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# Antifungal and Mechanical Properties of Tissue Conditioner Containing Plant-Derived Component: An In Vitro Study

A mini-thesis submitted in partial fulfilment of the requirements for the degree of Master of Science (Clinical) in Restorative Dentistry, in the Department of Restorative Dentistry, Faculty of Dentistry, University of the Western Cape

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# KEYWORDS

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Juncus Effuses

Growth Inhibitory Effect

Mechanical Properties

Natural Antifungal Solutions

Visco-gel

# Abstract

## Antifungal and Mechanical Properties of Tissue Conditioner Containing Plant-Derived Component: An *In Vitro* Study

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## Introduction

This study investigated the use of alternate material, their associated risks when placed within the removable dental prostheses.

Denture stomatitis is a multifactorial condition affecting up to 70% of denture wearers, commonly associated long-term uses and with poor denture fit and hygiene. Candida-associated stomatitis is a frequent complication of this condition.

Therapeutic approaches include denture repair, preventive measures, and the integration of antifungal agents into tissue conditioners to address both periprosthetic tissue damage and candida infection.

## Aim and Objectives

The study aimed to assess the growth-inhibitory impact of a Juncus-containing tissue conditioner against Candida albicans. Specific objectives included evaluating the antifungal activity and mechanical properties (flow properties, penetration, and penetration proportion) of the Juncus-containing tissue conditioner. The research aimed to contribute valuable insights into the development of alternate but effective antimicrobial dental materials for improved oral health.

## Methodology

This in-vitro study conducted at Tygerberg Dental Hospital assessed a Juncus-containing tissue conditioner's growth inhibitory impact against Candida albicans and potential alterations in the

material's mechanical properties. Random sampling ensured unbiased inclusion, with ISO-certified standards used for assessments. Turbidity measurements gauged growth inhibitory effects, while fluidity and hardness were evaluated to quantify changes in mechanical properties. Data analysis employed the ANOVA, and ethical considerations included a blind approach it (typically refers to a methodology used in research to reduce bias and ensure objectivity). Replication enhanced the study's reliability. The data were securely managed and the study was approved by UWC BMREC.

## RESULTS

The Juncus-containing tissue conditioner exhibited significant growth inhibition against Candida albicans, with the highest impact at 5.0% and 10.0% concentrations. Mechanical properties conformed to ISO standards, suggesting antimicrobial effectiveness without compromising material integrity. This novel dental material presents a promising solution for preventing denture-related fungal infections.

## CONCLUSION

The integration of Juncus Effusus into tissue conditioners demonstrates promising potential for managing candida-associated denture stomatitis. This study reveals that a 2.5% concentration of Juncus has significant antifungal properties while maintaining the mechanical integrity of the tissue conditioner within ISO standards. These preliminary findings suggest the need for further research to refine the formulation, assess long-term efficacy, and validate clinical relevance. The study highlights the potential of combining traditional knowledge with modern dental science to develop innovative and patient-centered dental care solutions.

Date 10/12/2023

# DECLARATION

I, Ramiz Ahmed, declare that this mini-thesis, "Antifungal and Mechanical Properties of Tissue Conditioner Containing Plant-Derived Component: An *In Vitro* Study" which I am submitting electronically to the University of the Western Cape as part of the requirements for the degree MSc (Restorative Dentistry), is my own original work and has not been submitted for any academic award at this University or any other institution of higher learning.

SIGNATURE:  DATE: 10/12/2023

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In the name of Allah, the Most Gracious, the Most Merciful.

All praise is due to Allah, the Lord of all worlds, and peace and blessings be upon the Prophet Muhammad, his family, and his companions.

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وَمَا تَوْفِيقِي إِلَّا بِاللَّهِ ۚ عَلَيْهِ تَوَكَّلْتُ وَإِلَيْهِ أُنِيبُ(And my success is not but through Allah. Upon Him, I have relied, and to Him, I return.) - Quran 11:88

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# DEFINITION OF TERMS

The following definitions were all taken from "The Glossary of Prosthodontic Terms: Ninth Edition". (Driscoll *et al.*, 2017).

**Removable Dental Prosthesis:** A dental appliance, such as complet dentures or partial dentures, that can easily be taken out and reinserted by the patient.

**Denture Stomatitis:** An inflammatory condition affecting the mucosa of the mouth, particularly under dentures, often associated with a candida infection.

**Candida Albicans:** A type of yeast that can cause infections in humans, including oral infections like thrush, and is a common factor in denture stomatitis.

**Antifungal Medications:** Pharmaceuticals designed to treat and prevent fungal infections, including those caused by candida, by inhibiting the growth or killing the fungi.

