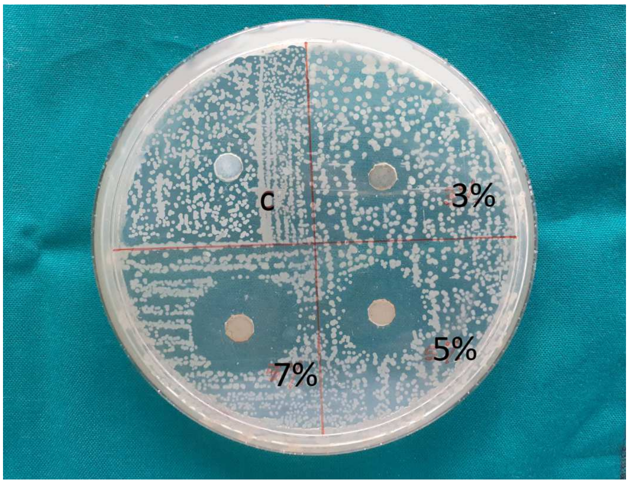


Figure 13: Soft liner with control, 3%, 5%, and 7% on day 3

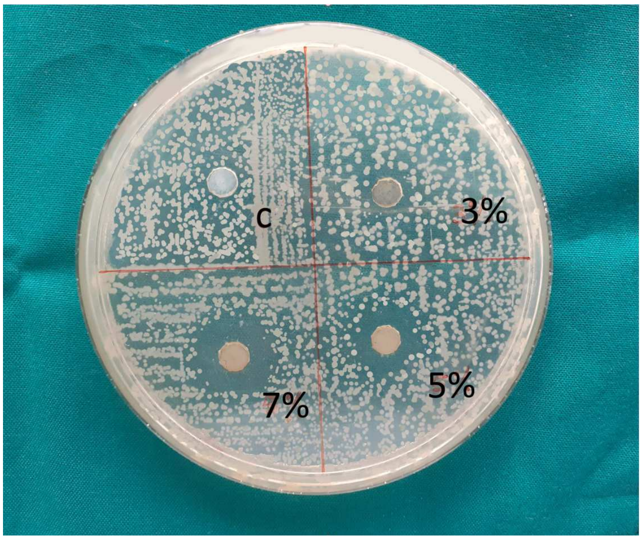


Figure 14: Soft liner with control, 3%, 5%, and 7% on day 7

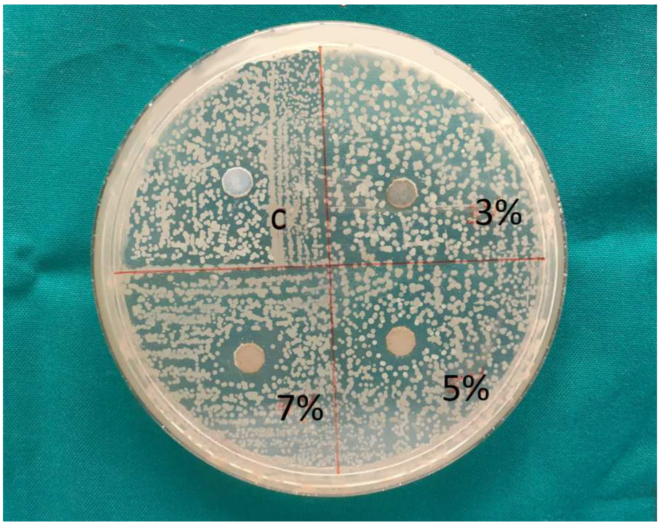


Figure 15: Soft liner with control, 3%, 5%, and 7% on day 12

Figure 16: Specimens of control group for water sorption and solubility for day 1

Figure 17: Specimens of control group for water sorption and solubility for day 7

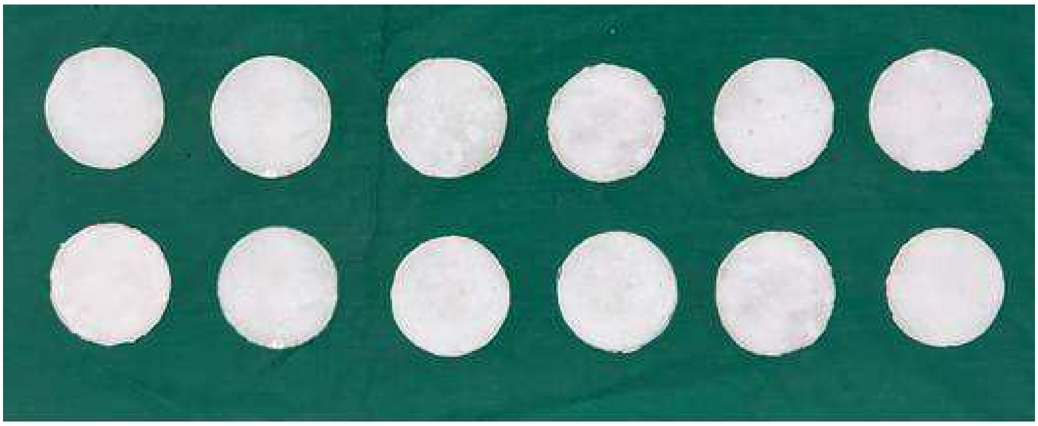


Figure 18: Specimens of control group for water sorption and solubility for day 14

Figure 19: Specimens of 3% thyme-montmorillonite group for water sorption and solubility for day 1

Figure 20: Specimens of 3% thyme-montmorillonite group for water sorption and solubility for day 7

Figure 21: Specimens of 3% thyme-montmorillonite group for water sorption and solubility for day 14

Figure 22: Specimens of 5% thyme-montmorillonite group for water sorption and solubility for day 1

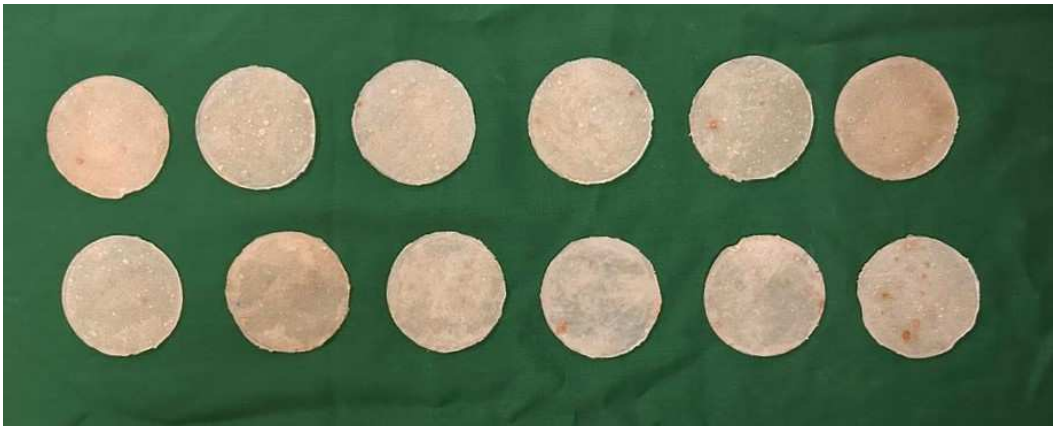


Figure 23: Specimens of 5% thyme-montmorillonite group for water sorption and solubility for day 7

Figure 24: Specimens of 5% thyme-montmorillonite group for water sorption and solubility for day 14

Figure 25: Specimens of 7% thyme-montmorillonite group for water sorption and solubility for day 1

Figure 26: Specimens of 7% thyme-montmorillonite group for water sorption and solubility for day 7

Figure 27: Specimens of 7% thyme-montmorillonite group for water sorption and solubility for day 14

Figure 28: Weighing of the specimens in digital analytic balance

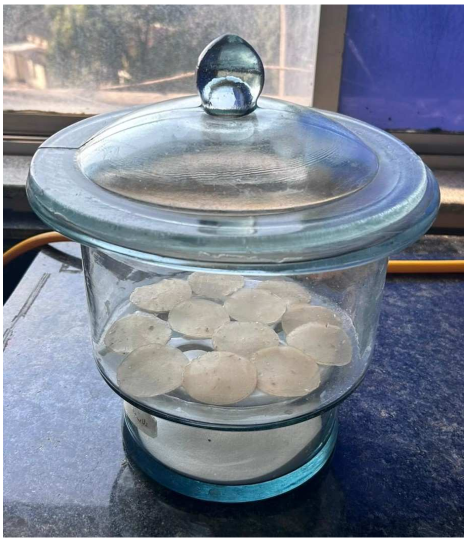


Figure 29: Specimens placed in the desiccator

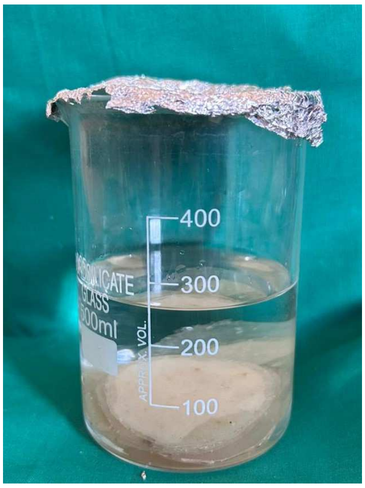


Figure 30: Specimens in artificial saliva

RESULTS

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

Statistical Analysis

Data obtained from the study was entered in Microsoft excel sheet and SPSS version 20 software was used to carry out the statistical analysis. Descriptive statistics were applied to describe the basic features of the data.

Dependent t-test was used for comparison of 3 groups with MIZ in millimetres (mm) at different time intervals and pair-wise comparison of 4 groups (control, 3%, 5%, 7%) with MIZ scores at different time points by Newman-keuls multiple post hoc procedures. Comparison of the 3 experiment groups with the mean antifungal score at different time points (days 3, 7, and 12) was done by One-Way ANOVA.

