"COMPARATIVE EVALUATION OF ANTIFUNGAL ACTIVITY, SURFACE ROUGHNESS AND TENSILE BOND STRENGTH OF GINGER GRASS OIL INCORPORATED IN TISSUE CONDITIONER– AN VITRO STUDY"

BY

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Dissertation

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PROSTHODONTICS AND CROWN & BRIDGE
(BRANCH – I)

Under the Guidance of
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DEPARTMENT OF PROSTHODONTICS
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To

My parents

and

My guide

ACKNOWLEDGEMENT

No endeavor can start, continue and complete without the blessings of ***LORD GANESHA and GODDESS JAMUNA.*** *I thank them for blessing me with the strength and patience to complete the task entrusted.*

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"The task of the excellent teacher is to stimulate "apparently ordinary" people to unusual effort. The tough problem is not in identifying winners: it is in making winners out of ordinary people."

I feel honored to be a student of my respected teacher and guide ***Dr. MAHANTESH BEMBALAGI*** ***MDS,*** *professor, Department of Prosthodontics and Crown and Bridge, KAHER Vishwanath Katti Institute of Dental Sciences, Belagavi, without whose everlasting inspiration, incessant encouragement, constructive criticism, and with valuable suggestions for improvement, the completion of this study would not have been possible. His unlimited patience, meticulous supervision at every step and everlasting zeal for perfection has not only enabled me to complete the dissertation, but has also helped me tremendously during the postgraduate programme.*

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Thank you, one and all.

Dr. VISHAKHA DARE

LIST OF ABBREVIATIONS USED IN THE STUDY

DS	DENTURE STOMATITIS
SR	SURFACE ROUGHNESS
TC	TISSUE CONDITIONER
TBS	TENSILE BOND STRENGTH
MMIZ-D	MEAN MINIMUM INHIBITION ZONE DIAMETER
MIZ-D	MINIMUM INHIBITION ZONE DIAMETER
TC WITH GGO	TISSUE CONDITIONER WITH GINGER GRASS OIL
TC WITHOUT GGO	TISSUE CONDITIONER WITHOUT GINGER GRASS OIL
CM	CYMBOPOGAN MARTINI

ABSTRACT

STATEMENT OF PROBLEM

Tissue conditioners are resilient materials condition the denture bearing sore mucosa caused by Denture induced stomatitis. Medicinal plants extracts and essential oils incorporated in tissue conditioners have been used in developing countries as alternative treatments to this problems and its effect on mechanical properties of material.

PURPOSE

To evaluate antifungal efficacy, surface roughness and tensile bond strength of *Ginger grass oil* incorporated in tissue conditioner.

METHODS

A total of 150 samples were taken in the present study. Minimum inhibition zone diameter of 90 samples were used to evaluate the antifungal activity using well diffusion method. Then, experimental group of 45 samples was further subdivided into 3 groups of 30%,40%,50% concentration (n = 15) which was used to measure minimum inhibition zone diameter and evaluate the antifungal activity at two different time intervals i.e. after 1 day and 1 week which was compared with comparative group containing 45 samples of fluconazole with tissue conditioner i.e. 15 samples in each group of 1%,3%, 5% concentration.

The antifungal activity of mean minimum inhibition zone diameter of ginger grass oil and fluconazole in tissue conditioner at different concentrations were compared. The surface roughness and tensile bond strength of tissue conditioner with optimized ginger grass oil concentration were evaluated.

RESULT

The collected data was subjected to statistical analysis using Dependent t test and Independent t test. There was statistically significant difference between the control and experimental group. ($P < 0.05$)

CONCLUSION

The commercially available ginger grass oil can serve as one of the potential antifungal agent for treatment against denture stomatitis. Mean minimum inhibition zone diameter of ginger grass oil with tissue conditioner was less compared to fluconazole with tissue conditioner but it was comparable as fluconazole is potent antifungal agent. Additionally, systemic administered antifungal agents has harmful effect on humans when they are misused. Therefore herbal essential oil can be better option to treat denture stomatitis. Surface roughness decreased statistically after incorporation of ginger grass oil and increased after 1 week of time interval. Tensile bond strength decreased statistically after incorporation of ginger grass oil and increased with 1 week of time intervals.

KEYWORDS

Ginger grass oil, Fluconazole, Tissue conditioner, Denture stomatitis, Surface roughness, Tensile bond strength, Disc diffusion test.

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INTRODUCTION

Prosthodontic treatment for the elderly and edentulous population is rapidly increasing in developed countries, particularly removable dentures.[1-2] In 2011, a national dental survey found that 89% of adults aged 75 and older had removable dental prostheses, with edentulism increasing with age.[3] Polymers are the only material widely used in removable denture fabrication (methyl methacrylate).

Polymethyl meth acrylate (PMMA) as the denture base resin has multiple benefits, including ease of manipulation, repair, aesthetics, and dimensional stability.[4-6] Due to its high-water absorption, it is prone to denture plaque contamination. All these factors appear to increase the ability of candida albicance to colonize both the denture and the mucosal surfaces, where it act as an opportunistic pathogen resulting in a variety of clinical issues such as denture stomatitis (DS) and angular cheilitis.[7-9]

Denture stomatitis (DS) is a common oral mucosal condition where trauma from ill-fitting dentures causes erythema or inflammation of the palatal surface, results pain and discomfort to the acrylic denture wearers.[10]Approximately 72% of denture wearers are affected by this condition.[11] Denture-induced stomatitis is often referred as Candida-associated stomatitis because among the fungal species, mainly the candida albicance, are thought to be the primary cause. DS can be caused by a variety of factors other than fungal infections.[12]

While Candida albicans will only adhere under situations of poor denture hygiene and continuous wearing, risk factors such as denture trauma, poor oral and denture hygiene, continuous and nocturnal denture use, xerostomia, and changes in salivary pH have all been linked to denture stomatitis. Denture material themselves

can contribute to the risk for denture stomatitis, as area of surface roughness(SR) and the hydrophobicity of denture surfaces can promote attachment of microorganism and development of biofilm. It is important to reduce the risk for development of DS. The treatment of DS is mainly aimed at identifying and eliminating possible contributing factors, prevent systemic dissemination and eliminate any associate discomfort. [13,14]

Mechanical cleaning of dental prostheses was reported to be underutilised, resulting in increased denture plaque retention and microorganism accumulation on the denture surface. The fact remains that oral health care has traditionally been a low priority. [15,16] Chemical cleaning with denture cleansers may allow residual biofilm retention, which could lead to microorganism regrowth and denture colonisation. Other than these techniques antifungal agents, relining with tissue conditioners, tissue conditioners in combination with antifungal agents, chemical agents such as chlorhexidine and sodium hypochlorite, and physical techniques such as microwaves are all examples to treat DS.[17]

The most important factor to be considered in DS treatment are concentration of antifungal agent, susceptibility of the strain and the source of the mucosal surface. Most commonly used antifungal agents are either polyenes or azoles for example amphotericin B, nystatin or fluconazole, itraconazole, ketoconazole. However they do not eliminate or inhibit the denture colonized microorganisms. Nonetheless, the development of resistant species is the greatest issue related with the long-term or repeated use of antifungal medicines.[18] This entails the search for novel therapeutic approaches. Topical antifungal medication for Denture stomatitis "is difficult to apply in elderly denture wearers due to decreased "motor activity", "cognitive impairment", and "memory loss". Due to circumstances such as frequent consumption

and continual wash out by salivary flow, the administered antifungal drugs fail to attach and remain in touch with oral mucosal tissue.[19]

The main problem in applying these drugs are their high toxicity and side effects also the potential development of drug resistance in the species. Additionally these medication are typically effective against fungal species, but DS can caused by variety of microbes.[20]

One of the primary strategies for overcoming these problems is the use of herbal essential oils and probiotics in place of antifungal and antibiotic medications. Due to their organic composition, they can exhibit both high antibacterial activity and low toxicity. Another advantage of these essential oils is their broad spectrum of antimicrobial and antifungal activity.[21]

Tissue conditioners (TC) are resilient materials usually used as a temporary relining material to prevent the transfer of masticatory load and evenly distribute the external stresses on the mucosa of the basal seat are resilient materials that are often used for conditioning the denture bearing sore mucosa, by ill-fitting dentures. They act as a cushion under the dentures. Denture reliners have a variety of applications in the field of prosthodontics. Denture liner use in dentistry is not new and has been practised for many years and has many desirable properties like long-term stable viscoelastic behaviour, low water sorption, improved colour stability, stain resistance, tear resistance, good bond strength to denture base, dimensional stability, resistance to fungal and bacterial growth, ease of processing, good shelf life, biocompatibility, good resiliency, special rheological properties.

TC have been also used as drug delivery tools in the oral cavity for example, antifungal agents delivered through tissue conditioners inhibited c. albicance proliferation.[22] But there are many studies concerning the adhesion mechanisms of C. albicans to denture base materials as well as factors affecting these mechanisms and surface properties . Because the adhesion of microorganisms to a surface is prerequisite for the colonization at that surface, the denture may function as a reservoir of infection C. albicans has been found on both hard denture base acrylic resins based resilient liner materials in vivo and in vitro .[23]

The recent resurgence of natural health has contributed to an increase in interest in commercially available naturopathic therapies. Medicinal plants extracts and essential oils like Melaleuca alternifolia oil (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.[24,25]

Binomial name of Ginger grass oil (GGO) is Cymbopogon martinii (CM). The essential oils of CM are rich in monoterpenes. Essential oils from Cymbopogon species and their components are known for their antifungal, antimicrobial, antihelmintic, antiparasitic, anticonvulsant and antioxidant activities. GGO can also help manage minor pain and reduces inflammation and act as an anti-inflammatory agent.[56]

Therefore aim of this in vitro study is to compare and evaluate the antifungal activity of the Ginger grass oil incorporating in tissue conditioner, and its effect on SR property and tensile bond strength (TBS) between tissue conditioner and acrylic

resin material. Clinically, adequate bond strength, reduced Candida albicans adhesion, and reduced SR may be beneficial.

However, the properties of TC with essential oil were found to decrease considerably with time. Therefore, they must be replaced regularly as with conventional tissue conditioner by using different essential oils in TC one should to realize its potential clinical application.

NEED FOR THE STUDY

One of the most common types of treatment in prosthetic dentistry is a removable dental prosthesis. However, long-term use without rest to underlying oral tissues may have a negative impact on their integrity. To allow the deformed tissues of the residual ridges to return to normal form, both rest and tissue conditioners have been advocated. Tissue conditioners(TC) are soft, resilient temporary liners that have been widely used in dentistry to treat a wide range of patient issues and clinical applications.[26] They can also be used for temporary relining of immediate dentures, in addition to reducing and evenly distributing stresses on the mucosa of the basal seat. [27]

Elimination of the local irritating factors, treatment of the abused tissue, diet counselling, systemic evaluation, replacement of the old prosthesis, establishment of proper occlusal scheme and correction of any occlusal prematurity's, meticulous oral hygiene maintenance, and use of soft tissue liners are all part of a successful treatment plan for denture stomatitis. TC have recently been used as a drug delivery tool for elderly patients suffering from denture stomatitis caused by Candida infection, as the success of topical drug application in the oral cavity may be hampered by patient compliance. [28]

Furthermore, due to reduced motor skills, cognitive impairment, and memory loss, maintaining a regular and optimal dose of topical antifungal agents is difficult in geriatric denture wearers. To address these issues, antifungal agents have been incorporated into soft tissue liners and are being tested for their efficacy against Candida albicans.[29] Many studies have been conducted on antifungal agents such as

nystatin, azole group derivatives, chlorhexidine, metallic oxide powders, photocatalysts, and silver nanoparticles incorporated into tissue conditioners, with varying degrees of success along with toxicity with overuse.[30]

However, due to Candida species resistance to these drugs, the therapeutic effects of plant oils with antifungal properties are being investigated. A number of studies have been conducted to investigate the effect of natural and herbal antimicrobials, such as tea tree oil (TTO), lemongrass oil, Thai herbs, and Origanum oil, on C. albicans by incorporating them into TC.[31]

Binomial name of *Ginger grass oil* is Cymbopogon martinii. The essential oils of Cympopogan martini are rich in monoterpenes. Essential oils from Cymbopogon species and their components are known for their antifungal, antimicrobial, antihelmintic, antiparasitic, anticonvulsant and antioxidant activities. *GGO* can also help manage minor pain and reduces inflammation and act as an anti-inflammatory agent.[56]

Antifungal effectiveness and potency of *GGO* has been proven but its effect in tissue conditioner is not yet done so the aim of this study is to investigate the role of antifungal agents incorporated in the TC to investigate their effectiveness on the growth of C. albicans for the treatment of denture induced stomatitis. And further its comparison with Fluconazole in tissue conditioner will be observed.

After incorporating GGO in TC, its effect on surface roughness and tensile bond strength in which the TBS of the acrylic resin-based liner will be checked as, reliable bond between denture base and soft liner which is required for the denture to function properly.