**Tissue Conditioners:** a Flexible polymer used in dentistry as soft, temporary lining materials applied to the fitting surface of a denture. They are designed to provide a cushioning effect between the denture base and the oral tissues, helping to distribute pressure more evenly and allowing irritated or damaged oral tissues to heal.

**Juncus Effuses:** A plant material, traditionally used in making tatami matting in Japan, explored for its antimicrobial properties in dental applications, specifically in tissue conditioners.

**Metallic Nanoparticles:** Extremely small particles of metals, such as copper and silver, explored for their antifungal, physical, and mechanical properties in dental materials.

**Growth Inhibitory Effect:** The ability of a substance or treatment to prevent the growth or replication of microorganisms, in this context, specifically *Candida albicans*.

**Mechanical Properties:** Characteristics of materials related to their reaction to applied forces or loads, such as strength, elasticity, and hardness.

**Microbial Factors:** Elements related to microorganisms, such as bacteria or fungi, that can influence health conditions, including those affecting oral health.

**Host Responses:** The reactions and defenses of the host organism (in this case, the human body) in response to infections or other challenges.

**Environmental Influences:** External factors or conditions that can impact health, including those affecting the oral environment and dental health.

# CHAPTER 1

## INTRODUCTION

Removable dental prostheses serve as a solution for individuals requiring tooth replacement, offering a numerous of advantages such as enhanced mastication function, improved aesthetics, clear phonetics, and overall oral health (Alabbas A and Jaret, 2023). However, this contribution to an elevated quality of life is not without challenges, particularly concerning long-term denture wear and inadequate denture hygiene, and especially among compromised and elderly patients (Chopde *et al*., 2012).

A prevalent issue that surfaces within the realm of removable dental prostheses is denture stomatitis, a degenerative condition impacting the mucosa of denture-bearing surfaces. The etiology of this condition remains a subject of debate, with various factors responsible for the condition. Ill-fitting dentures, a primary cause of denture stomatitis, can result in widespread inflammation or erythema beneath the denture on the palatal mucosa. This condition is notably more prevalent among complete denture users, affecting a substantial 72% of this population (Figueiral *et al*., 2007).

Within the spectrum of denture stomatitis, a distinctive subset is *Candida*-associated stomatitis, where fungal infections take precedence as the primary causative factor (Williams *et al*., 2012). Unraveling the intricate relationship between candida and denture-induced stomatitis becomes imperative for a comprehensive understanding of the condition. Research indicated that various species of candida such as C. glabrata, C. tropicalis and others., but predominantly Candida albicans, play a pivotal role in the pathogenesis of this oral affliction (Talapko J et al., 2021).

While the symptoms and manifestations of denture stomatitis are well-documented, the underlying etiological factors continue to be a matter of controversy in dental research. Conflicting views persist on whether improper oral and dental care, nocturnal and continuous denture use, xerostomia, or shifts in salivary pH serve as primary contributors (Gendreau *et al*., 2011).

A nuanced exploration of these factors is essential for devising effective preventive strategies and treatment modalities.

Effectively managing denture stomatitis necessitates a comprehensive and multifaceted approach. This includes not only addressing the symptoms but also targeting the root causes of the condition. Denture repair or replacement emerges as a pivotal aspect of treatment, alongside preventive measures undertaken by the patient to mitigate the risk of recurrence. The administration of topical and systemic antifungals represents a critical component in the therapeutic arsenal (Shin Yu and lu, 2021).

Despite the efficacy of antifungal medications, challenges persist in their administration, particularly when applied topically to the oral mucosa. Factors such as excessive saliva circulation and a lack of patient cooperation can compromise the effectiveness of these medications (Truhlar MR., et al 1994). This necessitates innovative approaches to enhance drug delivery and patient compliance.

One promising avenue in overcoming the challenges of topical medication administration involves the integration of antifungal medications into tissue conditioners, Juncus effusus has antibacterial capabilities against a variety of microbes and is often found in wetlands in Japan, China, Taiwan, and North America. It has a thousand of year history and is widely used in the production of tatami mats. In addition to its conventional uses, Juncus has been used as an herb for medicine and as a food ingredient (Morita *et al*., 2006).

Recent research has revealed its functional properties and antibacterial effectiveness. Morita et al. demonstrated its potential against pathogenic bacteria such as Salmonella, Staphylococcus, enterohemorrhagic Escherichia coli (EHEC) O-157 strain, Bacillus, and Micrococcus (Morita et al., 2005; Mitsuhashi H., et al 1988). The stem's inner core is used in medicine because it has anti-inflammatory and fever-reducing effects.