Comparison of 4 groups and 3 different time points (days 1, 7, and 14) with mean water sorption and solubility was done by Two-Way ANOVA. Pair-wise comparison of 4 groups and different time points was done by Newman-keuls multiple post hoc procedure.

Table 4: Comparison of three experiment groups (3%, 5%, and 7%) with mean Antifungal scores at different treatment time points by One-Way ANOVA followed by Newman-Keuls post hoc procedures

Time points	Summary	Experiment group			F-value	p-value	Pair-wise comparisons		
		Experiment 3%	Experiment 5%	Experiment 7%			3% vs 5%	3% vs 7%	5% vs 7%
Day 3	Mean	8.83	13.75	20.50	266.8265	0.0001*	p=0.0001*	p=0.0001*	p=0.0001*
	SD	1.11	1.29	1.31					
Day 7	Mean	3.25	6.17	14.92	419.0789	0.0001*	p=0.0001*	p=0.0001*	p=0.0001*
	SD	0.62	1.19	1.16					
Day 12	Mean	0.00	3.00	6.83	236.2203	0.0001*	p=0.0001*	p=0.0001*	p=0.0001*
	SD	0.00	0.95	0.94					
Day 3-Day 7	Mean	5.58	7.58	5.58	5.6927	0.0324*	p=0.0168*	P=1.0000	p=0.0064*
	SD	1.24	1.93	1.78					
Day 3-Day 12	Mean	8.83	10.75	13.67	29.8505	0.0001*	p=0.0047*	p=0.0001*	p=0.0002*
	SD	1.11	1.60	1.83					
Day 7-Day 12	Mean	3.25	3.17	8.08	61.7262	0.0001*	p=0.8705	p=0.0001*	p=0.0001*
	SD	0.62	1.75	1.08					

*p<0.05

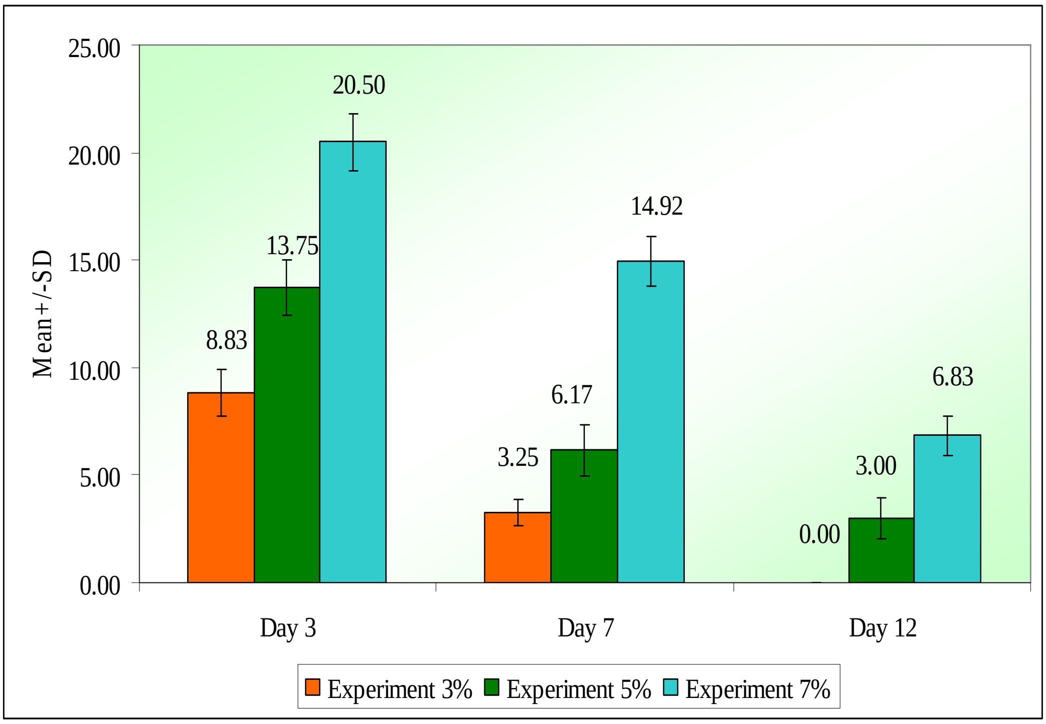
Antifungal activity was evaluated for soft liner incorporated with *thyme-montmorillonite* for three different concentrations of 3%, 5%, and 7% at three different time intervals i.e., at 3 days, 7 days, and 12 days. The control group showed no antifungal activity and therefore was not considered for statistical analysis.

Mean and standard deviation was calculated. The mean value for the minimum inhibition zone calculated in millimeters for the 3% *thyme-montmorillonite* group on days 3, 7, and 12 were 8.83, 3.25, and 0 respectively. The mean value for the 5% thyme-montmorillonite group on days 3, 7, and 12 were 13.75mm, 6.17mm, and 3mm.

For the 7% *thyme-montmorillonite* group on days 3, 7, and 12 were 20.50mm, 14.92mm, and 6.83mm.

A pair wise comparison of the 3 groups with MIZ scores at different time points was done by a Newman-keuls post hoc procedure. The table 4 compares antifungal activity measured by the zone of inhibition between the groups at different concentrations. Thus, there was a statistically significant difference ($p < 0.05$) in zone of inhibition at different concentrations within the groups. The highest zone of inhibition (antifungal activity) was seen in 7% group and the lowest in 3% group.

Graph 1: Comparison of three experiment groups (3%, 5%, and 7%) with mean Antifungal scores at different treatment time points



In accordance with graph 1, on the same day, the zone of inhibition (mm) grew as the concentration of thyme-montmorillonite did. The increase in zone of inhibition in ascending order according to this graph is as follows 3%< 5%< 7%.

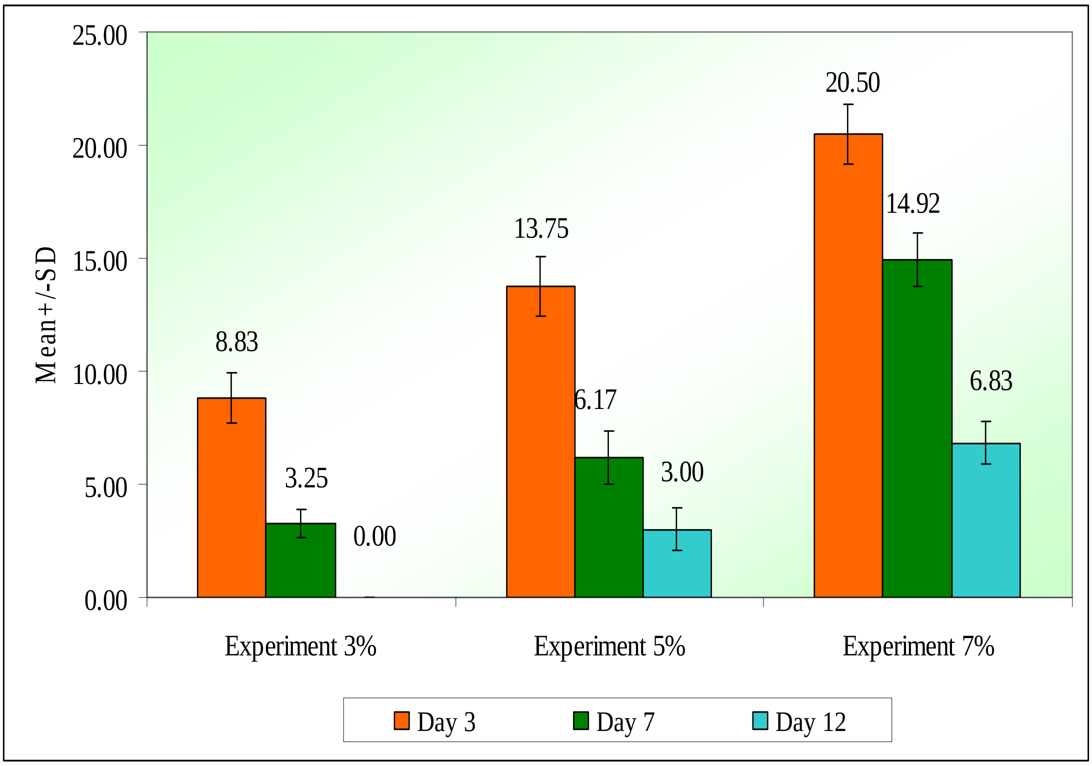
Table 5: Comparison of different treatment time points with mean Antifungal scores in three experiment groups (3%, 5% and 7%) by dependent t test

Groups	Times	Mean	SD	Mean Diff.	SD Diff.	% Of change	t-value	p-value
Experiment 3%	Day 3	8.83	1.11	5.58	1.24	63.19	15.5964	0.0001*
	Day 7	3.25	0.62					
	Day 3	8.83	1.11	8.83	1.11	100.00	27.4524	0.0001*
	Day 12	0.00	0.00					
	Day 7	3.25	0.62	3.25	0.62	100.00	18.1124	0.0001*
	Day 12	0.00	0.00					
Experiment 5%	Day 3	13.75	1.29	7.58	1.93	55.13	13.6206	0.0001*
	Day 7	6.17	1.19					
	Day 3	13.75	1.29	10.75	1.60	78.18	23.2373	0.0001*
	Day 12	3.00	0.95					
	Day 7	6.17	1.19	3.17	1.75	51.38	6.2703	0.0001*
	Day 12	3.00	0.95					
Experiment 7%	Day 3	20.50	1.31	5.58	1.78	27.22	10.8559	0.0001*
	Day 7	14.92	1.16					
	Day 3	20.50	1.31	13.67	1.83	66.68	25.9307	0.0001*
	Day 12	6.83	0.94					
	Day 7	14.92	1.16	8.08	1.08	54.16	25.8406	0.0001*
	Day 12	6.83	0.94					

*$p<0.05$

Comparison of the different days on days 3, 7 and 12 with respect to MIZ in all 3 groups (3%, 5%, 7%) was done by dependent t test (Table 5). Statistically, significant difference was seen between all the groups on all the days ($p<0.05$).