HYPOTHESIS

NULL HYPOTHESIS

There is no difference in the antifungal property, surface roughness and tensile bond strength of the denture soft liner incorporated with ginger grass oil.

ALTERNATIVE HYPOTHESIS

There is difference in the antifungal property, surface roughness and tensile bond strength of the denture soft liner incorporated in the ginger grass oil.

AIM AND OBJECTIVES

AIM OF THE STUDY

To evaluate antifungal efficacy, surface roughness and tensile bond strength of *Ginger grass oil* incorporated in tissue conditioner.

OBJECTIVES

1. To evaluate antifungal activity of tissue conditioner incorporated with Ginger grass oil and Fluconazole.
2. To evaluate surface roughness and tensile bond strength of tissue conditioner incorporated with Ginger grass oil.
3. To compare antifungal activity of Ginger grass oil with Fluconazole incorporated with tissue conditioner.

REVIEW OF LITERATURE

1. **Amany 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. On the basis of lower water sorption and solubility, and higher tensile bond strength (TBS), Luci-sof may improve clinical outcomes and success.[32]

2. **Y. kulak ozkan et al (2002)** The goal of this study was to find out about elderly people oral hygiene practises, denture cleanliness, yeast presence, and denture stomatitis. Swabs from the palate were obtained and mycologically examined in order to identify the yeast colonies. There was no statistical correlation between denture stomatitis, denture (DS) brushing frequency, or denture cleaning procedures. However, there was a statistically significant relationship between DS, the presence of yeasts, and the cleanliness of dentures. [33]

3. **V. Naik and J. L. Jabade (2005)** Determination of the tensile bond strength of three commercially available soft liners to a polymethyl methacrylate denture base resin to assist clinicians in selecting the liner for their patients. The tensile test used in this study was effective in comparing the three liners' tensile bond strength. The TBS of Supersoft was better compared to Molloplast B, which was in turn, better than Mucopren. [34]

4. **Blanca Liliana Torres Leo'n et al. (2005)** investigated and compared the water sorption, solubility, and TBS 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 TBS. The adhesive/cohesive failure of materials polymerized using microwave radiation and visible light was noticeable. [35]

5. **T. Baena-Monror et al. (2005)** studied the presence of Candida albicans, Staphylococcus aureus, and Streptococcus mutans on the prosthesis and mucosal membrane in individuals with and without denture stomatitis. The prevalence of Candida albicans, Staphylococcus aureus, and Streptococcus mutans was found to be 52.4%, 53.4%, and 68.6%, respectively, according to the study. Candida albicans was found in 86% of denture stomatitis patients, but Streptococcus mutans was found in just 16% of these individuals. They concluded that Candida albicans, Staphylococcus aureus, and Streptococcus mutans colonised the oral cavity of individuals using dental prosthesis on a regular basis, and that denture stomatitis was more common in these patients. [36]

6. **Mustafa Murat Mutluay and I. Eystein Ruyter (2006)** To assess the initial bonding properties of new and old soft relining materials to denture base polymers with varying polymerization techniques and water contents. The tensile bond strength results varied significantly. A plasticized PMMA soft liner (Vertex Soft) and a poly(organosiloxane) soft liner (Mollosil Plus) had statistically similar bond strength results for different denture base polymers. Poly(organosiloxane) based materials had slightly higher bond strength with water immersed than dry specimens. [37]

7. **Ayse Mese et al (2008)** The bond between the acrylic resin and the resilient liner material can fail, and the resilient liner material can lose its resiliency over time. Resistant liners made of acrylic resin and silicone that were heat or auto polymerized onto denture base acrylic resin were evaluated for their tensile bond strength and hardness. The results showed that the hardness and bond strength of resilient liners varied significantly. Less hardness and higher bond strength than the other silicone-based heat-polymerized (Molloplast-B) resilient liners. Long-term exposure to water increased hardness and decreased bond strength. [38]
8. **E. Emami et al (2008)** For the first time, edentulous elderly people with maxillary full dentures and mandibular two-implant overdentures or conventional dentures were studied for denture stomatitis. The incidence of DS and risk factors were also studied. Study hypothesis was that elders with mandibular implant-retained overdentures have less denture stomatitis than those with conventional dentures.[39]
9. **Abbas Falah-Tafti et al (2010)** Candida albicans were used to test the efficacy of two commonly used antifungal agents mixed with TC. Candida albicans cannot adhere to tissue conditioner with 1% to 10% nystatin or 10% fluconazole. [40]
10. **Jose Francisco Ho fling and others (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 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. [41]

11. **Buket Akalin 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. [42]
12. **Narendra Chopde et al (2012)** Analyze the antifungal activity of two tissue conditioners with nystatin, miconazole, and fluconazole. Combining tissue conditioner with antifungal agents effectively inhibited candida albicance. [43]
13. **Vinaya Bhat et al (2012)** According to the findings, a denture wearer is more likely to acquire candida associated denture stomatitis as a result of the typical oral commensal candida species being converted into a pathogen under favourable settings. Immunocompromised state, prosthesis-related injuries, other systemic illnesses, and patient-maintained dentures are only a few of the causal factors that change the oral balance into an unhealthy and inadequate basis for prosthetic usage. Denture stomatitis caused by the fungus Candida has been found to affect 65 to 70% of denture users over the world. Candida albicance has been the most common source of infection, but there has lately been a change to non-candida albicance (NCAC). [44]
14. **Guang Hong and et al (2012)** The goal of this investigation was to check if commercial tissue conditioners have dynamic viscoelasticity and plasticizer leachability. COE Comfort (CC), Fit Softer (FS), Hydro-Cast (HC), Soft

Conditioner (SC), and Visco-Gel were the five commercial tissue conditioners tested in this investigation (VG). In 37°C distilled water, five specimens of each substance were preserved. Using a dynamic mechanical analyser and high performance liquid chromatography, the dynamic viscoelasticity and plasticizer leaching of each specimen were assessed at 0, 1, 3, 7, and 14 days after immersion. The materials have significant variances in dynamic viscoelasticity and plasticizer leaching. All materials' dynamic viscoelasticity increased or decreased dramatically over time. On the first day following water immersion, rapid changes in dynamic viscoelasticity were visible. The greatest degree of plasticizer leaching was found in the substance CC. These findings imply that plasticizer leaching affects the dynamic viscoelasticity of commercial tissue conditioners. These effects, however, are restricted. [45]

15. **Akanksha Srivatstava et al (2013)** The antifungal activity, surface roughness, and tensile bond strength of TC were evaluated after adding Origanum oil. So, Origanum oil in tissue conditioner reduces candida albicans adherence without affecting its bond strength to heat polymerised acrylic resin. [46]
16. **Sunanda Sharma et al (2014)** The in vitro study compare the antifungal activity of melaleuca alterfolia oil and fluconazole in tissue conditioner. Study concluded that 30% melaleuca alterfolia oil was superior to 5% fluconazole in tissue conditioner as an antifungal agent. Both showed antifungal activity against Candida albicans for 24 hours. [47]
17. **Pokpong Amornvit et al (2014)** After prolonged use, tissue conditioner becomes a reservoir of oral microbiota, especially Candida species. Many antifungal drugs are mixed with TC to control fungal activity. The

anticandidal efficacy of COE-COMFORT tissue conditioner with lemongrass essential oil was added in this study. Lemongrass essential oil illustrated vivid inhibition zone against *C. albicans* and its MIC value was 0.625 µl/ml [0.0625% (v/v)]. In contrast, neither reference nor clinical strain of *C. albicans* was inhibited by the tissue conditioner primarily supplemented with the tested oil at its MIC or without supplement (negative control). [48]

18. **Anna Mertas et al (2014)** Study tested fluconazole's activity against 32 clinical Candida albicans strains and the ATCC 10231 reference strain after they were exposed to sublethal concentrations of tea tree oil (TTO) or its main bioactive component terpinen-4-ol. TTO and terpinen-4-ol MICs ranged from 0.06 percent to 0.5 percent for all tested fluconazole-resistant C. albicans strains. After 24 hours, fluconazole with sublethal dose TTO increased fluconazole activity against fluconazole-resistant C. albicans strains. [49]
19. **Koteswara Rao and Pachva (2015):** The study tested whether tea tree oil in soft denture line would inhibit candida albicance growth and shown activity up to 60 days. These results suggest that TTO could be used to treat DS and possibly other oral infections. [50]
20. **Aparna H. Gopikrishna et al (2016)** The antifungal activity of Centratherum anthelminticum and Ocimum sanctum seed oils was evaluated against six pathogenic Candida strains. Researchers looked into how major oil constituents work together. The antifungal activity of Centratherum anthelminticum and Ocimum sanctum seed oils exhibited strong activity against six species of Candida.[51]
21. **Zahid Iqbal et al (2016)** This review will examine 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 commercial available tissue conditioners can help treat denture-induced stomatitis. [52]

22. **Wander José da Silva et al (2016)** The goal of the study was to check how the surface roughness (SR) of denture base and liner materials affected Candida albicans biofilm development. Higher surface roughness was obtained with finished acrylic resin and denture liner discs compared to finished + polished discs ($P<0.001$). No metabolic activity differences were seen between biofilms formed on both groups ($P>0.05$). Bulky, thick and less rough biofilms were formed on only finished denture materials ($P<0.05$). Confocal images reveal increased presence of black spaces for biofilms developed on finished + polished discs. Reduced SR resulted in decreased *C. albicans* biofilm accumulation on both denture materials. [53]
23. **Seshagiri Muttagi et al (2017)** investigated SR, antifungal property, wettability, glucose sorption, and weight change. They concluded that adding Linum usitatissimum, Ocimum sanctum, and Centratherum anthelminticum seed oils to soft liners remarkably reduced Candida albicans progression, SR, wettability, and glucose absorption. [54]
24. **Pragati Rawat (2017)** Tissue conditioners are used to heal abused oral tissues. Microorganisms compromises patient health and cause oral diseases like candidiasis.. Addition of antifungal agents in tissue conditioner may also change its properties. This study compares the antifungal and mechanical properties of different . This can be used as an effective alternative to systemic or topical synthetic antifungal agents. [55]

25. **Promila (2018)** According to a review article, Cymbopogon martini is medicinal and aromatic plant rich in essential oils. Oil has some antimicrobial, antifungal, antiviral, anthelmintic, and antioxidant properties. [56]

26. **Seenivasan Madhan Kumar et al. (2018)** investigated the in vitro growth inhibition of Candida albicans in soft-liner materials derived from resin-based denture soft lining materials treated with neem or garlic. Resin discs were put on agar plates infected with Candida albicans and tested for antifungal activity using the streaking method after 2, 4, and 7 days. C. albicans was inhibited by neem and garlic applied to a PMMA 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. [57]

27. **Gayathri Krishnamoorthy et al. (2019)** conducted a study to assess the antifungal efficacy and tensile strength of tissue conditioners containing 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. [58]

28. **Hejazi et al (2021)** Study findings suggest that the C. copticum L. EO-loaded tissue conditioner can treat both injured mucosa and denture stomatitis. As a novel treatment for denture stomatitis, the Carum copticum L. essential oil-loaded tissue conditioner exhibited suitable physical, biological, and release properties. [59]

MATERIAL AND METHODOLOGY

SOURCE OF DATA:

This in-vitro study was conducted in-

- KLE Academy of Higher Education and Research, KLE V. K. Institute of Dental Sciences, Belagavi:
    1. Department of Prosthodontics and Crown & Bridge
    2. Department of Microbiology
- Department of Mechanical Engineering, K.L.S. Gogte Institute of Technology, Belagavi.

INCLUSION CRITERIA:

- Specimens with identical size and shape.
- Specimens free of any voids.

EXCLUSION CRITERIA:

- Specimens with surface defects and deformities.
- Specimens with inaccurate dimensions.