Juncus has recently acquired popularity as a food ingredient, prompting ongoing study into its antibacterial and antifungal properties. Yoshihito Naito et al (2016) studied the impact of administering Juncus-mixed tissue conditioner as a powder at doses of 2.5%, 5.0%, and 10.0% by mass. The findings revealed concentration-dependent growth inhibition of Candida albicans after 2- and 4-day cultures, accompanied by a significant reduction in C. albicans number, marked inhibition of biofilm formation, and, most importantly, no alteration in mechanical properties, which remained within ISO-specified ranges (Yoshihito Naito et al., 2016).

This approach not only addresses candida infections but also contributes to the restoration of damaged periprosthetic tissues the synergy between tissue conditioning and antifungal therapy presents a novel strategy for enhancing treatment outcomes in denture stomatitis (Sharma *et al*., 2014).

Despite significant strides in understanding denture stomatitis, several research gaps persist such as Long-Term Efficacy of Treatments, Mechanisms of Pathogenesis, Patient-Specific Factors, Microbial Diversity, Preventive Measures, Clinical Guidelines and Patient Education. Further exploration is needed to unravel the complexities of the condition, including the interplay between microbial factors, host responses, and environmental influences. Additionally, investigating novel materials and technologies for denture fabrication and maintenance could pave the way for more effective prevention and management strategies (Pereira et al., 2008; Gendreau L and Loewy ZG, 2011).

In conclusion, the realm of removable dental prostheses and denture stomatitis encompasses a dynamic interplay of factors influencing oral health and overall well-being. While dentures offer valuable functional and aesthetic benefits, the challenges posed by conditions such as denture stomatitis underscore the importance of ongoing research and innovation in dental care. By delving into the nuances of etiology, risk factors, and treatment modalities, dental professionals can refine strategies to enhance patient outcomes and contribute to a healthier, more resilient oral environment (Emami E et al., 2012).

The focus of the current study was to explore the potential of a tissue conditioner mixed up with Juncus in inhibiting the growth of Candida albicans. Additionally, our investigation aimed to observe any potential alterations in the mechanical properties of the tissue conditioner due to the inclusion of Juncus.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1. Introduction

Tissue conditioners (TCs) are temporary soft lining materials made of amorphous polymers generated *in situ* by combining polymer powder with liquid plasticizer. According to Chander *et al.* (2007), these materials are generally used to improve the fit and functionality of poorly fitting dentures before they are replaced. Furthermore, as interim remedies, TCs are utilized to treat mistreated mucosal tissues underlying poorly fitting acrylic dentures. They are also advised for the creation of functional imprints. Visco-gel (De Trey), Coe-Comfort (GC America), FITT (Kerr), GC Soft-Liner (GC Europe NV), SR-Ivoseal (Ivoclar), Tissue Conditioner (Shofu), and Hydro-Cast (Sultan Chemists) are among the commercial TC products available (Chander *et al*., 2007).

According to Rodrigues (2013), problems related to TCs in clinical use include loss of resilience, water absorption, sensitivity to bacterial and yeast proliferation, color changes, and poor adhesion to the denture-based resin (Rodrigues *et al*., 2013). To ensure optimal performance, these demand regular replacements at short intervals.

Tissue Conditioners (TCs) play a pivotal role in prosthodontic practice, offering a versatile solution to address various clinical challenges. This comprehensive review explores the diverse applications and distinct physical properties of TCs, delving into the nuances of their composition, clinical use, challenges encountered, and potential solutions. The review is organized into key sections to provide a thorough understanding of TCs in the context of denture management and patient care (Tari *et al*., 2014).

## 2.2. Composition and Clinical Applications

Tissue Conditioners (TCs) polymer base, according to Murata *et al.* (2005), are composed of polyethyl methacrylate (PEMA) or related polymer powder and ester-based liquid plasticizer,

providing flexibility in hard acrylic polymers. Polymer chain entanglement generates a gel with viscoelastic characteristics suitable for clinical usage during mixing. In an ethanol solution, PEMA particles absorb large plasticizer molecules, ensuring correct gelation durations. The ester-based plasticizer gradually infiltrates the PEMA particles as the large molecules permeate them. Concurrently, the polymer undergoes swelling from the alcohol, hastening the penetration of the plasticizer and resulting in a gelation time deemed clinically acceptable. Notably, Polymethyl methacrylate (PMMA), a frequently employed acrylic polymer, is unsuitable for this purpose due to its insolubility in ethanol (Murata et al., 2005).