Graph 2: Comparison of different treatment time points with mean Antifungal scores in three experiment groups (3%, 5% and 7%)



According to graph 2 there was a decrease in the zone of inhibition (mm) as the time interval (Days 3, 7, and 12) increased for the same group. All the groups had the highest zone of inhibition on day 3 followed by day 7 and day 12. 7% thyme-montmorillonite group had the highest zone of inhibition on all the days.

Table 6: Summary of water sorption in four groups and three-time points

Factors	Level of Factor	Mean	SD	SE	95% CI for mean	
					Lower	Upper
Group	Control group	73.42	20.02	3.34	66.64	80.19
	Experiment 3%	148.83	39.06	6.51	135.61	162.04
	Experiment 5%	175.34	32.93	5.49	164.20	186.48
	Experiment 7%	221.70	32.55	5.42	210.69	232.71
Times	Day 1	118.48	48.54	7.01	104.38	132.57
	Day 7	154.64	56.64	8.18	138.20	171.09
	Day 14	191.34	60.07	8.67	173.90	208.78
Group*Time	Control group with Day 1	54.05	5.46	1.58	50.58	57.52
	Control group with Day 7	67.53	7.82	2.26	62.56	72.50
	Control group with Day 14	98.66	6.46	1.86	94.56	102.77
	Experiment 3%with Day 1	99.81	5.11	1.47	96.57	103.06
	Experiment 3%with Day 7	154.94	10.01	2.89	148.58	161.29
	Experiment 3%with Day 14	191.73	7.71	2.23	186.83	196.63
	Experiment 5%with Day 1	136.06	5.73	1.65	132.41	139.70
	Experiment 5%with Day 7	175.88	6.09	1.76	172.01	179.75
	Experiment 5%with Day 14	214.08	7.72	2.23	209.18	218.99
	Experiment 7%with Day 1	183.99	7.09	2.05	179.49	188.49
	Experiment 7%with Day 7	220.22	6.82	1.97	215.89	224.55
	Experiment 7%with Day 14	260.89	6.71	1.94	256.63	265.16

Table 6 shows the mean, Standard Deviation (SD), and Standard Error (SE) of water sorption values of all the groups independent of time, the mean, SD and SE water sorption values of days independent of groups, and the mean, SD and SE water sorption values of all the groups (3%, 5%, 7%) on all the days (1,7, and 14).

Table 7: Comparison of four groups and three time points with mean water sorption by Two-Way ANOVA

Sources of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	p-value
Main effects					
Groups	416045.53	3	138681.84	2821.8579	0.0001*
Times	127421.32	2	63710.66	1296.3661	0.0001*
2-way interaction effects					
Groups* Times	8562.16	6	1427.03	29.0367	0.0001*
Error	6487.22	132	49.15		
Total	558516.23	143			

*p<0.05

A comparison of four groups and three time points was done by Two-Way ANOVA. Table 7 depicts that there was a statistically significant difference ($p<0.05$) between all the groups at all the time points.

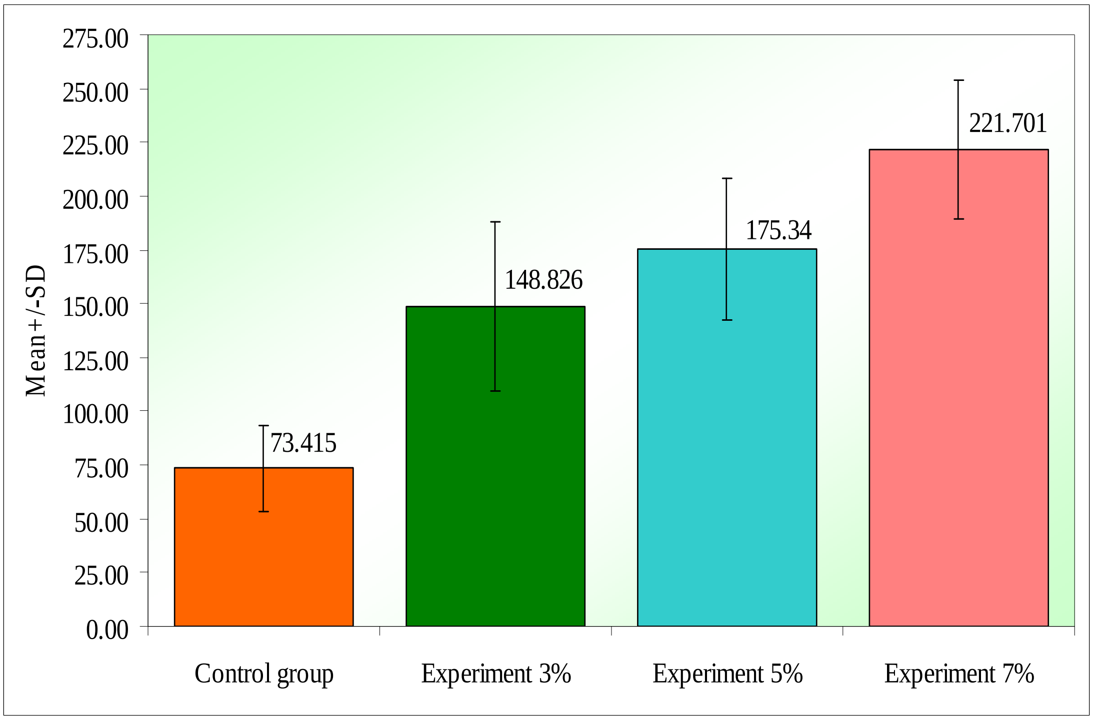
Table 8: Pair-wise comparison of four groups with mean water sorption by Newman-Keuls multiple post hoc procedures

Groups	Control group	Experiment 3%	Experiment 5%	Experiment 7%
Mean	73.415	148.826	175.340	221.701
SD	20.017	39.063	32.932	32.550
Control group	-			
Experiment 3%	p=0.0001*	-		
Experiment 5%	p=0.0001*	p=0.0001*	-	
Experiment 7%	p=0.0001*	p=0.0001*	p=0.0001*	-

*p<0.05

Pair-wise comparison of four groups with mean water sorption was done by Newman-Keuls multiple post hoc procedures. Table 8 compares the water sorption of 4 groups (control, 3%, 5%, and 7%) independent of the time points. The water sorption values increased as follows in ascending order: control< 3%< 5%< 7%.

Graph 3: Comparison of four groups with mean water sorption by Newman-Keuls multiple post hoc procedures



According to graph 3, there was an increase in the mean water sorption values of all the groups irrespective of the time points. 7% has the highest water sorption value and the control group had the lowest water sorption value.

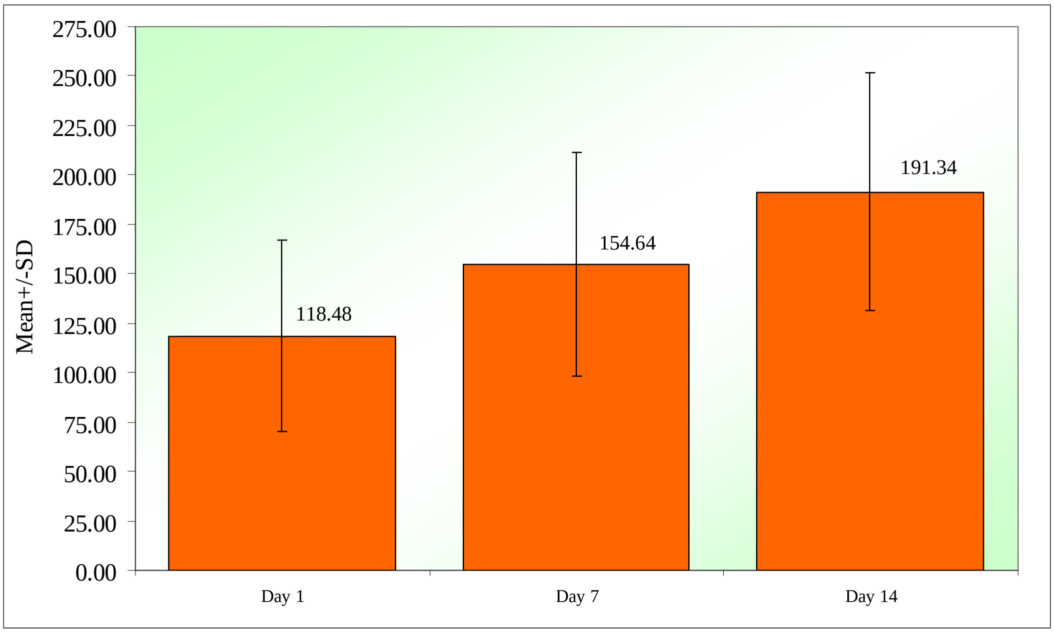
Table 9: Pair-wise comparison of three time points with mean water sorption by Newman-Keuls multiple post hoc procedures

Times	Day 1	Day 7	Day 14
Mean	118.48	154.64	191.34
SD	48.54	56.64	60.07
Day 1	-		
Day 7	P=0.0001*	-	
Day 14	P=0.0001*	P=0.0001*	-

*p<0.05

Pair-wise comparison of three-time points with mean water sorption was done by Newman-Keuls multiple post hoc procedures. Table 9 compares water sorption at 3 different time points (days 1, 7, and 14) independent of the groups (control, 3%, 5%, and 7%). The water sorption values increased as follows in ascending order: day 1< day 7 < day 14.

Graph 4: Comparison of 3 time points with mean water sorption by Newman-Keuls multiple post hoc procedures



According to graph 4, there was an increase in the mean water sorption values at 3 different time points (days 1, 7, and 14) irrespective of the groups (control, 3%, 5%, and 7%). Day 14 showed the highest water sorption while day 1 had the lowest water sorption.