PERMISSIONS TO BE TAKEN:

Permissions to be taken from –

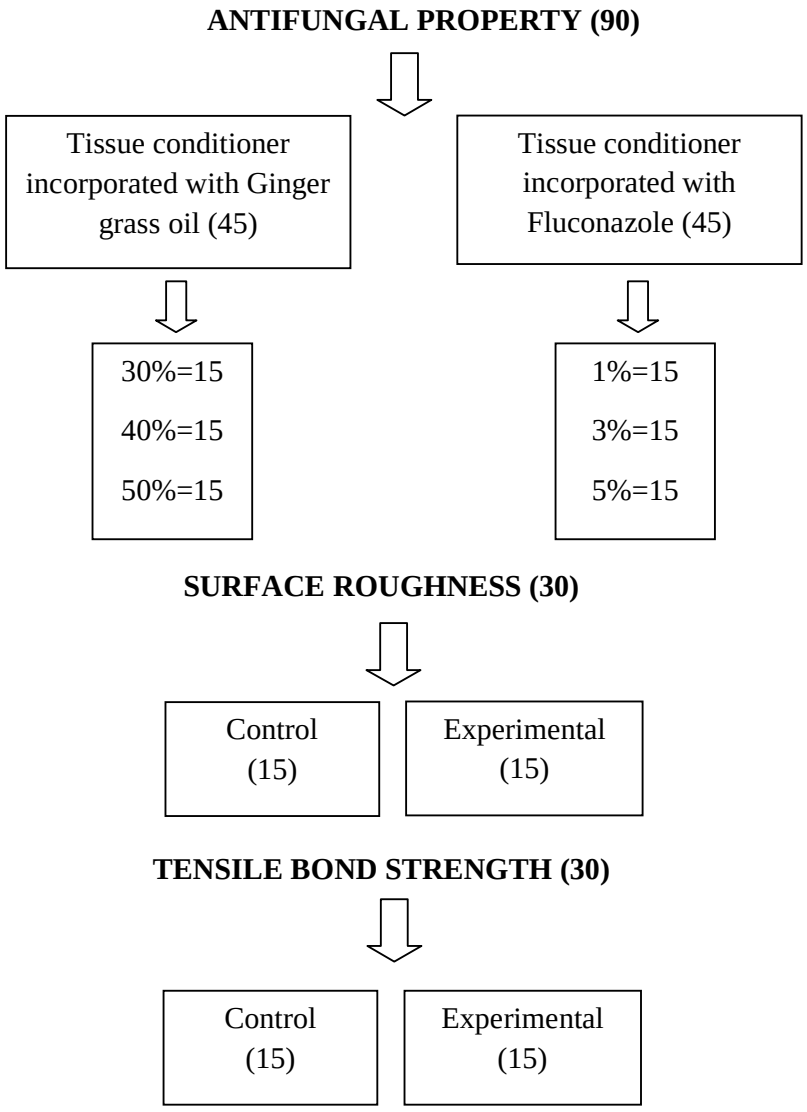
- KLE Academy of Higher Education and Research, KLE V. K. Institute of Dental Sciences, Belagavi:

1. Department of Prosthodontics and Crown & Bridge

2. Department of Microbiology

- Department of Mechanical Engineering, K.L.S. Gogte Institute, of Technology Belagavi – To use Profilometer and Universal testing machine.

TOTAL SAMPLE SIZE:



MATERIALS AND ARMAMENTARIUM

MATERIALS

- Candida albicans -ATCC catalogue number 90028 strains
- Sabouraud dextrose agar
- Commercially available antifungal additive- Ginger Grass Oil
- Tissue conditioner- Acrylic based
- Pure powder form of fluconazole
- Acrylic denture base resin

Table 1: Materials used in the study.

MATERIALS	DESCRIPTION	MANUFACTURER
Ginger grass oil	**Essential Oil**	**Commercially Available**
Sabouraud dextrose agar	**LOT D18JI4200- TR- 13C**	**Hi media, Mumbai**
***Candida albicans* strain**	**ATCC 90028**	**MTCC**
GC Soft liner	**1609031**	**GC Corp. Tokyo, Japan**
Denture base resin	**Heat Cure Resin**	**DPI Heat Cure**
Petri plates	**PW011**	**Hi- Media**
Fluconazole	**Powder Form**	**Hindustan Chemicals and Pharmaceuticals**

ARMAMENTARIUM

- Dapen dish
- Cement spatula
- Petri dishes
- Test tubes
- Metallic scale
- Sterile cork borer
- Incubator
- A brass metal pattern mould of dimension 40 mm x 10 mm x 7 mm and 10 mm x 7 mm x 3 mm
- Profilometer
- Universal testing machine

Table 2: Armamentarium used in the study.

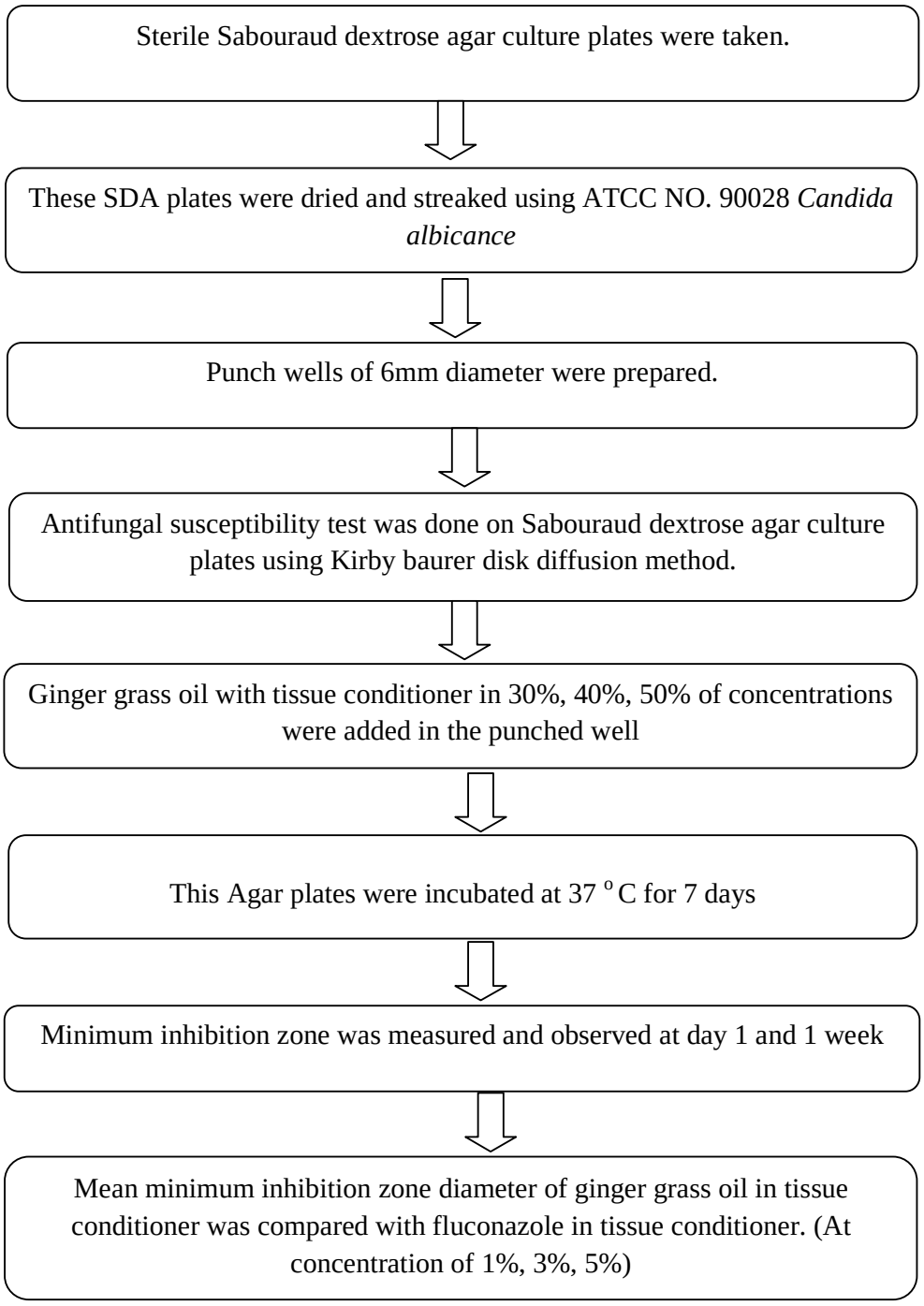
MATERIALS	DESCRIPTION	MANUFACTURER
Incubator	**Sr no. ZBCT-08444**	**Remi elektrotechnik ltd India**
Profilometer	**Surfcom Flex 50A**	**Zeiss India**
Universal testing machine	**Unitest -10**	**ACME Engineers, India**

A. To check the minimum inhibition zone diameter to evaluate antifungal property of Ginger grass oil in tissue conditioner and its comparison with fluconazole.

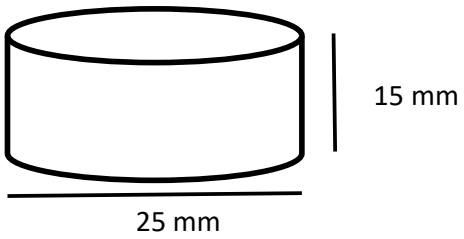
- Albicans (American type culture collection catalogue number 90028 strains) was obtained from the Department of Microbiology, Jawaharlal Nehru's medical college, Belagavi. Sterile Sabouraud dextrose agar culture plates were taken for the study. These SDA plates were dried and streaked using *Candida albicance* maintaining all sterilization and safety protocols. Antifungal susceptibility test was done on Sabouraud dextrose agar culture plates using Kirby baurer disk diffusion method. For that punch wells of 6mm were made.
- These wells were filled with different concentration of Ginger grass oil mixed with tissue conditioner to check the minimum inhibition zone diameter to evaluate antifungal activity against *Candida albicans* after 1 day and 1 week of time intervals. The minimum inhibition zone diameter of Ginger grass oil at different concentration (5%, 10%, 20%, 30%, 40%, 60% and 80%) in tissue conditioner were checked. (The pilot study was conducted and study concluded that, 30% of Ginger grass oil in tissue conditioner showed the minimum inhibition zone against candida albicans. The minimum inhibition zone diameter after 24 hours was 12 mm and similar diameter was observed after 1 week, only growth around the zone was increased.)
- In the present study 30%, 40%, 50% concentrations of Ginger grass oil with tissue conditioner (experimental group) were taken considering the pilot study. A total of 150 samples, minimum inhibition zone diameter of 90 samples were used to evaluate the antifungal activity using well diffusion method.

- Experimental group of 45 samples was further subdivided into 3 groups of 30%,40%,50% concentration (n = 15) which was used to measure mean minimum inhibition zone diameter and evaluate the antifungal activity which was compared with group containing 45 samples of fluconazole with tissue conditioner (comparative group) i.e. 15 samples in each group of 1%,3%, 5% concentration. These Agar plates were incubated at 37 ^{o} c for 7 days. Minimum inhibition zone diameter was measured and observed at day 1 and 1week. Later, mean minimum inhibition zone diameter of experimental group and comparative group were evaluated.