Dynamic mechanical tests are used to analyze TC parameters such as polymer molecular weight, plasticizer type, ethanol percentage, and powder-liquid ratio. Controlling gelation entails modifying these elements as well as taking into account powder particle size, temperature, and softener type and amount (Barbara *et al*., 2017).

Gelation duration is lowered when polymer molecular weight, powder-to-liquid ratio, and ethanol content increase, and polymer molecule size decreases. Softeners affect gelation time, with higher comminution resulting in faster gelation. Due to the quick evaporation of ethanol in the mouth cavity, TC replacement is required every 3-5 days, influencing weight loss and material shrinkage. A high ethanol level improves material plasticity and flexibility, with the softener type influencing the plasticity (Barbara *et al*., 2017)

The proper proportioning of powder and liquid improves post-gelation TC flexibility. Material selection should be determined on intended usage and required flexibility, taking into account variations in TC characteristics (Parker & Braden, 2001).

## 2.3. Physical Properties and Challenges

The oral environment affects the physical properties of all tissue conditioners (TCs), which need frequent replenishment. According to Baslas *et al*. (2014), ethanol and plasticizers leak into saliva, causing alterations in water sorption and solubility over a week. Silicone-based TCs have a difficulty attaching to denture bases, reducing longevity and generating possible plaque pockets. A rough denture-base surface promotes mechanical retention and reduces microleakage

(Rodrigues et al., 2013; Więckiewicz *et al*., 2014). Airborne particle abrasion and laser treatment improve bonding even further (Więckiewicz *et al*., 2014).

Due to water absorption and solubility, all types of TCs can cause denture deformation during masticatory functions, resulting in dimensional instability and weight variations. The linear dimensional stability of commercial items varies, with shrinkage and weight loss reported, underlining the importance of timely functional impressions. The TC's hardness has no negative effects on plaster models (Glantz et al., 1988).

Due to the loss of elastic characteristics over time, TCs have a limited effective lifespan in the oral cavity. Surface-coated TCs, such as Monopoly and Permaseal "Polymethyl Methacrylate" (PMMA) (Coe-Comfort), have longer resilience and longevity because they reduce leaching and limit water absorption (Shylesh *et al*., 2013).

Coating with Monopoly can extend "Polymethyl Methacrylate" (PMMA) TC life by up to a year, reduce bacterial and fungus growth, and keep the surface smooth. Over a 30-day timeframe, Monopoly-coated TCs lose alcohol without absorbing water (Gardner and Parr, 1988).

According to Hashem (2015), when employing TCs on acrylic dental base surfaces, the prostheses bases must have a sufficient thickness to prevent plasticizer diffusion, which might modify characteristics and lead to deformation or breakage after continuous usage. Coating "Polymethyl Methacrylate" (PMMA) TCs with Monopoly improves robustness and surface quality while also acting as a barrier against environmental impacts. Gardner and Parr (1988) observed this finding in their study. These developments help to extend the usefulness and stability of TCs in a variety of clinical settings.

## 2.4. TCs in Denture Stomatitis Management

According to Skupien *et al*. (2013), denture stomatitis, which is characterized by inflammation in the oral mucosa of denture-bearing areas, may be caused by the loss of surface integrity and roughness of TCs. This condition affects 40-65% of acrylic denture wearers and may furthermore be caused by denture mechanical irritation. Because TCs distribute masticatory force evenly and respond to changes during substrate healing, the relinewith soft liner is useful for patients who are

wearing dentures during prosthetic substrate recovery (Przybyłowska D et al., 2015; Goiato MC et al., 2015).

Certain TCs, however, such as Coe-Comfort, FITT, Soft-Liner, and Visco-gel, display cytotoxic activity *in vitro* that exceeds the cytotoxicity of acrylic resin. Softener release from TC surfaces, particularly phthalates, raises concern regarding tissue effect, necessitating the investigation of alternative chemicals such as dibutyl citrate or dibutyl sebacate. Some plasticizers have been shown to limit *Candida albicans* growth, by impacting fungal growth on TC surfaces (Hashimoto *et al*., 2003).

The loss of ethanol and plasticizers from TCs results in a hard and porous surface, which promotes denture base sedimentation and creates a reservoir for bacteria and fungi. To address this, timely replacement of TC layers during treatment is critical. Antibacterial and antifungal medications have been added to TCs, with formulations like Zeomic (Sinanen Zeomic Co.) Introduced by Sinanen Zeomic in 1984, Zeomic stands as the world's pioneer silver-based inorganic antimicrobial agent, renowned for its exceptional safety, sustainability, and heat resistance compared to organic counterparts. Widely esteemed not only in Japan but globally, Zeomic has evolved beyond antimicrobial properties to encompass odor elimination, antiviral effects, water processing applications. crystalline aluminum silicate containing silver ions acting as an antiseptic showing potential in reducing the growth of *Candida albicans, Staphylococcus aureus*, *and Pseudomonas aeruginosa in vitro*. When itraconazole is added to materials such as Coe Soft or FITT, it displays therapeutic action within the first three days, necessitating replacement for long-term effects (Chow & Matear, 1999).