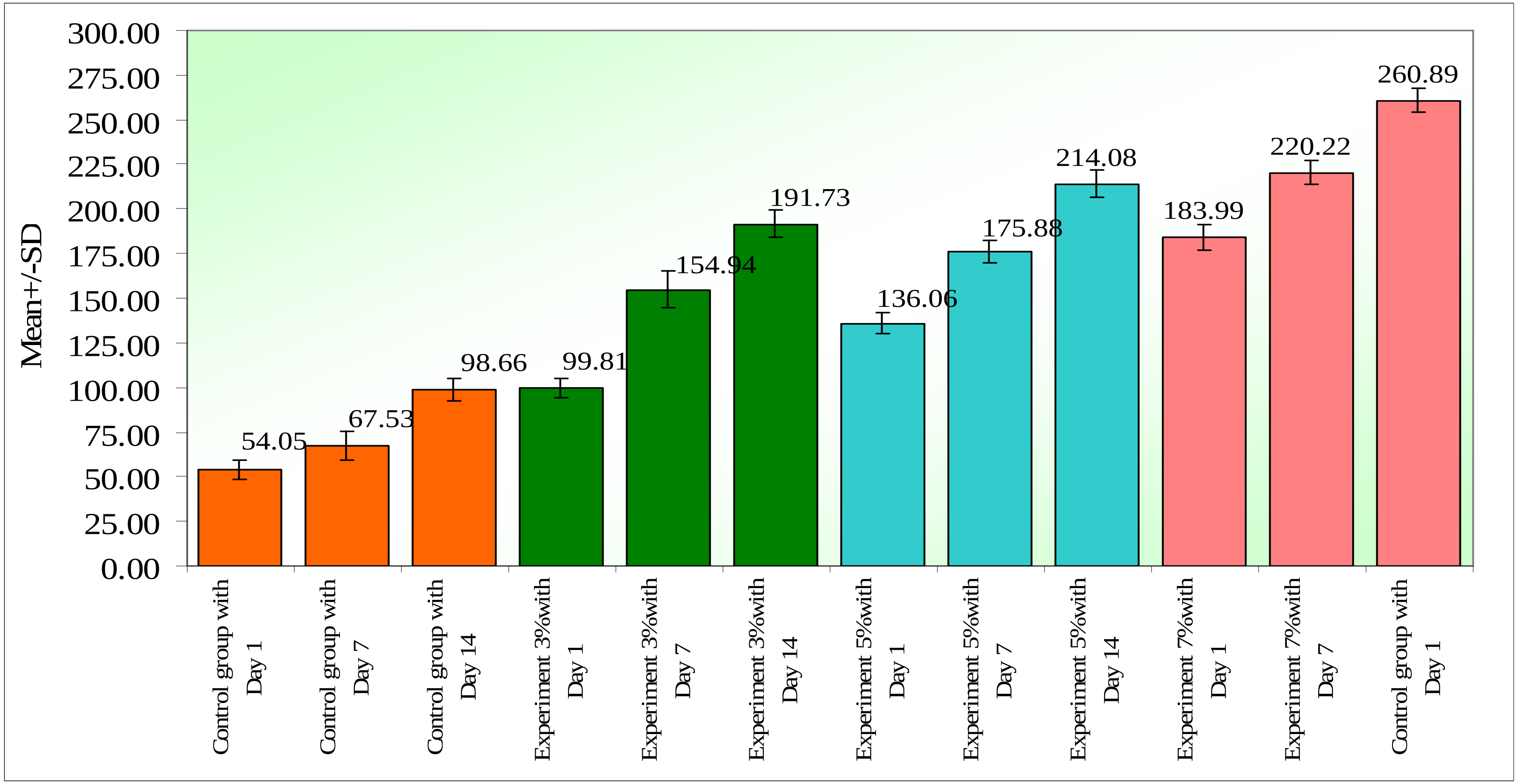
Table 10: Pair-wise comparison of four groups and three time points with mean water sorption with mean water sorption

Interactions	Control group with Day 1	Control group with Day 7	Control group with Day 14	Experiment 3%with Day 1	Experiment 3%with Day 7	Experiment 3%with Day 14	Experiment 5%with Day 1	Experiment 5%with Day 7	Experiment 5%with Day 14	Experiment 7%with Day 1	Experiment 7%with Day 7	Control group with Day 1
Mean	54.05	67.53	98.66	99.81	154.94	191.73	136.06	175.88	214.08	183.99	220.22	260.89
SD	5.46	7.82	6.46	5.11	10.01	7.71	5.73	6.09	7.72	7.09	6.82	6.71
Control group with Day 1	-											
Control group with Day 7	p=0.0001*	-										
Control group with Day 14	p=0.0001*	p=0.0001*	-									
Experiment 3%with Day 1	p=0.0001*	p=0.0001*	P=0.6880	-								
Experiment 3%with Day 7	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	-							
Experiment 3%with Day 14	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	-						
Experiment 5%with Day 1	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	-					
Experiment 5%with Day 7	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	-				
Experiment 5%with Day 14	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	-			
Experiment 7%with Day 1	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	P=0.0069*	p=0.0001*	P=0.0046*	p=0.0001*	-		
Experiment 7%with Day 7	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	P=0.0320*	p=0.0001*	-	
Experiment 7%with Day 14	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	-

*p<0.05

Table 10: When pair-wise comparison of four groups and three time points with mean water sorption, all the groups had shown statistically significant differences at all the time points except with the control group on day 14 and 3% group on day 1.

Graph 5: Comparison of four groups and three time points with mean water sorption with mean water sorption



According to graph 5, the mean water sorption values increased significantly at all different time points (day 1, 7 and 14) for all the groups.

Table 11: Summary of solubility in four groups and three time points

Factors	Level of Factor	Mean	SD	SE	95% CI for mean	
					Lower	Upper
Group	Control group	21.58	3.00	0.50	20.56	22.59
	Experiment 3%	26.43	2.89	0.48	25.46	27.41
	Experiment 5%	29.40	2.99	0.50	28.39	30.41
	Experiment 7%	33.92	3.22	0.54	32.83	35.01
Times	Day 1	24.48	4.73	0.68	23.10	25.85
	Day 7	27.92	4.68	0.67	26.56	29.28
	Day 14	31.10	4.73	0.68	29.73	32.47
Group*Time	Control group with Day 1	18.16	1.10	0.32	17.46	18.86
	Control group with Day 7	21.77	1.32	0.38	20.93	22.61
	Control group with Day 14	24.80	1.27	0.37	24.00	25.61
	Experiment 3%with Day 1	23.14	1.22	0.35	22.36	23.92
	Experiment 3%with Day 7	26.58	1.12	0.32	25.87	27.28
	Experiment 3%with Day 14	29.59	1.04	0.30	28.93	30.25
	Experiment 5%with Day 1	26.06	0.93	0.27	25.47	26.65
	Experiment 5%with Day 7	29.30	1.11	0.32	28.60	30.01
	Experiment 5%with Day 14	32.85	1.06	0.30	32.17	33.52
	Experiment 7%with Day 1	30.56	1.92	0.55	29.33	31.78
	Experiment 7%with Day 7	34.04	1.72	0.50	32.95	35.14
	Experiment 7%with Day 14	37.16	1.60	0.46	36.14	38.17

Table 11 shows the mean, SD and SE of solubility values of all the groups independent of time,the mean, SD and SE of solubility values of days independent of groups and, the mean, SD and SE solubility values of all the groups (3%, 5%, 7%) on all the days (1,7, and 14)

Table 12: Comparison of four groups and three time points with mean solubility by Two-way ANOVA

Sources of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	p-value
Main effects					
Groups	2900.23	3	966.74	557.3713	0.0001*
Times	1052.43	2	526.22	303.3869	0.0001*
2-way interaction effects					
Groups* Times	1.25	6	0.21	0.1204	0.9938
Error	228.95	132	1.73		
Total	4182.86	143			

*p<0.05

Table 12: Comparison of four groups and three time points was done by Two-Way ANOVA. When only groups were compared there was a statistically significant difference and when only time points were compared there was a statistically significant difference but when groups were compared along with time points there was no significant difference.

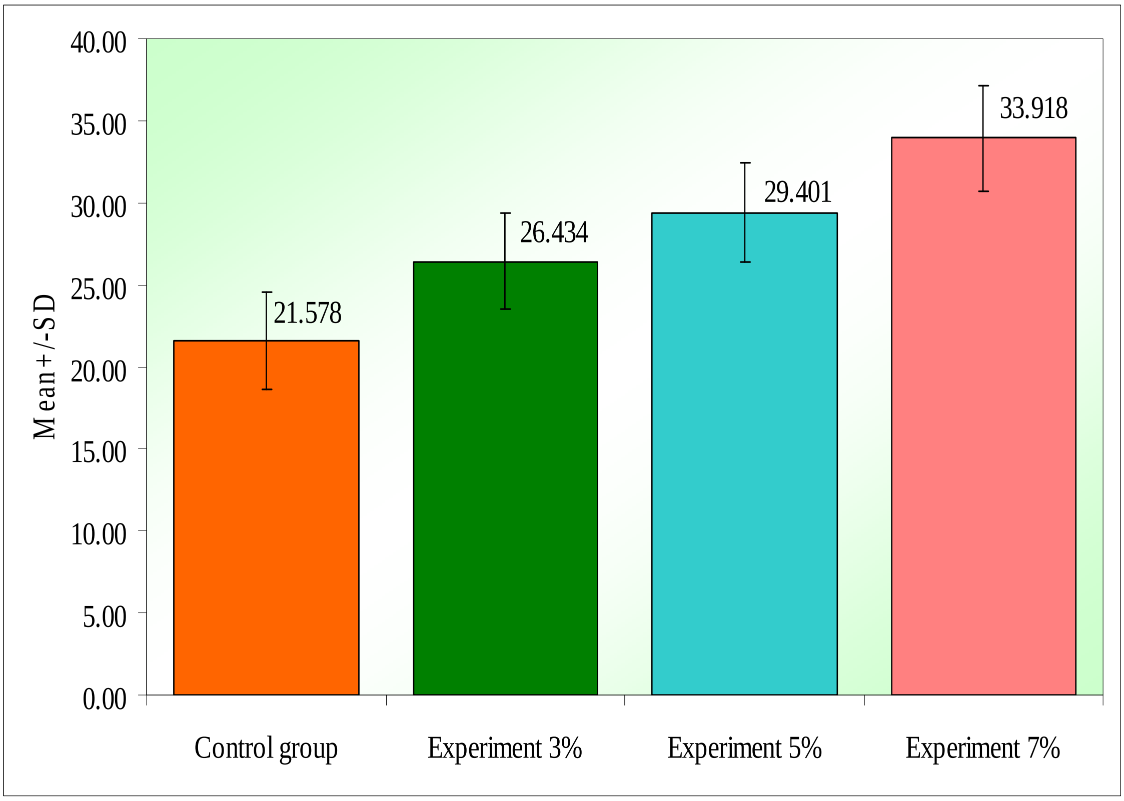
Table 13: Pair-wise comparison of four groups with mean solubility by Newman-Keuls multiple post hoc procedures