FLOWCHART-TO CHECK THE ANTIFUNGAL ACTIVITY

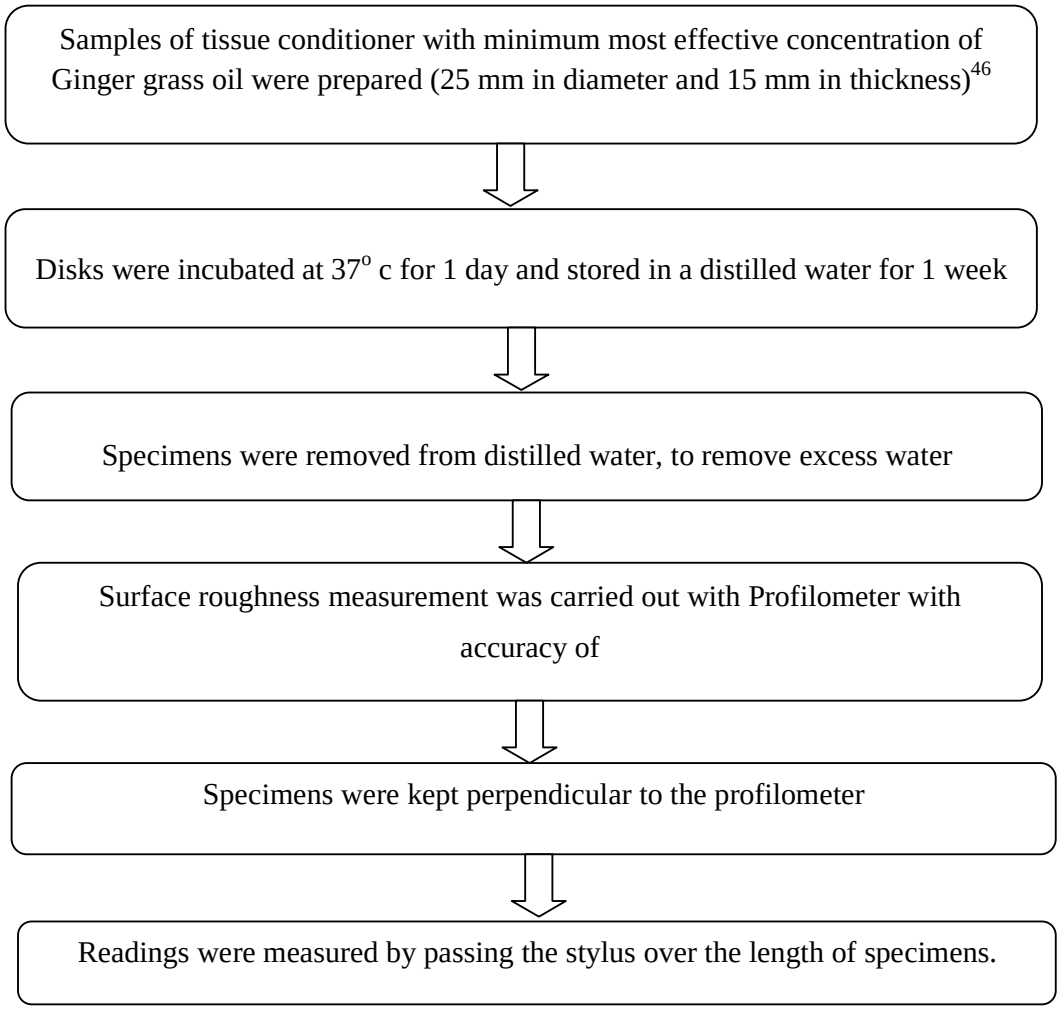


B. To check the surface roughness

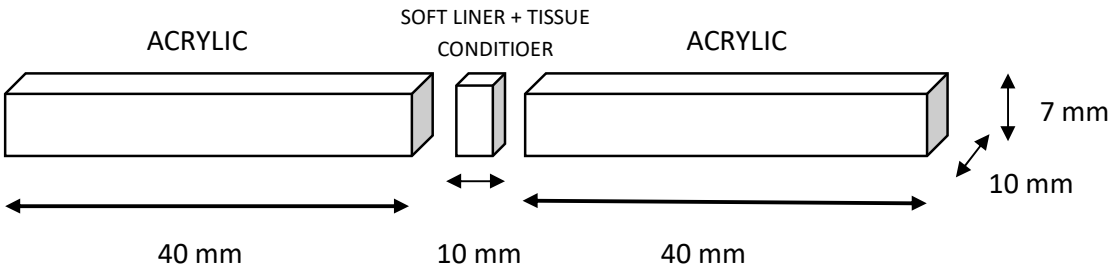


- 30 samples of 30% tissue conditioner with ginger grass oil (experimental group) and without ginger grass oil were prepared of dimension 25 mm in diameter and 15 mm in thickness.[46] 15 samples in each group
- Disks were incubated at 37^{o} c for 1 day and stored in distilled water for 1 week . Excess water was removed from the specimens. Surface roughness measurement was carried out with Profilometer with accuracy of ±0.01 µm (at 1 day and 1 week).
- Specimens were kept perpendicular to the profilometer. Surface roughness readings of experimental, control group at 1 day and 1 week in um were obtained by passing the stylus over the length of specimens.

FLOWCHART- TO CHECK THE SURFACE ROUGHNESS



C. To check tensile bond strength



- 30 samples of 30% tissue conditioner with ginger grass oil (experimental group) and without ginger grass oil were prepared using rectangular brass metal pattern mould for a test specimen of size 40 mm x 10 mm x 7 mm. Wax blocks were prepared by pouring molten wax into the mould cavity.[34]

- Two blocks was invested with a brass spacer of dimensions 10 mm x 7 mm x 3 mm in between the wax blocks. Dewaxing was done.

- Heat-cured PMMA (DPI – heat cure material) was used to fabricate the acrylic blocks.

- Trial packing was done, and excess was trimmed. After packing, the flasks were compressed with the help of hydro press.

- Then it was processed in a water bath at 75°C for 1½ hour, followed by 100°C for 1 hour.

- After polymerization of acrylic blocks, the metal spacer was retrieved. The required tissue conditioner was manipulated with minimum most effective concentration of ginger grass oil, trial packing done, and it was cured according to the manufacturer's instructions.

- After setting specimens were stored in 200 ml of distilled water and incubated at 37^{o} c for 1 day and 1week.

- The specimen were fixed to the grip of the Universal Testing Machine and pulled in either way. The maximum load, which the specimen can take till the break point, was noted kg/cm^2

- The bond strength was calculated by = $\frac{\text{Stress at failure (kg)}}{\text{Cross sectional area of the sample } (cm^2)}$,

TO CHECK THE TENSILE BOND STRENGTH

30 samples of 30% tissue conditioner with ginger grass oil (experimental group) and without ginger grass oil were prepared using rectangular brass metal pattern mould for a test specimen of size 40 mm x 10 mm x 7 mm. Wax blocks were prepared by pouring molten wax into the mould cavity.[34]

⇩

Two blocks were invested with a brass spacer of dimensions 10 mm x 7 mm x 3 mm in between the wax blocks. Dewaxing was done.

⇩

Heat-cured PMMA (DPI – heat cure material) was used to fabricate the acrylic blocks.

⇩

Trial packing was done, and excess was trimmed. After packing, the flasks was compressed with the help of hydro press.

⇩

Then it was processed in a water bath at 75°C for 1½ hour, followed by 100°C for1 hour

⇩

After polymerization of acrylic blocks, the metal spacer was retrieved. The minimum most effective concentration of Ginger grass oil in tissue conditioner was manipulated; trial packing done and it was cured according to manufacturers instructions.

⇩

After setting specimens were stored in 200 ml of distilled water and incubated for 1 day and simulate the oral environment.

⇩

The specimen was fixed to the grip of the Universal Testing Machine and pulled in either way. The maximum load, which the specimen was noted till the break point in kg/cm.^{2}

⇩

The bond strength calculated by =

$$\frac{\text{Stress at failure (kg)}}{\text{Cross sectional area of the sample (cm}^2\text{)}}$$

Figure1: Commercially available Ginger grass oil

Figure 2:Tissue conditioner and relining material

Figure 3:Pure form of fluconazole powder

Figure 4: Sabouraud Dextrose Agar

Figure 5: Heat cure denture base material

Figure 6: An Incubator

Figure 7: Candida Albicance Streaking on Sabouraud Dextros Agar

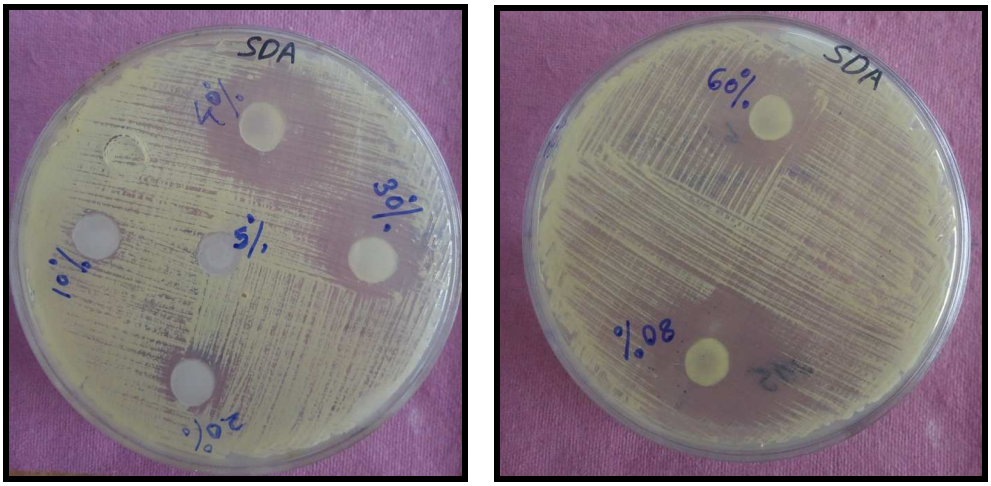


Figure 8 : The minimum inhibition zone diameter of Ginger grass oil at different concentration (5%, 10%, 20%, 30%, 40%, 60% and 80%) in tissue conditioner.

Figure 9: Different Concentrations (30%, 40%, 50%) of Ginger Grass Oil Mixed with Tissue Conditioner.

Figure 10: Different Concentrations (1%, 3%, 5%) of Fluconazole Mixed with Tissue Conditioner

Different Concentrations (30%, 40%, 50%) of Ginger Grass Oil Mixed with Tissue Conditioner

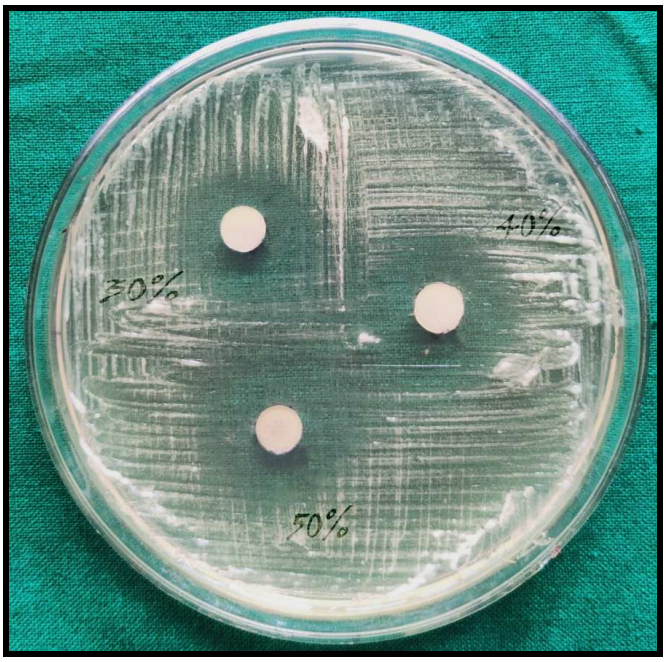


Figure 11: At 1st Day

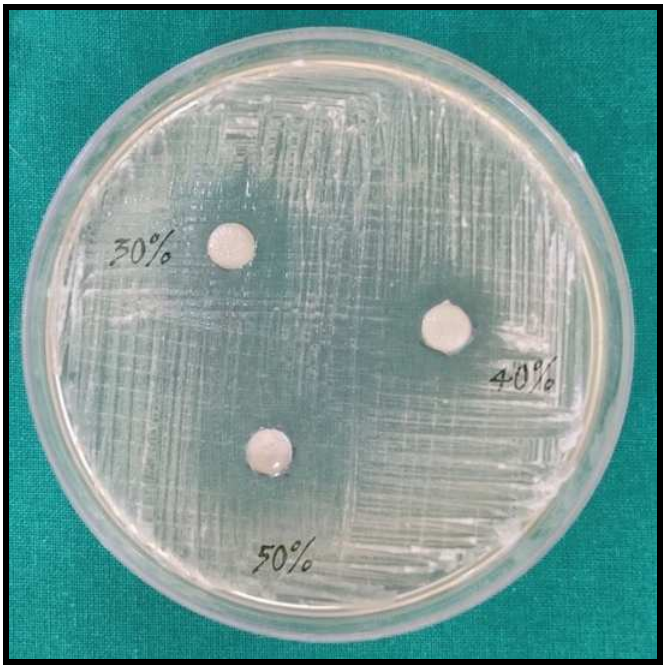


Figure 12: At 1 Week

Different Concentrations (1%, 3%, 5%) of Ginger Grass Oil Mixed with Tissue Conditioner

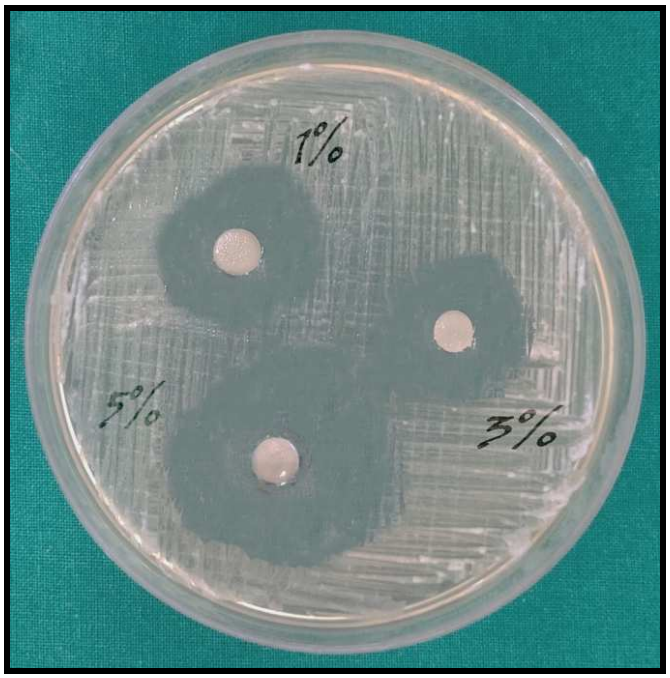


Figure 13: At 1st Day

Figure 14: At 1 Week

Figure 15: Samples of tissue conditioner to evaluate surface roughness

Figure 16: Profilometer Used To Check Surface Roughness

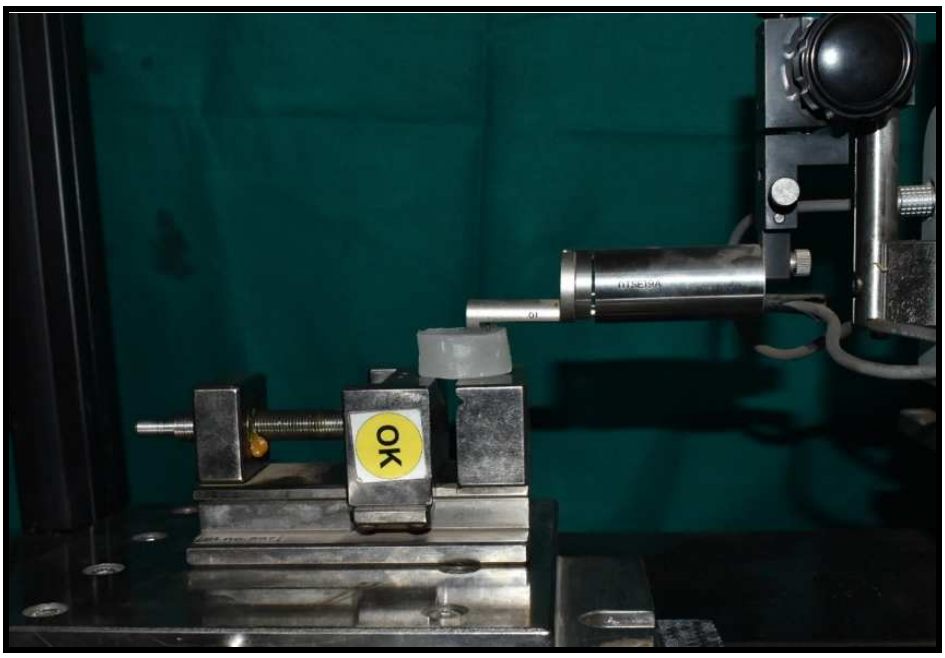


Figure 17: Disk of Tissue conditioner incorporated with Ginger grass oil prepared of dimension 25 mm in diameter and 15 mm in thickness.