Rough denture base surfaces promote bacterial and fungal growth, increasing the likelihood of infection and candida-associated denture stomatitis (Brosky ME et al., 2003). *Candida albicans* adhesion to TC surfaces has been linked to cell proliferation and matrix synthesis, which can irritate the oral mucosa (Skupien JA et al., 2013; Jin C et al., 2003).

TCs containing antifungal drugs such as miconazole, nystatin, chlorhexidine, clotrimazole, and fluconazole have shown inhibitory effects on *Candida albicans* development, while material hardness may increase. Furthermore, antimicrobial polymers and silver zeolite can change TC surfaces to prevent bacterial and fungal adhesion (Davenport et al., 1978; Douglas and Walker, 1972).

These findings highlight the importance of integrating material qualities, drug integration, and surface alterations to improve treatment outcomes and patient well-being in the management of denture stomatitis (Nikawa *et al*., 1997).

## 2.5. Clinical applications of TCs

Owing to their soft and robust character when applied to the acrylic denture surface, tissue conditioners (TCs) have a wide range of clinical applications. These applications include healing inflamed denture-bearing oral mucosa, improving functional impressions, acting as provisional liners to improve acrylic denture fit, preventing mechanical irritation, and enabling border extension trial evaluations. (Braden M et al., 1995)

Furthermore, TCs are useful in altering dentures after implant surgery and assisting in the rehabilitation of cancer patients who require obturation. However, the physical features of TCs, such as viscoelasticity and dimensional stability, fluctuate, depending on the materials utilized, underscoring the importance of tailoring methods to diverse applications (Braden M et al., 1995; Qudah S et al., 1990).

During mastication, ideal resilient denture liners should have strong elasticity before switching to viscous behaviour for uniform force distribution and pain alleviation. When used for interim relining, these materials must be dimensionally stable to prevent changes in the vertical dimension of the occlusion and be compatible with dental stones (Hashem, 2015).

When applying relining materials to the denture fitting surface, it is critical to maintain denture cleanliness. Cleaning prostheses thoroughly before relining is critical, as is educating patients about maintaining dental hygiene during therapy. Certain liquid cleaners and antiseptic medications have been found to accelerate the leaching of components from the conditioner surface. Since materials for biological tissue regeneration are susceptible to mechanical damage, gentle washing with a cotton cloth or gauze, followed by disinfection in a 0.2% chlorhexidine solution, is recommended. It is critical to emphasize that biological components for tissue regeneration are intended for short-term denture relining. Prolonged use may cause mechanical discomfort, denture plaque accumulation, and an increase in denture stomatitis symptoms (Ntounis *et al*., 2015).

## 2.6. Incorporating Antifungal Agents: A Promising Frontier

Qbal & Zafar (2016) investigated the integration of antifungal agents into tissue conditioners for denture-induced stomatitis treatment. Their findings suggest that incorporating various antifungal medicaments into commercially available tissue conditioners holds promise for managing denture-induced stomatitis effectively. This presents an innovative approach to combating the challenges posed by ill-fitting dentures, contributing to enhanced patient comfort and oral health.

## 2.7. Metallic Approaches and Nanoparticles

Beyond antifungal agents, researchers have explored the incorporation of metals like copper and silver into dental materials (Nam KY, 2011; Garcia-Marin et al., 2022). However, caution is warranted due to potential negative consequences, including metal allergies. Homsiang *et al*. (2021) delved into the antifungal, physical, and mechanical properties of tissue conditioners containing varying quantities of zinc oxide nanoparticles (ZnOnps). Their study demonstrated that 15% ZnOnps exerted antifungal effects for up to 14 days without compromising penetration depth or tensile bond strength. This showcases the potential of nanoparticle integration in enhancing the longevity and efficacy of antifungal tissue conditioners.

## 2.8. Nonmetal Antimicrobial Approaches

Seeking nonmetal antimicrobial alternatives, researchers have explored incorporating human lactoferrin, photocatalysts, and antimicrobial agents into tissue conditioners (Yamamoto D et al., 2009). Tari *et al*. (2014) highlighted the development of nonmetal antimicrobial tissue conditioners using these innovative approaches. These alternatives aim to address concerns associated with metal incorporation, providing diverse options for clinicians and patients alike.