Groups	Control group	Experiment 3%	Experiment 5%	Experiment 7%
Mean	21.578	26.434	29.401	33.918
SD	3.002	2.889	2.986	3.221
Control group	-			
Experiment 3%	p=0.0001*	-		
Experiment 5%	p=0.0001*	p=0.0001*	-	
Experiment 7%	p=0.0001*	p=0.0001*	p=0.0001*	-

*p<0.05

Pair-wise comparison of four groups with mean solubility was done by Newman-Keuls multiple post hoc procedures. Table 13 compares the solubility of 4 groups (control, 3%, 5% and 7%) independent of the time points. The solubility values increase as following in an ascending order: Control< 3%< 5%< 7%.

Graph 6: Comparison of four groups with mean solubility by Newman-Keuls multiple post hoc procedures



According to graph 6, there was an increase in the mean solubility values of all the groups irrespective of the time points. 7% has the highest solubility value and the control group had the lowest solubility value.

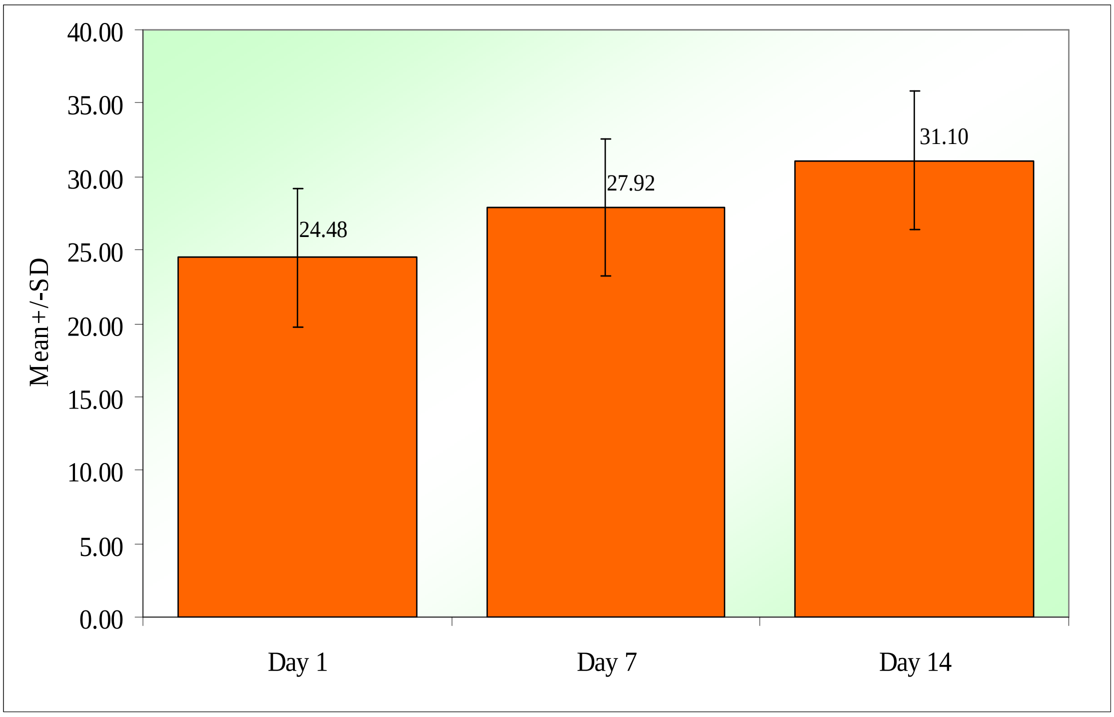
Table 14: Pair-wise comparison of three time points with mean solubility by Newman-Keuls multiple post hoc procedures

Times	Day 1	Day 7	Day 14
Mean	24.48	27.92	31.10
SD	4.73	4.68	4.73
Day 1	-		
Day 7	P=0.0001*	-	
Day 14	P=0.0001*	P=0.0001*	-

*p<0.05

Pair-wise comparison of three-time points with mean solubility was done by Newman-Keuls multiple post hoc procedures. Table 14 compares solubility at 3 different time points (days 1, 7, and 14) independent of the groups (control, 3%, 5%, and 7%). The solubility values increased as follows in ascending order: day 1< day 7 < day 14.

Graph 7: Comparison of 3 time points with mean solubility by Newman-Keuls multiple post hoc procedures



According to graph 7, there was an increase in the mean solubility values at 3 different time points (days 1, 7, and 14) irrespective of the groups (control, 3%, 5%, and 7%). Day 14 showed the highest solubility while day 1 had the lowest solubility.

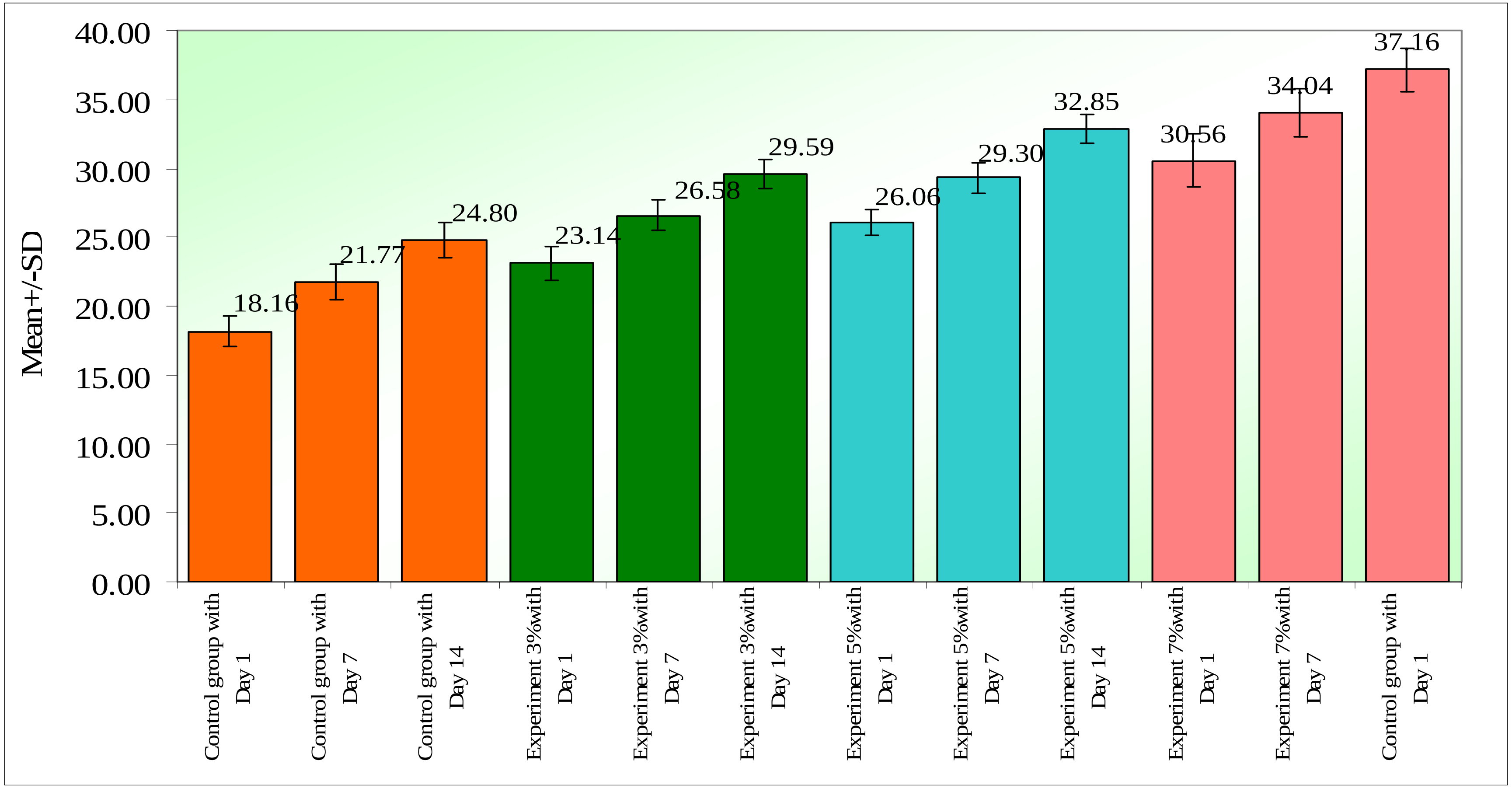
Table 15: Pair-wise comparison of four groups and three time points with mean solubility