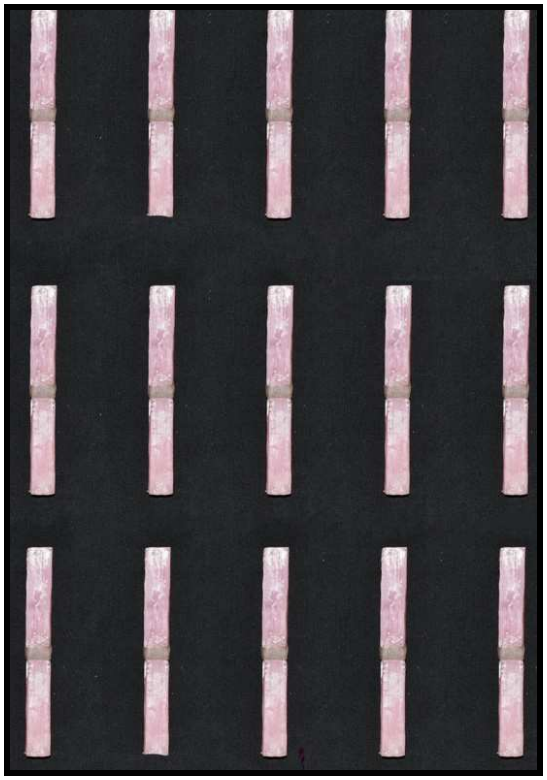


Figure 18: Samples of heat cure with tissue conditioner to evaluate tensile bond strength

Figure 19: A rectangular Test Specimen of Size 40 mm x10 mm x7 mm with a brass spacer of dimensions 10 mm x 7 mm x 3 mm in between the wax blocks fabrication.

Figure 20: A rectangular Test Specimen of Size 40 mm x10 mm x7 mm.

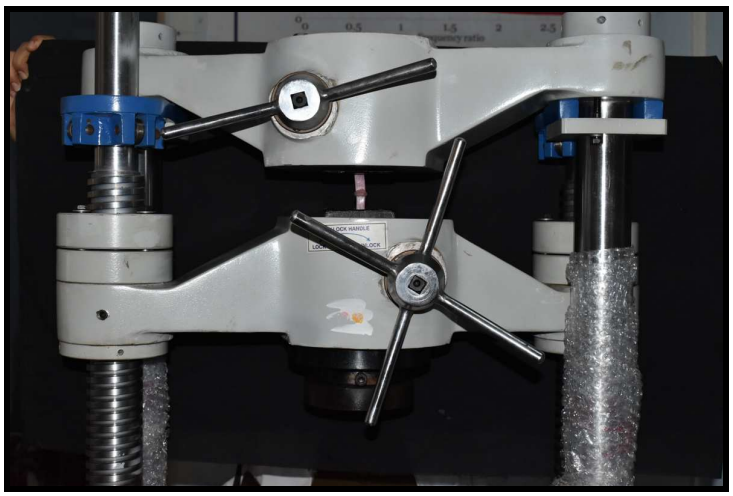


Figure 21: Universal Testing Machine Used To Check Tensile Bond Strength

RESULTS

The present study was carried out to evaluate the comparative evaluation of antifungal activity, surface roughness and tensile bond strength of ginger grass oil incorporated in tissue conditioner**.**

Statistical Analysis

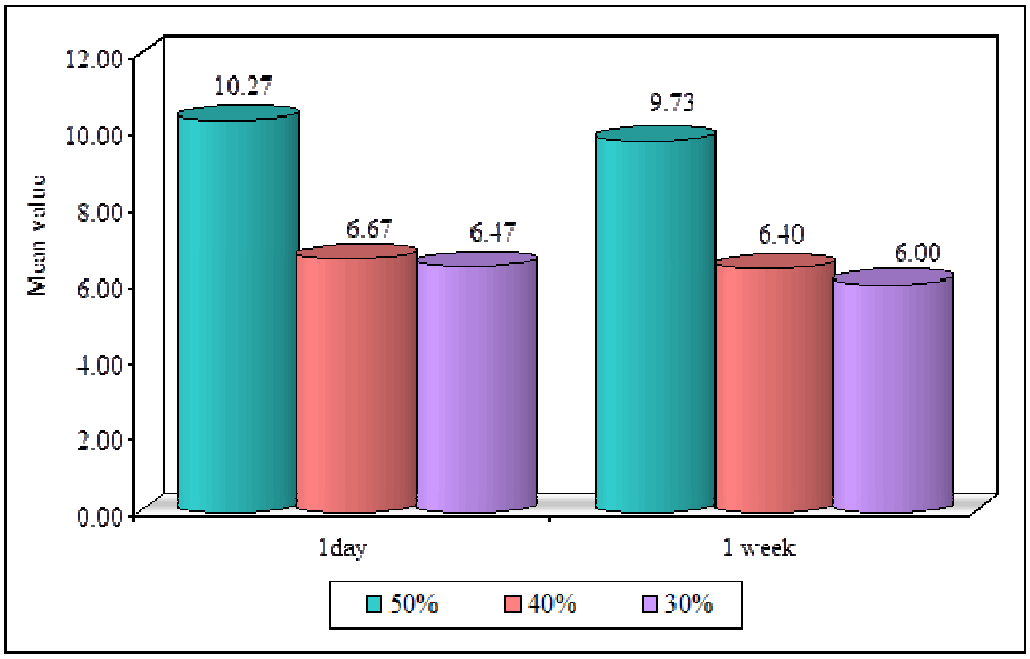
Data obtained from the study was entered in Microsoft excel sheet and SPSS version 20 software was used to carry out the statistical analysis. Descriptive statistics were applied to describe the basic features of the data.

"Dependent t test" was used for comparison between 1day in experimental and control group and 1week in experimental and control group and "Independent t test" was used for comparison between 1 day , 1 week in experimental group and 1 day,1 week in control group respectively.

Table 3: Summary of minimum inhibition zone diameter (MIZ-D) in three concentrations at 1 day and 1 week time intervals of Ginger grass oil with tissue conditioner (experiment group) to evaluate antifungal activity.

Time points	Concentration	Mean	SD	SE	95% CI for Mean	
					Lower Bound	Upper Bound
1day	30%	6.47	0.92	0.24	5.96	6.97
	40%	6.67	1.18	0.30	6.02	7.32
	50%	10.27	2.05	0.53	9.13	14.40
1 week	30%	6.00	0.85	0.22	5.33	6.47
	40%	6.40	1.12	0.29	5.78	7.02
	50%	9.73	1.94	0.50	8.66	10.81

Graph 1 : Summary of minimum inhibition zone diameter in three concentrations at 1 day and 1 week time intervals of Ginger grass oil with tissue conditioner (experiment group) to evaluate antifungal activity.



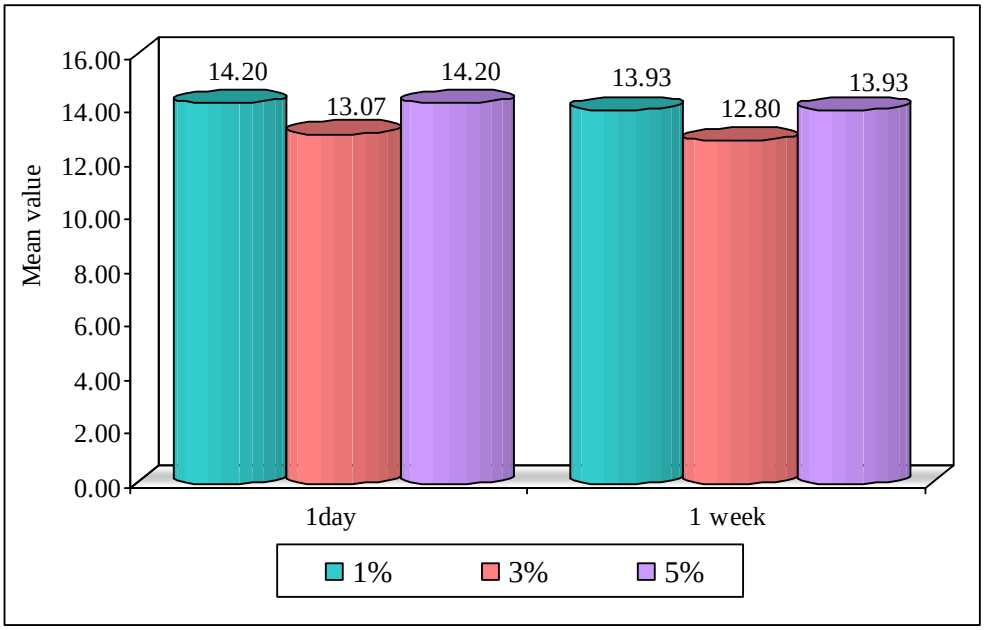
Minimum inhibition zone diameter (MIZ-D) of ginger grass oil with tissue conditioner at 30% after 1 day, 1week obtained were 6.47mm, 6.00mm, at 40% was 6.67mm, 6.40mm and at 50% was 10.27mm, 9.73mm, respectively. Thus there was a statistically significant difference in the zone of inhibition of experimental group at 1 day and 1 week.

According to the graph, there was a statistically significant difference in the zone of inhibition of experimental group at 1 day and 1 week. Zone of inhibition at 30% was less than other two concentrations taken in the study and this zone of inhibition increased with concentration and decreased with time.

Table 4: Summary of minimum inhibition zone diameter (MIZ-D) in three concentrations at 1 day and 1 week time intervals of Fluconazole with tissue conditioner (comparative group) to evaluate antifungal activity.

Time points	Concentration	Mean	SD	SE	95% CI for Mean	
					Lower Bound	Upper Bound
1day	1%	14.20	0.68	0.17	13.83	14.57
	3%	13.07	0.96	0.25	12.53	13.60
	5%	14.20	0.68	0.17	13.83	14.57
1 week	1%	13.93	0.70	0.18	13.54	14.32
	3%	12.80	1.01	0.26	12.24	13.36
	5%	13.93	0.70	0.18	13.54	14.32

Graph 2: Summary of minimum inhibition zone diameter (MIZ-D) in three concentrations at 1 day and 1 week time intervals of Fluconazole with tissue conditioner (comparative group) to evaluate antifungal activity.



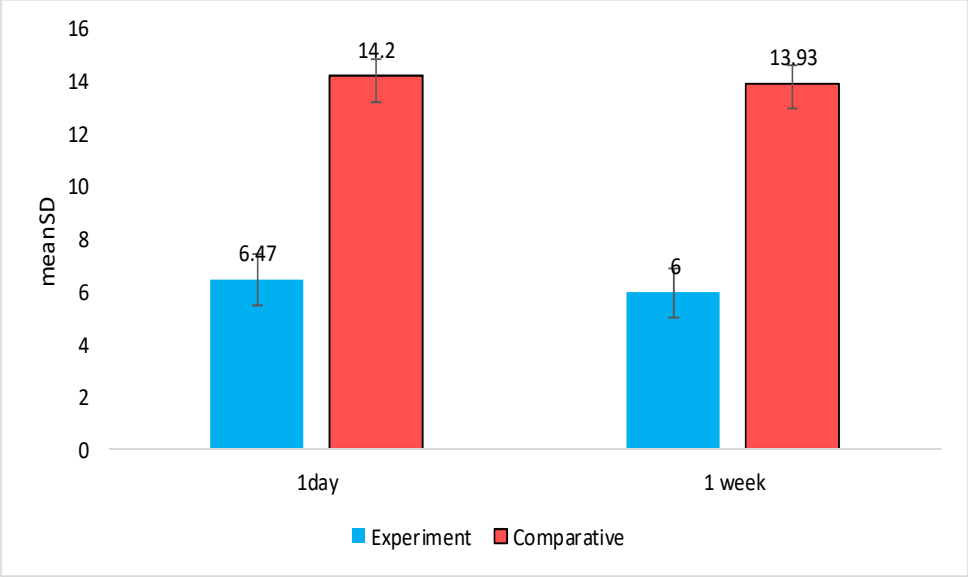
Minimum inhibition zone of Ginger grass oil with tissue conditioner (MIZ-D) at 1% after 1 day, 1week obtained were 14.20mm, 13.93mm, at 3% after was 13.07mm, 12.80mm, at 5% was 14.20mm, 13.93mm respectively. Above graph shows that mean minimum inhibition zone observed in 1% and 5% of fluconazole with tissue conditioner at 1 day and 1 week was similar.