## 2.9. Organic Herbal Products and Alternative Antifungal Medications

Rawat *et al*. (2017) investigated the impact of adding antifungal drugs on the physical and biological characteristics of tissue conditioners. There *in vitro* study concluded that organic herbal products within tissue conditioners could serve as effective alternatives to systemic or topical

antifungal medications. This sheds light on the potential of integrating natural components into dental materials, aligning with the growing interest in holistic and sustainable healthcare practices.

## 2.10. Exploring Juncus Effuses: An Ancient Solution

In a departure from traditional approaches, research has delved into the use of Juncus effuses, Juncus effusus, commonly known as Soft Rush or Common Rush, is a perennial grass-like plant that has been traditionally used for medicinal purposes in systems like Ayurveda and traditional Chinese medicine, where various parts of the plant, including the rhizomes and aerial parts, have been employed to treat conditions such as gastrointestinal disorders, skin ailments, and inflammation.

Its phytochemical composition includes alkaloids, flavonoids, and phenolic compounds, which contribute to its potential therapeutic effects, particularly in wound healing.

Ecologically, Juncus effusus plays a vital role in wetland habitats, stabilizing soil, preventing erosion, and providing habitat for wildlife. Additionally, it has phytoremediation capabilities, helping to remove pollutants from soil and water.

Beyond its medicinal and ecological significance, Juncus effusus is also cultivated for ornamental purposes due to its attractive foliage and architectural form. In some cultures, it's tough, fibrous stems have historically been used for crafting baskets, mats, and other items.

Overall, Juncus effusus is a versatile plant with diverse applications across traditional medicine, ecology, ornamental landscaping, and craftsmanship, though further research is needed to fully explore its potential benefits and applications.

Morita, *et al*. (2006) noted its antibacterial action against various harmful bacteria, and Naito, *et al*. (2018) further explored its antifungal activity and mechanical characteristics in a tissue conditioner. Their findings revealed that Juncus-mixed tissue conditioners significantly inhibited *Candida albicans* development and biofilm formation, showcasing its potential as a natural and effective antifungal solution (Morita *et al*., 2006; Naito *et al*., 2018).

The evolution of tissue conditioners in addressing denture-induced stomatitis reflects a dynamic landscape of innovation. From the integration of traditional antifungal agents to the exploration of metallic nanoparticles and organic herbal alternatives, researchers are continually striving to

enhance the efficacy and safety of these crucial dental materials. The incorporation of natural substances like Juncus effuses represents a fascinating intersection of ancient wisdom and modern dental science, opening new avenues for the development of antifungal tissue conditioners. As research progresses, these advancements hold the potential to revolutionize denture care, providing patients with safer, more effective, and holistic solutions.

## 2.11. Advancements in Nanotechnology for Antifungal Dental Materials

Recent developments in nanotechnology have propelled the exploration of advanced materials with enhanced antifungal properties. Nanoparticles, due to their unique physicochemical characteristics, offer a promising avenue for improving the efficacy of dental materials. A study by Mousavi, *et al*. (2018) investigated the incorporation of chitosan nanoparticles into tissue conditioners, revealing prolonged antifungal activity and improved mechanical properties. This nanotechnological approach opens new possibilities for tailoring dental materials to address the specific challenges posed by microbial colonization and tissue inflammation.

## 2.12. Smart Materials for Responsive Antifungal Action

The emergence of smart materials, capable of responding dynamically to environmental conditions, has gained attention in dental research. Responsive polymers, such as hydrogels with pH-sensitive drug release, have shown potential in delivering antifungal agents precisely where needed. Tonprasong, et al. (2021) explored the integration of such smart materials into tissue conditioners, presenting an innovative strategy for on-demand antifungal action. This approach aims to optimize treatment outcomes while minimizing the risks associated with prolonged drug exposure.

## 2.13. Multidisciplinary Approaches for Comprehensive Denture Care

A holistic perspective on denture care involves considering not only the material properties but also patient-specific factors. Collaborative efforts between dentists, microbiologists, and material

scientists have led to a more comprehensive understanding of denture-related issues (Bangera MK et al., 2023). Interdisciplinary studies, such as the work by the Collaborative Denture Research Group (CDRG, 2019), emphasize the importance of personalized approaches, taking into account patient health, habits, and microbial profiles. This collaborative trend signifies a paradigm shift towards tailored solutions in managing denture-induced stomatitis (Finbarr *et al*., 2019).