Interactions	Control group with Day 1	Control group with Day 7	Control group with Day 14	Experimen t 3%with Day 1	Experimen t 3%with Day 7	Experimen t 3%with Day 14	Experimen t 5%with Day 1	Experimen t 5%with Day 7	Experimen t 5%with Day 14	Experimen t 7%with Day 1	Experimen t 7%with Day 7	Control group with Day 1
Mean	18.16	21.77	24.80	23.14	26.58	29.59	26.06	29.30	32.85	30.56	34.04	37.16
SD	1.10	1.32	1.27	1.22	1.12	1.04	0.93	1.11	1.06	1.92	1.72	1.60
Control group with Day 1	-											
Control group with Day 7	p=0.0001*	-										
Control group with Day 14	p=0.0001*	p=0.0001*	-									
Experiment 3%with Day 1	p=0.0001*	p=0.0109*	p=0.0020*	-								
Experiment 3%with Day 7	p=0.0001*	p=0.0001*	p=0.0028*	p=0.0001*	-							
Experiment 3%with Day 14	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	-						
Experiment 5%with Day 1	p=0.0001*	p=0.0001*	p=0.0198*	p=0.0001*	p=0.3335	p=0.0001*	-					
Experiment 5%with Day 7	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.5918	p=0.0001*	-				
Experiment 5%with Day 14	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	-			
Experiment 7%with Day 1	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0722	p=0.0001*	p=0.0512	p=0.0001*	-		
Experiment 7%with Day 7	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0258*	p=0.0001*	-	
Experiment 7%with Day 14	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	-

*p<0.05

Table 15: When pair-wise comparison of four groups and three-time points with mean solubility was done, all the groups had shown statistically significant difference at all the time points except with 3% group on day 7 and 5% group on day 1: 3% group on day 14 and 5% on day 7 and 7% on day 1.

Graph 8: Comparison of four groups and three time points with mean solubility



According to graph 8, the mean solubility values increased for all the groups (Control, 3%, 5%, 7%) at all different time points (day 1, 7 and 14).

DISCUSSION

The oral cavity is a unique ecosystem with many ecological niches for microbial colonization.[39] The bacteria and fungi that dwell on the surfaces of the teeth and mucosa of the floor of the mouth, palate, gingival, buccal mucosa, tongue, and oral pharynx make up the human oral microbiome. *Streptococcus, Veillonella, Leptotrichia, Prevotella, Haemophilus, Capnocytophaga, Rothia, Gamella, Neisseria,* and *Lactococcus* were shown to be the ten most prevalent genera in the oral microbiome in a study of saliva from dentate subjects whereas in edentate patients with dentures are *Lactobacilli, Staphylococci, Streptococci,* and yeast.[39]

A rise in systemic and oral fungal infection has been outlined in the last few years. Candidiasis has become a threatful infection mainly in immunocompromised patients, older patients, and patients under medications.[22] Denture-induced Candidiasis is seen in almost 11% to 70% of all denture wearers. According to various articles, *Candida albicans* is the most common *Candida* species responsible for denture stomatitis followed *by C. tropicalis, C. krusei, and C. glabrata*.[41]

This increased incidence of fungal infection has led to a greater need of antifungal drugs.[16] The treatment of *Candida* infections is currently challenging owing to the inadequate number of available drugs, increased resistance to these drugs, high costs, tedious regimen, and toxicity. But the use of synthetic drugs over time has resulted to have many harmful side effects which in need led to the application of herbal medicines in various fields.[62]

To avoid the emergence of fungal resistance, novel alternative treatments that are better than standard antifungal drugs are necessary.[17] Few studies have assessed the anticandidal activity of herbal extracts (aloe vera, curcumin, and origanum essential oils as well as probiotics) to help overcome these challenges. Due to their organic nature, they have high antimicrobial efficacy and low toxicity.[15]

Numerous in vitro and in vivo studies showed that adding fungicidal ingredients to soft liners can help prevent biofilm formation and promote healing in denture stomatitis, improve denture retention and stability while distributing masticatory pressures to the underlying tissues to reduce denture-induced soft tissue trauma and hence be used as oral drug delivery tools.[52]

Because short-term soft liners have a 14-day life cycle, the time required to treat DS using these medication-modified materials is equivalent to the period required when using typical topical antifungal medications.[10,47] So a period of 14 days was chosen for this study.

Naoe T et al conducted a study to investigate the antibacterial activity, mechanical characteristics, and biocompatibility of a novel antimicrobial tissue conditioner combining cetylpyridinium chloride with montmorillonite. The addition of CPC-Mont to the tissue conditioner dramatically reduced the numbers of both adherent and free-floating *Candida albicans*, and this effect lasted for a week. Furthermore, the mechanical characteristics and biocompatibility were equivalent to commercially marketed tissue conditioners.[55]

Montmorillonite is a fairly safe material that has been used in several human studies. It is a soft phyllosilicate mineral group that generates layered clay. It has been widely used in catalytic processes to improve cation exchange and piqued researchers' interest due to its adsorption properties.[63] Its adsorption property frequently aids in the trapping and prolonged release of hydrophobic medicines. Heating a drug with montmorillonite beyond its melting point in the absence of an aqueous phase or homogeneously grinding both substances have been attempted and shown to be effective for adsorbing hydrophobic drugs.[27]

Thymus vulgaris or EO of thyme has been utilized in traditional medicine for many years.[24] It could be used as a mouth rinse, toothpaste to prevent and treat related oral infections due to its significant antibacterial action against *S. pyogenes, S. mutans, C. albicans, A. actinomycetemcomitans, and P. gingivalis.*[25]

In this study, the EO of *T. vulgaris* and the clay mineral called montmorillonite have been incorporated into the soft liner and were tested against *Candida albicans* for its antifungal efficacy using the well diffusion method. Introducing antifungal agents to these materials can effectively inhibit the growth of *C. albicans*, but it may also change their morphological structure and properties.[10] So the study also assessed the changes in the water sorption and solubility of these soft liners.

The preparation of *thyme-montmorillonite* was done by adsorption/evaporation a technique in which essential oils are loaded into montmorillonite without the use of organic solvents. This method was selected since the direct mixing of essential oils and clays results in the formation of clay/essential oil slurries.[61]

According to the findings of this study, the mean value for the MIZ calculated in millimeters for the 3% thyme-montmorillonite group on days 3, 7, and 12 were 8.83, 3.25, and 0 respectively. The mean value for the 5% group thyme-montmorillonite group on days 3, 7, and 12 were 13.75mm, 6.17mm, and 3mm. The

7% thyme-montmorillonite group on days 3, 7, and 12 were 20.50mm, 14.92mm, and 6.83mm respectively. The control group had no antifungal effect.

Thyme EO has already been shown to have antimicrobial and anti-biofilm effects against *S. pyogenes, S. mutans, C. albicans, A. actinomycetemcomitans, P. gingivalis, P. aeruginosa, E. faecalis*. The antifungal effect of thyme is mostly attributed to the direct interaction of thymol, carvacrol, and P-cymene with cytoplasmic membrane ergosterol, which results in fungal cell membrane breakdown and release of cellular contents.[24,26]

According to graph 1, there was an increase in the zone of inhibition (mm) as the concentration of *thyme-montmorillonite* increased, on the same day. The zone of inhibition increased as the concentration of the soft liner combined with thyme-montmorillonite increased, so the highest antifungal activity was seen with the 7% *thyme-montmorillonite* group followed by 5% and 3%. This might be ascribed to the fact that as concentration increased, more antifungal chemicals leached out of the soft liner.

According to graph 2, the zone of inhibition (mm) decreased as the time interval (days 3, 7, and 12) rose for the same group. This indicated that as time passed, the antifungal action of thyme EO diminished, either owing to the leaching of the EO components or because the active constituent thymol was depleted.

Hence, this meant that the antifungal activity was both concentration and time dependent. Even though thyme oil is essentially non-toxic, larger amounts may cause tissue irritation.

This study yielded similar results to **Srivastava et al**[42], who integrated different concentrations (10%, 20%, 30%, 40%, 50%, 55%, 57%, 60%, 65%) of Origanum oil in Viscogel and tested for the antifungal activity for one week. **Muttagi et al**[16] incorporated *Centratherum anthelminticum, Linum usitatissimum, and Ocimum sanctum* into soft liner and tested for antifungal activity for 1 week, as did **Schneid et al**[11], who incorporated nystatin, chlorhexidine, itraconazole, and fluconazole into an interim resilient liner and tested for the anticandidal activity for 14 days.

In all the studies the zone of inhibition decreased as the days progressed and the MIZ increased as the concentration of oil increased. This was due to the leaching of the drug or diffusion of the drug; it was also hypothesized that the ethanol content on the liner could also influence the antifungal activity. Nevertheless, all the authors concluded that the minimal zone of inhibition is concentration and time dependent.

However, different results were seen in the case of **Sharma et al**[43] who had incorporated *Melaleuca* oil (1%, 5%, 10%, 20%, 25%, 27.5%, 30%, 35% w/w) and Fluconazole (1%, 3%, 5%, 10% w/w) in soft liner where antifungal activity was assessed up to 60 days. He concluded that 30%w/w Melaleuca oil had longer and better antifungal activity than 5% w/w Fluconazole and it had sustained its efficacy for up to 60 days. He reasoned that this could be because, the rates of release of each antifungal agent differ, with fluconazole releasing more quickly in Visco-gel than melaleuca oil, explaining the difference. Another discrepant finding from this study was that even at greater fluconazole concentrations, the MIZ showed no significant variation after 3 days, suggesting that the concentration may not raise the MIZ.

Kumar et al[64] incorporated *Azadirachta indica, Cocos nucifera, and Melaleuca alternifolia* in Visco gel and concluded that there was no significant change in the MIZ after the incubation period of 7 days.

Both **Sharma et al**[43] and **Kumar et al**[64] conclude that for the antifungal activity to persist even after 7 days the oil has to be active in the polymeric structure of soft liner.

All the studies agree that the leaching of antifungal agents is slow, consistent, and gradual, which is consistent with the study by Graham et al[20] who stated in their in-vitro study that tissue conditioners flow for 7 days and are clinically effective during this time.