Table 5: Comparison of experiment group and comparative group with mean minimum inhibitory zone diameter (MMIZ-D) at 1 day and 1 week time points by independent t test

Time points	Experiment group (30%)		Comparative group (5%)		Mean Difference	t-value	F value	p-value
	Mean	SD	Mean	SD				
1day	6.47	0.915	14.20	0.68	-7.73	-26.32	2.199	0.000*
1 week	6.00	0.845	13.93	0.70	-7.93	-27.94	2.104	0.000*

*p<0.05

Graph 3 : Comparison of experiment group and comparative group with mean minimum inhibitory zone diameter (MMIZ-D) at 1 day and 1 week time points by independent t test



Mean minimum inhibition zone diameter (MMIZ-D) was obtained i.e. 30% of ginger grass oil in tissue conditioner (experimental group) with the MMIZ-D i.e. 5% of fluconazole in tissue conditioner (comparative group) were compared after 1 day and 1 week of time period.

MMIZ-D at 1 day in experimental group, comparative group was 6.47mm, 14.20mm respectively. MMIZ-D at 1 week in experimental group, comparative group was 6.00mm, 13.93mm respectively.

Comparison of experimental and comparative group with MMIZ-D at 1 day and 1 week time intervals by independent t test. Thus, there was a statistically significant difference ($p<0.05$) seen at different time intervals between the groups.

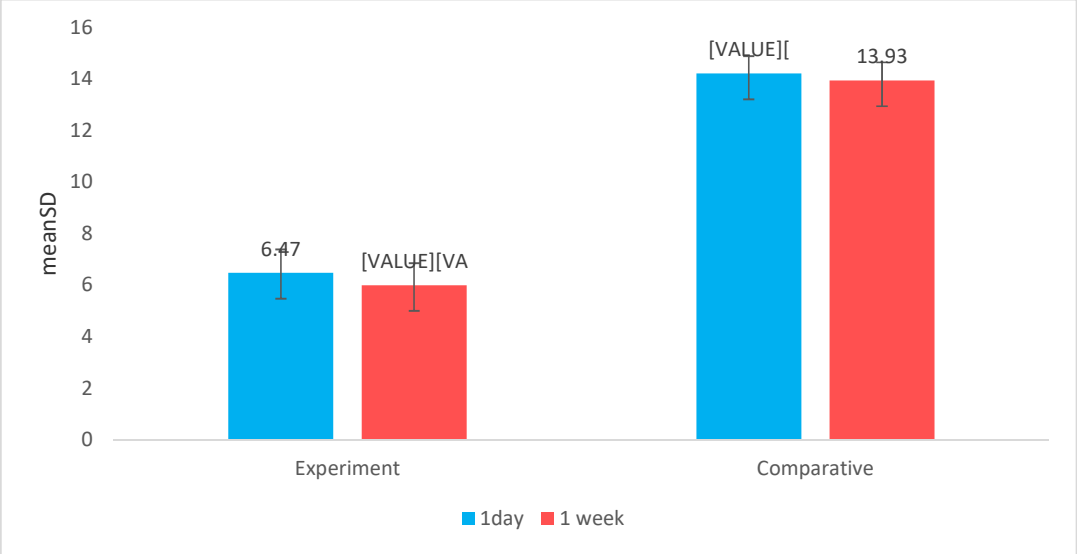
According to above graph, mean minimum inhibition zone diameter at 30% of oil in tissue conditioner was less compared to the comparative group (5% fluconazole in tissue conditioner) after 1 day, 1week.

Table 6: Comparison of experiment group and comparative group with mean minimum inhibitory zone diameter (MMIZ-D) at 1 day and 1 week time points by dependent t test

Groups	Time points	Mean	SD	Mean Diff.	SD Diff.	t-value	p-value
Experiment group	1day	6.47	0.915	0.165	0.64	2.824	0.014*
	1 week	6.00	0.845				
Comparative group	1day	14.20	0.68	0.27	0.46	2.2563	0.0406*
	1 week	13.93	0.70				

*p<0.05

Graph 4 : Comparison of experiment group and comparative group with mean minimum inhibitory zone diameter (MMIZ-D) at 1 day and 1 week time points by dependent t test



Mean minimum inhibition zone diameter (MMIZ-D) in experimental group at 1 day and 1 week was 6.47mm and 6.00mm respectively. In comparative group at 1 day and 1 week was 14.20mm and 13.93 respectively. Comparison of zone of MMIZ-D at different time intervals in experimental and comparative group was done by dependent t test. Thus, there was a statistically significant difference ($p<0.05$) seen at different time intervals within the groups.

According to above graph, mean minimum inhibition zone diameter (MMIZ-D)at 30% of oil in tissue conditioner at 1 day was more compared to 1week. Similar findings were obtained with the comparative group.

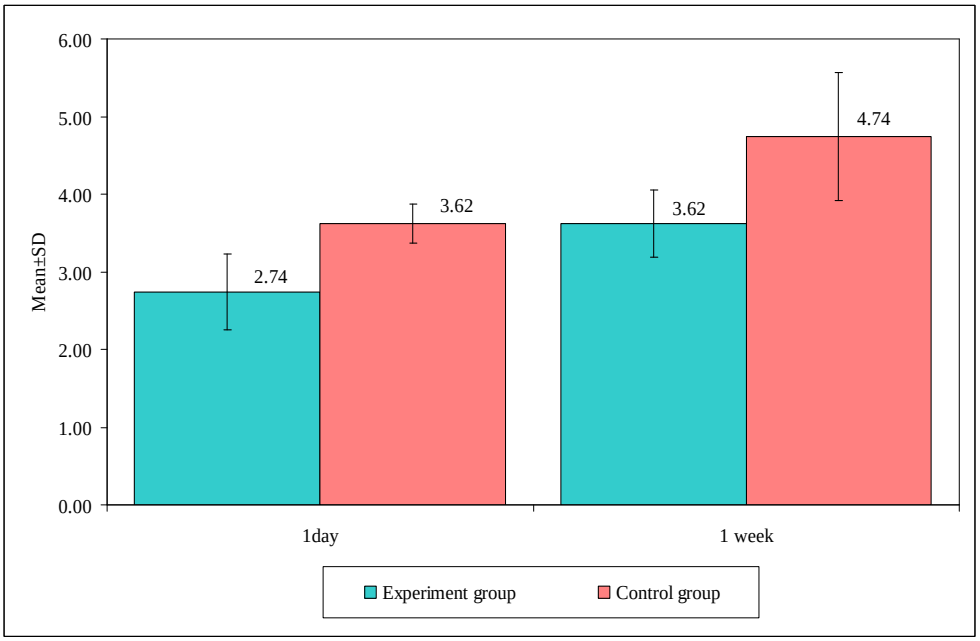
Surface roughness

Table 7: Comparison of tissue conditioner with Ginger grass oil (experiment group) and tissue conditioner without Ginger grass oil (control group) with mean Surface roughness scores at 1 day and 1 week time points by independent t test

Time points	Experiment group		Control group		Mean Difference	t-value	p-value
	Mean	SD	Mean	SD			
1day	2.74	0.49	3.62	0.25	-0.88	-6.1682	0.0001*
1 week	3.62	0.43	4.74	0.82	-1.13	-4.6891	0.0001*
Difference	0.87	0.67	1.12	0.87	-0.25	-0.8852	0.3836

*p<0.0

Graph 5: Comparison of tissue conditioner with Ginger grass oil (experiment group) and tissue conditioner without Ginger grass oil (control group) with mean Surface roughness scores at 1 day and 1 week time points by independent t test



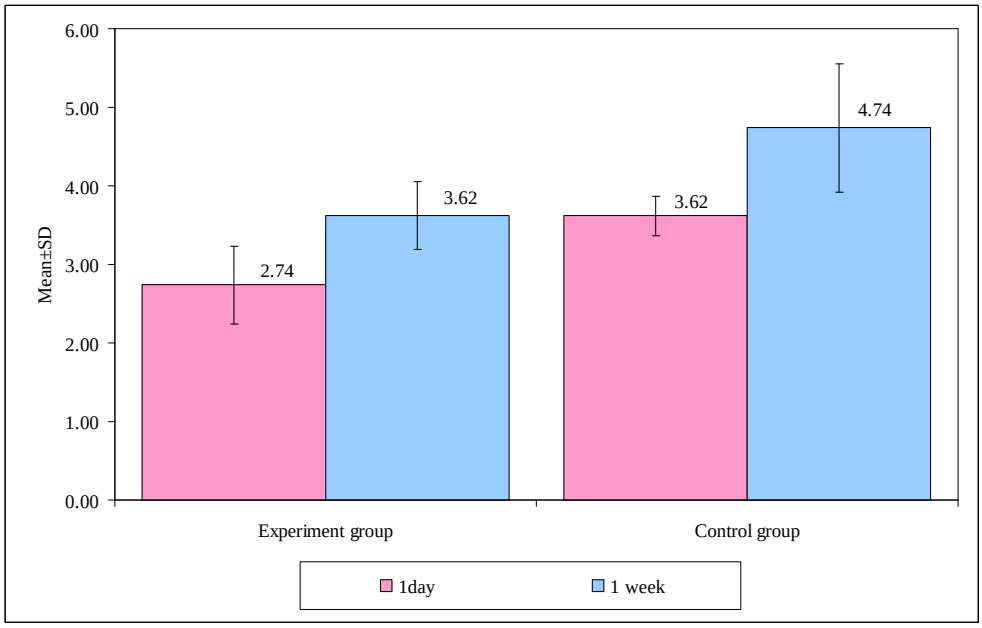
Comparison at 1 day and 1 week with respect to surface roughness of experimental group and control group was done by independent t test. Statistically significant difference was seen between the groups. ($p<0.05$) Surface roughness at 1 day, 1week in experimental group and control group was 2.74um, 3.62um, 3.62um, 4.47um respectively.

According to above graph there was significant increase in surface roughness with respect to time intervals in both the groups.

Table 8 : Comparison of tissue conditioner with Ginger grass oil (experiment group) and tissue conditioner without Ginger grass oil (control group) with mean Surface roughness scores at 1 day and 1 week time points by dependent t test

Groups	Time points	Mean	SD	Mean Diff.	SD Diff.	% of change	t-value	p-value
Experiment group	1day	2.74	0.49	-0.87	0.67	-31.71	-5.0427	0.0002*
	1 week	3.62	0.43					
Control group	1day	3.62	0.25	-1.12	0.87	-30.99	-4.9767	0.0002*
	1 week	4.74	0.82					

Graph 6 : Comparison of tissue conditioner with Ginger grass oil (experiment group) and tissue conditioner without Ginger grass oil (control group) with mean Surface roughness scores at 1 day and 1 week time points by independent t test



Comparison at 1 day and 1 week with respect to surface roughness within the experimental group and control group was done by dependent t test. Statistically significant difference was seen between the groups. ($p<0.05$), According to above graph increase in surface roughness was seen in experimental group at 1 day was 2.75um , after 1 week was 3.62um and in control group at 1 day was 3.62 um , after 1 week was 4.75um.