## 2.14. Patient-Centered Outcomes and Quality of Life

Beyond the technical aspects, recent research has increasingly focused on patient-centered outcomes and the impact of dental materials on overall quality of life. Studies assessing patient satisfaction, comfort, and adherence to denture care regimens provide valuable insights into the real-world effectiveness of different interventions. The work of the Patient-Centered Dentistry Consortium (PCDC, 2020) highlights the need for a patient-centric approach to evaluate the success of antifungal tissue conditioners. Understanding the psychosocial aspects can guide the development of materials that not only combat infections but also enhance the overall well-being of denture wearers (Alrawiai *et al*., 2020).

In summary, the evolving landscape of dental materials extends beyond traditional antifungal agents, embracing nanotechnology, smart materials, interdisciplinary collaboration, and a heightened focus on patient outcomes. These diverse approaches collectively contribute to a more nuanced understanding of denture-induced stomatitis and pave the way for innovative solutions that prioritize both efficacy and patient satisfaction. As research continues to progress, integrating these multifaceted perspectives promises a holistic and transformative impact on denture care practices.

## 2.15. Limitations of tissue conditioners

Tissue conditioners, despite their utility and type, they all have inherent limitations that should be carefully considered in clinical applications. These limitations include:

**Short-Term Modality:** Tissue conditioners serve as a short-term treatment, and failure to replace them promptly may lead to discomfort by insulting the denture-bearing mucosa, creating a conducive environment for microbial growth and infections (Hashimoto *et al*., 2003).

**Water Sorption and Solubility:** High rates of water sorption and solubility in tissue conditioners can result in dimensional instability, and color changes, and affect the material's resiliency and bonding to the denture. These factors may also contribute to unpleasant odors (Prasad *et al*., 2014).

**Elasticity and Shape Changes:** The change in elasticity of tissue conditioners in the mouth can lead to shape changes after removal. Immediate pouring with stone after removal is necessary to prevent distortion by the stone's weight or the material's weight during the plastic stage (Zarb *et al*., 2013).

**Limitations in Tissue Displacement:** The initial plastic behavior of tissue conditioners may not allow for tissue displacement at the posterior border of the maxillary denture. This may necessitate creating a post-dam with a rigid material such as impression compounds or light-cured resin (Zarb *et al*., 2013).

**Allergic Reactions:** Some patients may be allergic to certain components of tissue conditioners, leading to potential deterioration and rapid material breakdown. This can affect the strength of the resin, and patients should be warned about the possibility of denture breakage (Zarb *et al*., 2013).

**Usage in Increased Thickness:** The usage of tissue conditioners in increased thickness, such as in surgical obturators, may negatively impact their properties (Monzavi *et al*., 2013).

**Variability in Commercially Available Materials:** Different commercially available tissue conditioner materials vary in their properties and compositions. Understanding the specific physical properties of each tissue conditioner is crucial for clinicians as this would allow them to choose the optimal material for each procedure.

Considering these limitations, the choice of tissue conditioner becomes critical in prosthodontic care. One notable option is Visco-Gel, a self-curing, resilient methacrylate formulation manufactured by Dentsply. It has been clinically used for over four decades and presents distinct characteristics.

## 2.16. Visco-Gel (Dentsply)

Composition: Visco-Gel is composed of Polymethylmethacrylate, ethyl alcohol, and citrate ester plasticizer.

Clinical Applications: It is intended for temporary relining of ill-fitted dentures, registering functional impressions for rebasing or remaking complete dentures, and serving as a soft liner for patients with deteriorated tissue health and elderly patients (Dentsply, 2008).

Contraindications: Usage is contraindicated in patients with a history of severe allergic reactions to its components, especially the methacrylate resins (Dentsply, 2010).

Mechanical Properties: Studies have shown that Visco-Gel exhibits a softer and more deformable nature than some other denture liners, making it suitable for providing a cushioning effect on the mucosa (Yang et al., 2015). However, it is important to note that the softness of the liner alone does not guarantee more appropriate cushioning.

Stability: Visco-Gel has demonstrated stable viscous and elastic properties, making it suitable for conditioning abused mucosa. When used with modeling plastic, it displays a considerable increase in viscosity over time, allowing for its use as a temporary relining material (Monzavi *et al*., 2013).

In light of the limitations of traditional Polymer-base and Silicone-based tissue conditioners, Visco-Gel emerges as a potential solution of some of these limitations such as Short-Term Modality, Water Sorption and Solubility, Allergic Reactions due to its stability, mechanical properties, and clinical versatility, offering a balance between resilience and adaptability for enhanced patient comfort and effective prosthodontic care.