One of the serious challenges to employing essential oils as an antimicrobial agent is their unfavorable impact on the mechanical characteristics of tissue conditioners.

Solubility and water sorption are the most important physical properties of soft denture liner material. High rates of solubility and water sorption can lead to changes in the texture, loss of color, loss of resiliency, and the development of an unpleasant odor.[30] Increase in solubility and sorption of soft liners is also associated with swelling, deformation, stiffness, support of bacteria, and debonding of liners from denture bases. Therefore, water sorption and solubility are important properties to assess the lifespan of a particular liner.[33]

Water sorption follows Fickian diffusion kinetics.[65] When put in an aqueous environment, poly (methyl methacrylate) absorbs relatively little water. Nonetheless, this water has a major impact on the polymer's mechanical and dimensional characteristics. Although the polarity of poly (methyl methacrylate) molecules aids absorption, diffusion is the primary mechanism responsible for water infiltration. Water molecules penetrate the poly (methyl methacrylate) bulk after diffusion and become embedded between the polymer chains. First, it produces a modest expansion of the polymerized mass; second, water molecules interact with the intertwining polymer chains, acting as plasticizers and altering the physical characteristics of the resulting polymer. When this occurs, the polymer chains become more mobile, the tensions built during polymerization relax, and the physical and dimensional properties of the material change substantially.[29]

The present study was done in accordance with ISO specification #10139-2. This study compared the effects of thyme-montmorillonite at various concentrations on the solubility and water sorption of an acrylic-based self-curing soft denture liner material at intervals of 1, 7, and 14 days. Dentures with soft liners are in touch with saliva during clinical usage; to simulate oral conditions, artificial saliva was chosen instead of distilled water.

The mean water sorption values for the control group on days 1, 7, and 14 are 54.05 µg/mm^3, 67.53 µg/mm^3, and 98.66 µg/mm^3. The water sorption for 3% on days 1, 7, and 14 is 99.81 µg/mm^3 154.94 µg/mm^{3,} and 191.73 µg/mm^3. For 5% on days 1, 7, and 14 is 136.06 µg/mm^3, 175.88 µg/mm^3, and 214.08 µg/mm^3. For 7% on days 1, 7, and 14 is 183.99 µg/mm^3, 220.22 µg/mm^3, and 260.89 µg/mm^3.

According to graph 3, the mean water sorption values of all groups (control, 3%, 5%, 7%) increased regardless of time points (1, 7, 14 days). 7% had the greatest water sorption value, while the control group had the lowest.

Graph 4 shows that the mean water sorption values increased at three different time intervals (days 1, 7, and 14), regardless of the group (control, 3%, 5%, and 7%). The greatest water was absorbed on day 14, whereas the least was absorbed on day 1.

As per graph 5, the mean water sorption values rose considerably for all groups (control, 3%, 5%, and 7%) on all days (1, 7, and 14) and there was statistical significance.

Another important characteristic that indicates the acceptance of soft denture liner material is its solubility. Although plasticizers (dibutyl phthalate) make the soft liner more flexible, they also create certain challenges. Dentures with soft liners encounter saliva during clinical usage and are soaked in water or an aqueous disinfection solution during storage. Soft liner materials react in two ways during such immersion: plasticizers and other soluble elements are leached away, and water or saliva is absorbed. This behavior is hypothesized to be caused by plasticizers like dibutyl phthalate, contaminants in acrylic resin soft liners, and ethanol loss.[29]

The mean solubility values for the control group on days 1, 7, and 14 are 18.16 $\mu g/mm^3$, 21.77 $\mu g/mm^3$, and 24.80 $\mu g/mm^3$. The values for 3% on days 1, 7, and 14 are 23.14 $\mu g/mm^3$, 26.58 $\mu g/mm^3$, and 29.59 $\mu g/mm^3$. And 5%, the mean solubility values were 26.06 $\mu g/mm^3$, 29.30 $\mu g/mm^3$, and 32.85 $\mu g/mm^3$. And for 7% on day 1 was 30.56 $\mu g/mm^3$, on day 7 was 34.04 $\mu g/mm^3$, and on day 14 were 37.14 $\mu g/mm^3$.

The mean water sorption values of all groups rose independently of time point, according to graph 6. The lowest solubility value was found in the control group, while the highest was found in the 7% group.

According to graph 7, there was an increase in the mean solubility values at 3 different time points (days 1, 7, and 14) irrespective of the groups (control, 3%, 5%, and 7%). Day 14 showed the highest solubility while day 1 had the lowest solubility.

According to graph 8, the mean solubility values increased for all the groups (Control, 3%, 5%, 7%) at all different time points (day 1, 7 and 14) but there was no statistical significance.

It was seen that with the increase in the concentration and the number of days, the mean water sorption and solubility values increased. The reason for the increase in the values for the denture soft liner might be because the preparation of *thyme-montmorillonite* might have changed the diffusion coefficient of the denture soft liner and the other might be because of increased leaching of the drug from the denture soft liner and replacement with smaller molecules like water and components of artificial saliva.[66]

Montmorillonite which is hygroscopic in nature has a distinct propensity to swell and mold in water i.e., clay particles can absorb or lose water; when water is absorbed, it fills the spaces between the stacked silicate layers.[27] Or the essential oil compounds may have many secondary metabolites of varying molecular weights and sizes which might influence the solubility and water sorption.[65]

Another reason could be the interference of the antifungal agent with the polymeric structure causing an increased flow and diffusion through the channels and pores created in the polymeric matrix.[66]

Lima et al conducted a study to check the solubility and sorption of the nystatin, ketoconazole, and chlorhexidine in denture soft liners. For the 14-day interval, the water sorption and solubility increased with the incorporation of Chlorhexidine and ketoconazole when compared with the control group however, the solubility of the soft liner wasn't affected by the inclusion of nystatin. He reasoned that the tissue conditioner's greater levels of plasticizers and ethanol, which are connected to dibutyl phthalate's low molecular weight and fast release, as well as the leaching of the antifungal agent, create gaps in the polymeric structure and boost water solubility.[47]

A study conducted by **Jabbal et al** compared the solubility and water sorption of two soft liners namely Viscogel (acrylic-based soft liner) and Mollosil (silicone-based soft liner) at different time intervals of 7 days, 30 days, and 3 months in both artificial saliva and distilled water. They concluded that at all the intervals high rates of absorption and solubility were seen in Viscogel than Mollosil both in artificial saliva and distilled water and due to the higher crosslinking of acrylic soft liner more sorption was seen.[48]

In research by **Kazanji et al**, four of the commercially available soft lining materials demonstrated greater solubilities in artificial saliva than in distilled water. The reason is that since synthetic saliva is a blend of different salts and additives, there is a chance that it will interact with the soft lining material.[67]

Zeolite was shown to be a hygroscopic material in a study by **Sadeq et al.**, who added silver-zinc zeolite (0.5% and 0.75%) to acrylic soft liners. Its inclusion in the composition increased water absorption. The zeolite content, however, determines the hygroscopic characteristics of zeolite; as zeolite concentration increases, so do the water absorption and diffusion coefficient.[68]

These studies were in accordance with the present study where there was a gradual increase in water sorption and solubility as the concentration increased and the days progressed.

Thus, the null hypothesis was rejected as there was a significant difference in antifungal, water sorption, and solubility of soft liners incorporated with *thyme montmorillonite.*

SCOPE OF THE STUDY

The study evaluated and compared the antifungal activity, solubility, and water sorption of denture soft liner incorporated with thyme-montmorillonite.

Future research is suggested to evaluate the exact mechanism of action of thyme-montmorillonite.

Further investigations are required to assess the stability, cytotoxicity, and durability of the *thyme-montmorillonite.*

Additional research is required to investigate the effect of the incorporation of these antifungal agents on the physical and surface properties of acrylic based denture soft liner.

Further research is required to find out the reason for increase in water sorption and solubility after addition of thyme-montmorillonite to the soft liner.

More studies need to be carried out to assess different properties of the ‘acrylic based denture soft liner’ such as bond strength, hardness, color stability after the incorporation of these antifungal agents.

Since this is an in-vitro study, further in-vivo parameters should be considered with variable clinical conditions. Since other research have shown that presence of saliva, change in the temperature, pH and number of chewing cycles can affect the property of denture liner and potency of antifungal drugs.

LIMITATIONS OF THE STUDY

- Since this is an in-vitro study, the application of the results in clinical conditions might yield different results.
- Diameter of the inhibition zone of *thyme-montmorillonite* was evaluated against only one strain of *Candida albicans*. The prosthetic biofilm is complex, formed not only by fungi but also by bacteria, favoring the adhesion of fungal cells to the internal prosthesis surfaces by co-aggregation.
- Only one acrylic type of interim resilient liner was evaluated in this study. Different liners might yield different results.
- Because of the aggregation of thyme-montmorillonite particles, a homogeneous mixture could not be formed, potentially affecting the attributes evaluated.
- Incorporation of the *thyme-montmorillonite* caused discoloration of the soft liner which might gave a slight unaesthetic result. However, the discoloration was slight and can be masked with the acrylic resin denture surface.

CONCLUSION

The following findings may be derived within the restrictions of the current in-vitro investigation.

- The soft liner incorporated with *thyme-montmorillonite* showed antifungal efficacy at higher concentrations (7%) when compared with lower concentrations (3%,5%) till the 12^{th} day of the research study. The antifungal test revealed antifungal activity at 3%,5%, and 7% concentrations but the activity decreased with time for all concentrations.
- Water sorption increased significantly after the incorporation of *thyme-montmorillonite* at all the time intervals for all the groups. 7% showed the maximum water sorption and it increased with the progressing days.
- The inclusion of thyme-montmorillonite increased solubility at all time intervals for all groups. The highest solubility was 7%, and it increased with the progress of time.
- For clinical use of *thyme-montmorillonite,* further studies are required regarding biocompatibility and its effect on other properties.