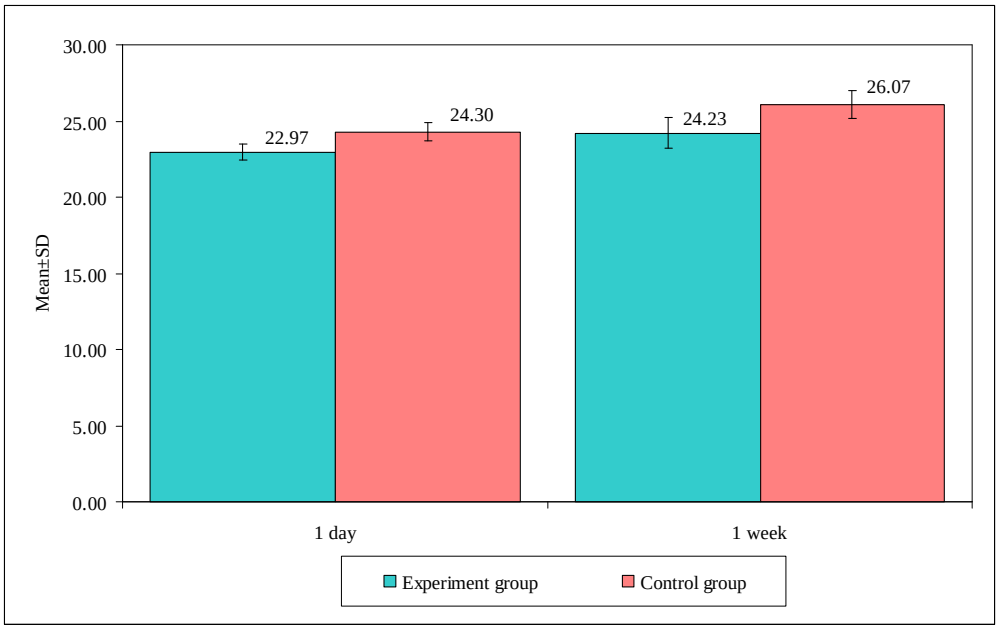
Tensile bond strength

Table 9: Comparison of tissue conditioner with Ginger grass oil (experiment group) and tissue conditioner without Ginger grass oil (control group) with mean tensile bond strength scores at 1 day and 1 week time points by independent t test

Time points	Experiment group		Control group		Mean Difference	t-value	p-value
	Mean	SD	Mean	SD			
1day	22.97	0.55	24.30	0.59	-1.33	-6.3934	0.0001*
1 week	24.23	0.98	26.07	0.92	-1.83	-5.2750	0.0001*
Difference	1.27	1.03	1.77	1.16	-0.50	-1.2451	0.2234

*$p<0.05$

Graph 7: Comparison of tissue conditioner with Ginger grass oil (experiment group) and tissue conditioner without Ginger grass oil (control group) with mean tensile bond strength scores at 1 day and 1 week time points by independent t test



Comparison at 1 day and 1 week with respect to tensile bond strength of experimental group and control group was done by independent t test. Statistically significant difference was seen between the groups. ($p<0.05$) Tensile bond strength at 1 day, 1week in experimental group and control group was 22.97 kg/cm^2, 24.23kg/cm^2, 24.30kg/cm^2, 26.07kg/cm^2 respectively.

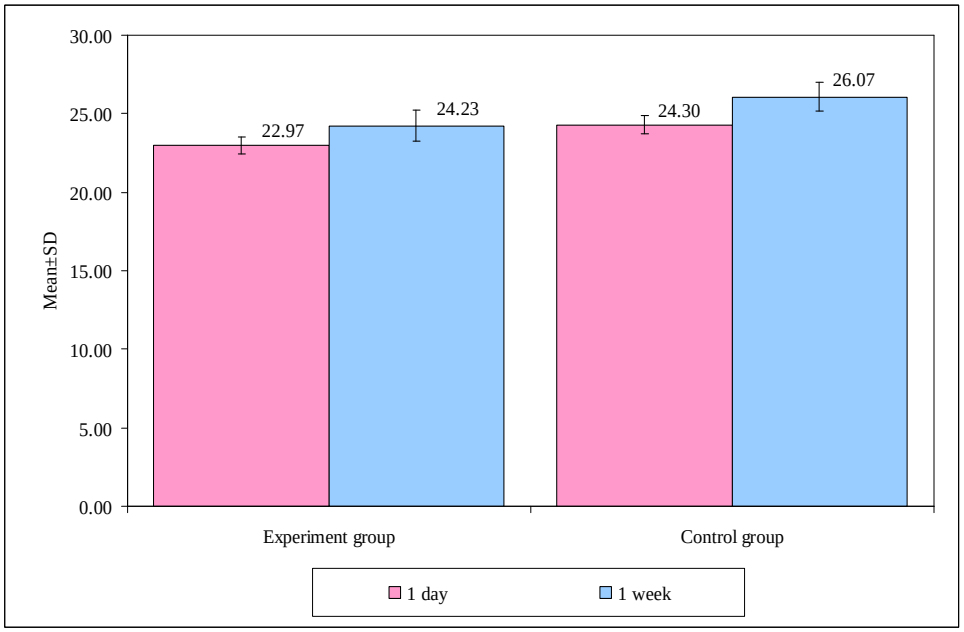
According to above graph there was significant increase in tensile bond strength with respect to time intervals in both the groups.

Table 10 : Comparison of tissue conditioner with Ginger grass oil (experiment group) and tissue conditioner without Ginger grass oil (control group) with mean tensile bond strength scores at 1 day and 1 week time points by dependent t test

Groups	Time points	Mean	SD	Mean Diff.	SD Diff.	% of change	t-value	p-value
Experiment group	1day	22.97	0.55					
	1 week	24.23	0.98	-1.27	1.03	-5.52	-4.7500	0.0003*
Control group	1day	24.30	0.59					
	1 week	26.07	0.92	-1.77	1.16	-7.27	-5.8837	0.0001*

*p<0.05

Graph 8 : Comparison of tissue conditioner with Ginger grass oil (experiment group) and tissue conditioner without Ginger grass oil (control group) with mean tensile bond strength scores at 1 day and 1 week time points by dependent t test



Comparison at 1 day and 1 week with respect to tensile bond strength within the experimental group and control group was done by dependent t test. Statistically significant difference was seen between the groups. ($p<0.05$)

According to the above graph, increase in tensile bond strength within the experimental group at 1 day was 22.97 kg/cm^2, after 1 week was 24.23 kg/cm^2 and in control group at 1 day was 24.30 kg/cm^2, after 1 week was 26.07 kg/cm^2.

DISCUSSION

The human oral cavity has been work as a unique ecosystem with many ecological niches for microbial colonization.[41] In the dentistry, PMMA acrylic resin is frequently used to make removable dentures because of its resilience, optical properties, aesthetics, and biocompatibility with oral tissues. Despite these benefits, Candida albicans colonization has been associated to PMMA and liner materials as well. The irregular surface increases the surface area and number of niches that the tongue or other orofacial musculature cannot easily clean. This is especially true of methacrylate resin oral appliances. Despite their appearance, these appliances have a pockmarked surface under a microscope. This is related to unpolymerized monomer bubbles in denture manufacturing.[18]

Candida albicans is a serious oral fungus that can colonize both hard and soft tissues and form complex biofilms. The material's porosity, roughness, surface free energy, and hydrophobicity would all influence this proliferation. Oral tissue health and patient comfort are affected by the surface finish of a removable prosthesis. Candida albicans is one of most medically important species that causes many oral fungal infections and Candida-associated denture stomatitis.[60] Ineffective denture care form biofilms on the acrylic denture surface without proper hygiene or antifungal treatment. This biofilms on denture acrylic are linked to denture-induced stomatitis as a consequence it results in local inflammation of the palatal mucosa. Effective denture cleaning is critical in treating this persistent infection, which many patients struggle with. **Budtz-Jorgensen & Bertram, 1970** claim that many elderly people are unaware of proper denture care. So, denture care is important in treating stomatitis. [61]

Denture biofilm prevention using mechanical brushing is a simple and common practice. While chemical immersion solutions have been recommended as a supplement to mechanical hygiene, it has been noted that these treatments also fail to eliminate Candida albicans biofilms.[33] Treatment with antifungal medications like nystatin, Clotrimazole, fluconazole, and miconazole. While systemic antifungals may help mucosal diseases, they do not help a Candida-infested denture fitting surface. Increased misuse of these drugs can also cause harm to humans. Again, geriatric denture wearers have reduced motor activity, cognitive impairment, memory loss, makes topical antifungal therapy difficult to apply and salivary washout prevent antifungal agents from remaining in contact with oral mucosal tissue.[33]

Natural antifungal and antibiotic alternatives such as melaleuca alterfolia and lemongrass essential oils as well as probiotics can help overcome these challenges. Due to their organic nature, they have high antimicrobial efficacy and low toxicity. [57] Numerous in vitro and in vivo studies showed that adding fungicidal ingredients to tissue conditioners can help prevent plaque formation and promote healing in denture stomatitis also improves denture retention and stability while distributing masticatory pressures to the underlying tissues to reduce denture induce soft tissue trauma and used as oral drug delivery tools.[58]

In continuation of the more serious issues with tissue conditioner is the lack of adhesion between the soft liner and the denture base. Bond failure also exposes a surface to bacterial, plaque, and calculus growth. Water sorption by the lining material causes a change in dimension and stress concentration at the liner-denture base interface, resulting in a decrease in bond strength. The following factors influence the bond between acrylic and liner: Geometry of the bond surface, whether

roughened or clinically etched, use of bonding agents, inherent bond strength, variable compliance, tear strength, and lining material thickness. When tissue conditioners are in constant contact with liquids such as oral fluids, the plasticizer and alcohol contents leach out and water or saliva fills the voids. The absorbed liquid acts as a plasticizer, lowering the mechanical properties of the polymer network and, as a result, the dynamic viscoelastic property. [22] Thus, surface properties should be evaluated during regular clinical evaluation so all these problems can be resolved.

Gruber *et al.* (1966) stated that silicone and methacrylate soft liners and tissue conditioners would sustain the growth of *C. albicans* in vitro. They reported that the growth on the surface of tissue conditioners revealed it starting to show on the third day of incubation. It has also been reported by Thomas & Nutt (1978), that there was no inhibitory effect for tissue conditioning material (Visco-gel) on *C. albicans* for periods of time varying from 3 days to 1 week. [33]

A study by **Duarte et al** found that the oils of Cymbopogon martini (Ginger grass oil) and Mentha arvensis were moderately antibacterial against Candida albicans. Cymbopogon martini is a highly valued medicinal and aromatic plant, according to Paula Cristina Anibale et al. In addition to antioxidant and cytotoxic properties derived from Palmarosa essential oils. [56] In the present study ginger grass oil has been used as it has moderate antifungal activity and can be used as herbal antifungal additive instead of using systemic antifungal medications.

In the pilot study, commercially available Ginger grass oil with acrylic based tissue conditioner (Relining and tissue conditioning- GC SOFT- LINER) was used as an antifungal agent for drug delivery. Commercially available Ginger grass oil mixed with acrylic based tissue conditioner at different concentrations i.e. 5%, 10%, 20%,

30%, 40%, 60%, 80% was used in the study to check the antifungal activity against *Candida albicans* and minimum most effective concentration after 1 day and 1 week of time period. The pilot study was conducted and study concluded that, 30% of *Ginger grass oil* in tissue conditioner showed the minimum inhibition zone against candida albicans. The minimum inhibition zone diameter at 1 day was 12 mm and similar diameter was observed after 1 week, only growth around the zone was increased.

In the present study, minimum inhibition zone diameter of ginger grass oil with tissue conditioner at three different concentrations 30%,40%,50% was evaluated to check the antifungal activity at two different time intervals i.e. after 1 day and 1 week which was compared with 1%, 3%, 5% concentrations of fluconazole with tissue conditioner.

Mean minimum inhibition zone diameter of ginger grass oil with tissue conditioner at 30% after 1 day, 1week obtained were 6.47mm, 6.00mm, at 40% was 6.67mm, 6.40mm and at 50% was 10.27mm, 9.73mm respectively. Thus there was a statistically significant difference in the zone of inhibition of experimental group at 1 day and 1 week.

Thus mean minimum inhibition zone diameter was found at lower concentration i.e. at 30% and this zone of inhibition was increased with the increased concentration. And in comparative group , minimum inhibition zone at 1% after 1 day, 1week obtained were 14.20mm, 13.93mm, at 3% after was 13.07mm, 12.80mm, at 5% was 14.20mm, 13.93mm respectively. Graph shows that mean minimum inhibition zone diameter observed in 1% and 5% of fluconazole with tissue conditioner at 1 day and 1 week was similar.

Mean minimum inhibition zone diameter (MMIZ-D) was obtained i.e. 30% of ginger grass oil in tissue conditioner (experimental group) with the MMIZ-D i.e. 5% of fluconazole in tissue conditioner (comparative group) were compared after 1 day and 1 week of time period.

MMIZ-D at 1 day in experimental group, comparative group was 6.47mm, 14.20mm respectively. MMIZ-D at 1 week in experimental group, comparative group was 6.00mm, 13.93mm respectively.

Comparison of experimental and comparative group with MMIZ-D at 1 day and 1 week time intervals by independent t test. Thus, there was a statistically significant difference ($p<0.05$) seen at different time intervals between the groups. Mean minimum inhibition zone diameter at 30% of oil in tissue conditioner was less compared to the comparative group (5% fluconazole in tissue conditioner) after 1 day, 1week. Incorporation of ginger grass oil in denture soft relining material inhibited the candida albicance growth, hence it can revolutionize the soft relining material with added benefit of anti-fungal activity.

Study conducted by Sunanda Sharma et al contradicted the present study when 5% w/w fluconazole was compared to 30% w/w melaleuca oil, the MIDs for fluconazole were some what lower than those for melaleuca oil at 24 hours. [46] According to the literature 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.[47]

Whereas Chow et al. evaluated that, fluconazole at a concentration of 5% w/w in Visco-gel exhibited the highest antifungal activity. This occurrence may be explained by the drug's greatest efficacy at that concentration and/or by an inhibitory effect at greater drug concentrations. Fluconazole, Chlorhexidine, and Clotrimazole in combination with Lynal'; and Zeolite in combination with a GC liner and Viscogel , demonstrated a range of growth inhibition outcomes superior to the control group.

According to Akanksha Srivatstava et al, the MIZ diameter obtained with various concentrations (10%, 20%, 30%, 40%, 50%, 55%, 57%, 60%, 65%) of origanum oil in tissue conditioner at 1 day and 1 week , which shows that the MIZ was dependent on the concentration of origanum oil. The control group did not show any antifungal activity against *Candida albicans*. Therefore 60% concentration of origanum oil was the most effective used as an natural additive to tissue conditioner to reduce the adherence of *Candida albicans.*[46]

In continuation with the similar study by Akanksha Shrivastava et al Origanum oil at 60v/v% oil concentrations incorporated into tissue conditioner (Visco-gel), and adherence of *Candida albicans*, surface roughness, of the tissue conditioner with an optimized Origanum oil concentration were evaluated. Results concluded that the surface roughness of tissue conditioners with Origanum oil was less than that for the control group. [46]

Whereas, the mean and standard deviation values for surface roughness in the present study were evaluated to find if incorporation of 30% ginger grass oil in tissue conditioner (experimental group) affect the surface roughness. Comparison at 1 day and 1 week with respect to surface roughness of experimental group and control

group was done by independent t test. Statistically significant difference was seen between the groups. ($p<0.05$)

Surface roughness at 1 day, 1week in experimental group and control group was 2.74um, 3.62um, 3.62um, 4.47um respectively. This comparison was done by independent t test showed that statistically significant difference was seen in between the groups. ($p<0.05$)

Surface roughness at 1 day, 1 week in experimental group was 2.74um, 3.62um and in control group was 3.62um, 4.47um respectively. This comparison was done by dependent t test showed that statistically significant difference was seen in between the groups. ($p<0.05$)

Result states that, surface roughness was less in experimental group than control group. There was significant increase in surface roughness with respect to time intervals in both the groups it could be due to the leaching of ginger grass oil which increased surface roughness of tissue conditioner. Therefore tissue conditioners should be replaced within short time of period which does not hamper the surface properties of the material.

Soft denture liners have several problems; one of the more serious problems with soft liners is the failure of adhesion between the soft denture liner and the denture base. Bond failure also creates a potential surface for bacterial growth, plaque and calculus formation. Therefore, frequent clinical evaluation and periodic replacement of the soft denture liner is required. Tests developed to evaluate adhesive strength of materials.

Akanksha Shrivastava et al Origanum oil at 60v/v% oil concentrations incorporated into tissue conditioner (Visco-gel), and tensile bond strength of the tissue conditioner with an optimized Origanum oil concentration were evaluated. Results concluded that incorporation of Origanum oil reduces tensile strength significantly, which could be due to the incomplete gelling of the tissue conditioner. However, after 1week tensile strength was found to be higher, indicating hardening of the tissue conditioner. In another study conducted by Thomas J Emmer et al, suggesting that water storage could also increases the bond strength. [46]

Whereas, in the present study, tensile bone strength was used to study the bond of the liner to the resin along with 30% of ginger grass oil (experimental group) .Comparison at 1 day and 1 week with respect to tensile bond strength of experimental group and control group was done by independent t test. Statistically significant difference was seen between the groups. ($p<0.05$)

Tensile bond strength at 1 day, 1week in experimental group and control group was 22.97 kg/cm^2, 24.23kg/cm^2, 24.30kg/cm^2, 26.07kg/cm^2 respectively. This comparison was done by independent t test showed that statistically significant difference was seen in between the groups. ($p<0.05$)

Tensile bond strength at 1 day, 1 week in experimental group was 22.97 kg/m^2, 24.23kg/cm^2 and in control group was 24.30kg/cm^2, 26.07kg/cm^2 respectively. This comparison was done by dependent t test showed that statistically significant difference was seen in between the groups. ($p<0.05$). graph shows decreased in the bond strength in experimental group compared to control group.

Thus there was significant increase in tensile bond strength with respect to time intervals which predicts the hardening of polymer or may be due to water storage of the material. Adequate bond strength is important factor to reduce the adhesion of *Candida albicans.* Therefore, addition of ginger grass oil in tissue conditioner decreases tensile bond strength was limitation of the study.

LIMITATIONS OF THE STUDY

- Since this is an in-vitro study, application of the results in clinical conditions might yield different result.
- Mean minimum inhibition zone diameter of *ginger grass oil* with tissue conditioner was less compared to *fluconazole* with tissue conditioner but it was comparable as fluconazole is potent antifungal agent. Additionally, systemically administered antifungal agents may be beneficial against mucosal diseases, these medications have been shown to have a harmful effect on humans when they are misused. Therefore herbal essential oil can be better option to treat denture stomatitis.
- Only one acrylic type of interim resilient liner was evaluated in this study. Different liners might yield different results.
- Incorporation ginger grass oil in tissue conditioner caused decrease in bond strength between tissue conditioner and denture base acrylic.

CLINICAL IMPLICATIONS

As denture soft liner can be used as a vehicle to deliver drugs, incorporation of antifungal drugs can be considered as a promising medicament against denture stomatitis to improve oral health status of the geriatric patients with cognitive disturbances, medically compromised conditions and reduced manual dexterity. Because of an increase in emergence of drug resistant microbes, herbal antifungal agents might prove beneficial. Though there is a remarkable reduction in the surface roughness of tissue conditioner after incorporation of antifungal oils. They can be clinically useful as the soft lining material if used for a short duration.

SCOPE OF THE STUDY

Study evaluated antifungal efficacy, surface roughness and tensile bond strength of *Ginger grass oil* incorporated in tissue conditioner.

Further research is suggested to evaluate exact mechanism of action of antifungal activity of *ginger grass oil* and other micro-organisms commonly seen in oral cavity.

Further investigations are required to assess the cytotoxicity and durability of the *ginger grass* oil.

Further research is required to find out the reason for increase in surface roughness and decrease in tensile bond strength after incorporation of antifungal herbal oils in tissue conditioner.

Further studies need to be carried out to assess different properties of the denture tissue conditioner such as hardness, color stability after the incorporation of herbal oils.

Since this is an in-vitro study, further in-vivo parameters should be considered with variable clinical conditions and outcome of the modification in the tissue conditioner since other researches have shown that change in duration and concentrations , change in the temperature, all affect the surface roughness, tensile bond strength.

CONCLUSION

Within the limitations of the present in-vitro study, the following conclusions can be drawn.

- The commercially available ginger grass oil can serve as one of the potential antifungal agent for treatment against denture stomatitis.
- Diameter of zone of inhibition of *ginger grass oil* with tissue conditioner was less compared to *fluconazole* with tissue conditioner but it was comparable as fluconazole is potent antifungal agent. Additionally, systemic administered antifungal agents has harmful effect on humans when they are misused. Therefore herbal essential oil can be better option to treat denture stomatitis.
- Surface roughness decreased statistically after incorporation of ginger grass oil and increased after 1 week of time interval.
- Tensile bond strength decreased statistically after incorporation of ginger grass oil and increased with 1 week of time intervals.

For clinical use of *ginger grass oil*, further studies are required to evaluate cell toxicity and durability.

SUMMARY

Study evaluated antifungal efficacy, surface roughness and tensile bond strength of *Ginger grass oil* incorporated in tissue conditioner.

A total of 150 samples were taken in the present study. Minimum inhibition zone diameter of 90 samples were used to evaluate the antifungal activity using well diffusion method. Then, experimental group of 45 samples was further subdivided into 3 groups of 30%,40%,50% concentration (n = 15) which was used to measure minimum inhibition zone diameter and evaluate the antifungal activity at two different time intervals i.e. after 1 day and 1 week which was compared with comparative group containing 45 samples of fluconazole with tissue conditioner i.e. 15 samples in each group of 1%,3%, 5% concentration.

The antifungal activity of mean minimum inhibition zone diameter of ginger grass oil and fluconazole in tissue conditioner at different concentrations were compared. The surface roughness and tensile bond strength of tissue conditioner with optimized ginger grass oil concentration were evaluated.

Mean minimum inhibition zone diameter was found at 30% concentration of ginger grass oil with tissue conditioner (experimental group) which was compared with the 5% of fluconazole in tissue conditioner (comparative group) as it was most effective concentration. The antifungal activity of 30% of ginger grass oil in tissue condition was less than 5% of fluconazole in tissue condition as 5% of fluconazole is potent antifungal additive used in various studies. Incorporation of ginger grass oil in denture soft relining material inhibited the candida albicance growth, hence it can revolutionize the soft relining material with added benefit of anti-fungal activity.

Surface roughness decreased statistically after incorporation of ginger grass oil and increased after 1 week of time interval. Tensile bond strength decreased statistically after incorporation of ginger grass oil and increased with 1 week of time intervals.The resultant data was tabulated and subjected to statistical analysis using SPSS software version 20. Antifungal activity and surface roughness, tensile bond strength properties “Dependent t test” was used to comparison between 1day in experimental and control group and 1week activity in experimental and control group and “Independent t test” was used to compare between 1 day , 1 week activity in experimental group and 1 day,1 week in control group respectively.

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ANNEXURE – I

ETHICAL CLEARANCE

Research and Ethics Committee
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CERTIFICATE

This is to Certify that the synopsis titled

COMPARATIVE EVALUATION OF SURFACE ROUGHNESS, TENSILE BOND STRENGTH AND ANTIFUNGAL ACTIVITY OF GINGER GRASS OIL INCORPORATED IN TISSUE CONDITIONER : IN-VITRO STUDY *Submitted by*

Dr. VISHAKHA DARE *P. G. Student / Staff, Guided by* DR. MAHANTESH BEMBALAGI *from Department of* PROSTHODONTICS & CROWN & BRIDGE *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date :

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

Research & Ethical Committee
KLEVK Institute of Dental Sciences
BELAGAVI.

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

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

ANNEXURE-II

Minimum inhibition zone of 30% concentration of ginger grass oil in tissue conditioner 15 samples at different time interval

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

Minimum inhibition zone of 40% concentration of ginger grass oil in tissue conditioner 15 samples at different time interval

SAMPLE NO.	1 DAY(mm)j	1WEEK (mm)
1.	6	5
2.	6	6
3.	7	6
4.	10	7
5.	7	9
6.	6	7
7.	7	6
8.	5	6
9.	6	5
10.	7	6
11.	6	7
12.	8	5
13.	7	8
14.	6	7
15.	6	6

Minimum inhibition zone of 50% concentration of ginger grass oil in tissue conditioner 15 samples at different time interval

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

Minimum inhibition zone of 1% concentration of fluconazole in tissue conditioner

15 samples at different time interval

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

Minimum inhibition zone of 3% concentration of fluconazole in tissue conditioner

15 samples at different time interval

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

Minimum inhibition zone of 5% concentration of fluconazole in tissue conditioner

15 samples at different time interval

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

Tissue conditioner with Ginger grass oil (experiment group) with surface roughness scores at 1 day and 1 week time points.

SAMPLE NO.	1 DAY (um)	1WEEK(um)
1.	2.04	3.55
2.	2.73	3.28
3.	2.88	2.97
4.	2.80	3.42
5.	2.70	3.42
6.	2.90	4.69
7.	3.24	3.98
8.	2.49	3.88
9.	2.77	3.32
10.	2.54	3.46
11.	3.49	4.03
12.	2.39	3.81
13.	2.38	3.76
14.	3.80	3.05
15.	2.03	3.61

Tissue conditioner without Ginger grass oil (control group) with tensile surface roughness scores at 1 day and 1 week time points

SAMPLE NO.	1 DAY (um)	1WEEK(um)
1.	3.33	4.58
2.	3.43	4.88
3.	3.77	4.88
4.	3.35	5.53
5.	3.70	3.41
6.	3.46	5.50
7.	3.33	4.98
8.	4.26	5.55
9.	3.75	3.90
10.	3.82	5.44
11.	3.55	3.45
12.	3.76	3.41
13.	3.80	4.83
14.	3.49	5.75
15.	3.52	5.02

Tissue conditioner with Ginger grass oil (experiment group) with tensile bond strength scores at 1 day and 1 week time points.

SAMPLE NO.	1 DAY (kg/cm^2)	1WEEK(kg/cm^2)
1.	24	24.50
2.	24	24.50
3.	23.50	24
4.	23	24
5.	23	24
6.	23	25
7.	22.50	25
8.	23	24.50
9.	22.50	21
10.	22.50	25
11.	22.50	24.50
12.	22	24
13.	23	24.50
14.	23	25
15.	23	24

Tissue conditioner without Ginger grass oil (control group) with tensile bond strength scores at 1 day and 1 week time points

SAMPLE NO.	1 DAY (kg/cm^2)	1WEEK(kg/cm^2)
1.	24	25
2.	24.50	25
3.	25	27
4.	24.50	26.50
5.	25	26
6.	24	26
7.	24	24
8.	24.50	27
9.	23.50	27.50
10.	23	27
11.	24	26.50
12.	24	26
13.	24.50	25.50
14.	25	26
15.	25	26