SUMMARY

The purpose of this in-vitro study was to test and compare the antifungal activity, sorption, and solubility of a denture soft liner infused with *thyme-montmorillonite.*

The powder extract of *thyme-montmorillonite.* was prepared by a process of evaporation/adsorption. A total of 216 samples were used in this study. 72 samples were assessed for antifungal activity using the well diffusion method. At 3, 7, and 12 days, 12 samples of thyme-montmorillonite at three different concentrations (3%, 5%, and 7%) mixed into the soft liner were evaluated for the zone of inhibition

To evaluate the sorption 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 *thyme- montmorillonite.* 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 20. For antifungal activity, comparison of 3 experiment groups (3%, 5%, and 7%) with mean antifungal scores at different treatment time points by One-Way ANOVA followed by Newman-Keuls post hoc procedures was done. The dependent t test was used to compare the mean zone of inhibition (mm) values in two research groups at 3 days, 7 days, and 12 days.

For water sorption and solubility: a dependent t-test was used for comparison of 1 day, 7 days, and 14 days time points with both mean water sorption and solubility (µg/mm^3) scores in the 4 study groups. Pair wise comparison of four groups and different time intervals with mean water sorption and solubility (µg/mm^3) was done by Newman-Keuls multiple posthoc procedures. And Two-way ANOVA was used to compare four groups and three time points with mean water sorption and solubility (µg/mm^3).

According to the results obtained, *thyme-montmorillonite* showed antifungal activity against *Candida albicans.* 7% concentration of *thyme-montmorillonite* showed the highest activity against C.albicans but its activity reduced gradually over the period of 12 days.

There was also a statistically significant increase in denture soft liner solubility and water sorption with the addition of *thyme-montmorillonite*. The maximum values in both attributes were found at a 7% concentration of thyme-montmorillonite, 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 incorporation of *thyme-montmorillonite* into the denture 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.

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ANNEXURE I

ETHICAL CLEARANCE

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

Sl. No. : 1460

CERTIFICATE

This is to Certify that the synopsis titled Comparative evaluation of antifungal activity against candida albicans, water sorption & solubility of a soft liner incorporated with thymus vulgaris (thyme) Montmorillonite - an in vitro study *Submitted by Dr.* _ *P. G. Student / Staff, Guided by* _ *from Department of* Prosthodontics & Crown & Bridge *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 5/5/21

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

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

ANNEXURE II

AUTHENTICATION FORM

Kanth Saanvi Essentials

GSTIN No.: 07AXGPV6000Q1ZY

House No. 41 Village Naharpur Sector-7, Rohini, Delhi-110085

CERTIFICATE OF ANALYSIS

Product	Thyme Oil
Lot No.	KS2522/ 2020
Manufacturing Date	September - 2020
Best before	September – 2022
Botanical Name	Thymus vulgaris
Method of Extraction	Steam Distillation
Thymol %	30% - 40%
Appearance	Fluid liquid
Colour	light Yellow colour
Odour	Warm, Spicy, Pungent and typical odour of Thyme

PHYSICO-CHEMICAL PROPERTIES:-	SPECIFICATION	RESULTS
Specific Gravity	0.890 to 0.950	0.901
Optical Rotation	-5 to +5	+0.5
Refractive Index	1.480 to1.499	1.493

Steam distillation and 100% soluble in alcohol

ANNEXURE III

AUTHENTICATION FORM

SIGMA-ALDRICH

3050 Spruce Street, Saint Louis, MO 63103 USA
Email USA: techserv@sial.com Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:	Montmorillonite K 10 surface area 220-270m^2/g
Product Number:	281522
Batch Number:	STBJ3312
Brand:	Aldrich
CAS Number:	1318-93-0
Formula:	
Formula Weight:	
Quality Release Date:	30 JUL 2019

TEST	SPECIFICATION	RESULT
APPEARANCE (COLOR)	OFF WHITE TO FAINT GREY	OFF-WHITE
APPEARANCE (FORM)	POWDER	POWDER
MISCELLANEOUS TESTS	K 10 GRADE CONFIRMED	CONFIRMED

Claudia Mayer
Manager Quality Control
Steinheim, Germany

ANNEXURE IV

Table 15 : Diameter of Inhibition Zone (mm) (antifungal activity) of control at the after 3, 7 and 12 days.

SAMPLE NO.	DAY 1	DAY 7	DAY 12
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

ANNEXURE V

Table 16: Diameter of inhibition zone (mm) (antifungal activity) of thyme-montmorillonite at 3% concentration after 3, 7 and 12 days.

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

ANNEXURE VI

Table 17: Diameter of inhibition zone (mm) (antifungal activity) of thyme-montmorillonite at 5% concentration after 3, 7 and 12 days.

SAMPLE NO.	DAY 3	DAY 7	DAY 12
1	12	5	4
2	14	6	3
3	14	8	3
4	16	6	4
5	15	5	3
6	14	6	2
7	12	6	4
8	12	8	3
9	15	6	4
10	14	7	1
11	13	7	2
12	14	4	3

ANNEXURE VII

Table 18: Diameter of inhibition zone (mm) (antifungal activity) of thyme-montmorillonite at 7% concentration after 3, 7 and 12 days.

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

ANNEXURE VIII

Table 19: Water sorption values of control on days 1, 7, and 14.

SAMPLE NO.	DAY 1	DAY 7	DAY 14
1	51.52	79.89	104.53
2	46.81	61.86	95.89
3	64.17	80.65	111.38
4	53.16	67.68	97.3
5	49.35	55.78	85.29
6	54.15	60.83	95.92
7	45.87	65.78	99.89
8	52.32	75.22	105.31
9	56.31	60.67	96.73
10	61.17	69.1	99.42
11	55.89	63.45	94.68
12	57.9	69.45	97.63

ANNEXURE IX

Table 20: Water sorption values of 3% *thyme.montmorillonite* on days 1, 7, and 14.

SAMPLE NO.	DAY 1	DAY 7	DAY 14
1	92.86	139.59	178.34
2	100.68	149.15	189.51
3	101.21	157.56	198.73
4	96.39	146.27	186.76
5	104.51	169.18	194.82
6	101.01	163.55	199.12
7	96.67	147.38	183.52
8	103.9	141.18	186.76
9	111.03	162.98	200.41
10	97.21	156.29	196.26
11	98.84	166.87	201.62
12	93.45	159.23	184.9

ANNEXURE X

Table 21: Water sorption values of 5% *thyme.montmorillonite* on days 1, 7, and 14.

SAMPLE NO.	DAY 1	DAY 7	DAY 14
1	128.68	164.69	200.56
2	136.79	182.15	212.78
3	144.93	180.78	216.77
4	132.84	172.97	206.87
5	134.29	178.43	217.08
6	139.63	185.36	225.67
7	133.45	179.83	204.37
8	147.63	167.82	209.34
9	131.67	170.45	217.99
10	135.54	174.56	220.83
11	137.45	177.39	223.6
12	129.78	176.15	213.13

ANNEXURE XI

Table 22: Water sorption values of 7% *thyme.montmorillonite* on days 1, 7, and 14.

SAMPLE NO.	DAY 1	DAY 7	DAY 14
1	174.78	210.89	256.99
2	182.97	221.67	260.98
3	180.39	215.84	250.76
4	177.54	211.87	273.84
5	185.28	232.67	269.46
6	183.98	229.35	266.24
7	189.74	225.89	264.52
8	194.78	223.42	253.48
9	175.35	220.56	259.39
10	179.36	219.27	261.73
11	196.39	217.78	255.98
12	187.33	213.44	257.33

ANNEXURE XII

Table 23: Solubility values of control on days 1, 7, and 14.

SAMPLE NO.	DAY 1	DAY 7	DAY 14
1	17.1	22.37	25.67
2	18.87	22.58	25.78
3	17.92	19.11	22.45
4	19.74	22.67	25.98
5	16.89	20.38	23.64
6	16.78	21.65	24.48
7	18.87	23.43	26.42
8	19.25	23.78	26.45
9	16.85	20.87	23.81
10	18.79	21.56	24.38
11	19.35	21.85	24.82
12	17.53	20.99	23.76

ANNEXURE XIII

Table 24: Solubility values of 3% *thyme.montmorillonite* on days 1, 7, and 14.

SAMPLE NO.	DAY 1	DAY 7	DAY 14
1	21.79	25.29	28.98
2	23.78	26.69	29.56
3	25.69	27.96	30.26
4	23.17	27.7	30.73
5	23.32	25.85	28.72
6	21.61	27.35	30.21
7	23.89	28.1	31.45
8	23.93	26.42	29.76
9	21.79	24.65	27.64
10	21.81	25.35	28.49
11	23.89	26.56	29.83
12	22.99	26.99	29.44

ANNEXURE XIV

Table 25: Solubility values of 5% *thyme.montmorillonite* on days 1, 7, and 14.

SAMPLE NO.	DAY 1	DAY 7	DAY 14
1	26.39	29.78	32.45
2	25.48	27.56	31.67
3	26.69	29.63	32.76
4	26.06	29.99	32.23
5	24.73	28.38	31.39
6	26.38	29.18	32.48
7	27.18	30.87	34.89
8	25.24	29.48	33.78
9	24.56	28.45	32.91
10	26.53	30.78	33.72
11	27.59	29.95	31.87
12	25.84	27.56	33.99

ANNEXURE XV

Table 26: Solubility values of 7% *thyme.montmorillonite* on days 1, 7, and 14.

SAMPLE NO.	DAY 1	DAY 7	DAY 14
1	33.31	36.45	39.78
2	27.51	30.45	33.72
3	29.83	34.67	37.65
4	29.48	34.23	37.46
5	27.12	31.37	35.93
6	30.86	33.69	36.34
7	31.86	34.28	37.44
8	33.15	36.35	39.6
9	31.57	34.52	37.04
10	30.18	33.85	36.43
11	30.48	33.82	36.82
12	31.32	34.84	37.65