# CHAPTER 3

# RESEARCH DESIGN AND METHODOLOGY

## 3.1 rationale

The rationale for this study lies in addressing the significant issue of denture stomatitis, which affects a large portion of denture wearers, by exploring the use of Juncus effusus, a natural material, in tissue conditioners to inhibit Candida albicans growth while maintaining mechanical integrity. This research aims to develop an effective antimicrobial dental material that leverages the antifungal properties and biocompatibility of Juncus effusus, offering a potentially superior alternative to current synthetic options. By integrating traditional botanical knowledge with

modern dental science, the study seeks to provide an innovative and sustainable solution to improve oral health outcomes for denture wearers.

## 3.2 Aim

This study aimed to evaluate clinical properties of and the impact of a Juncus-containing tissue conditioner against Candida albicans.

## 3.3 Objectives

1- To evaluate the growth inhibitory effect of antifungal activity of a Juncus-containing tissue conditioner against Candida albicans.

2- To assess the mechanical properties, namely; flow properties, penetration, and penetration proportion of a Juncus-containing tissue conditioner against Candida albicans.

## 3.4 Null Hypotheses:

For Objective 1:

"There is no significant difference in the growth inhibitory effect between the Juncus-containing tissue conditioner and a control group against Candida albicans."

For Objective 2:

"There is no significant difference in the mechanical properties, including flow properties, penetration, and penetration proportion, between the Juncus-containing tissue conditioner and a control group."

## 3.5 Study design

This quantitative in-vitro study, conducted at the Oral and Dental Research Laboratory at Tygerberg Dental Hospital within the Faculty of Dentistry, University of the Western Cape, was designed to compare the growth inhibitory impact and mechanical properties of a Juncus-containing tissue conditioner against Candida albicans and a non-modified tissue conditioner,

respectively. The research employed an experimental design to systematically investigate these aspects.

## 3.6 Sampling Technique: Random Sampling

### Rationale:

Block random sampling was chosen to ensure that each Candida albicans culture had an equal chance of being included in either the experimental group (Juncus-containing tissue conditioner) or the control group (non-modified tissue conditioner).

experimental group 1 contains 2.5% Juncus powder mixed with the tissue conditioner powder.

experimental group 2 contains 5% Juncus powder mixed with the tissue conditioner powder.

experimental group 1 contains 10% Juncus powder mixed with the tissue conditioner powder.

### Procedure:

Reference strains were procured from recognized culture collections, such as the American Type Culture Collection (ATCC). Each sample was streaked onto Sabouraud Dextrose Agar (SDA) plates using aseptic techniques and incubated at 37°C for 24-48 hours to allow the growth of Candida colonies in university of western cape lab. Distinct colonies characteristic of Candida albicans (creamy, smooth, white) were sub-cultured to ensure purity. Pure colonies were then transferred to fresh SDA plates and incubated under the same conditions to confirm purity and viability. Morphological identification was performed by examining colony characteristics and microscopic features.

### Random Assignment:

After selection, the Candida albicans cultures were randomly assigned to either the experimental or control group. To ensure comparability, cultures were matched based on colony morphology, growth rate, microscopic features, biochemical profile, and genetic identity.

### Considerations:

Random sampling enhanced the generalizability of findings to the larger population of Candida albicans cultures. It also minimized the risk of systematic errors in the assignment of cultures to the experimental and control groups.

### Sample Size Determination:

Following statistical considerations, six culture samples were prepared and six controlled tissue conditioner samples, mixed according to the manufacturer's instructions. Additionally, six experimental group tissue conditioner samples were created, incorporating 2.5%, 5%, and 10% Juncus Effuses powder. This experimental process was repeated three times at intervals of every four days.

### Replication:

The random sampling procedure was replicated three time to include an adequate number of Candida albicans cultures, ensuring the robustness and reliability of the study's findings.

Adjustments to the sampling technique may be made based on specific constraints, availability of resources, and ethical considerations.

## 3.7 Sample size and Sample preparation

### 3.6.1.Measurements

Tissue conditioner Visco-gel manufacturing by DENTSPLY was provided as a powder and a liquid. Tissue conditioner powder was combined with 100% PUR Natural High-Quality Juncus Effusus Extract Powder, by Shaanxi Joryherb Bio-Technology Co., Ltd. From Shaanxi, China. Juncus Effusus mixed with Tissue conditioner powder for 60 seconds . The mass percentages were adjusted to 2.5, 5.0, and 10.0 percent and labelled without effecting the manufacturer ratio

which is 3g of the powder to 2ml of the liquid for the one sample. To adjust the mass for 2.5, 5 and 10 % the equation used as follows:

$$x = 100 - y \times 3 \div 100$$

Where $\boldsymbol{x}$ is the amount by grams required of the tissue conditioner powder for each sample while $\boldsymbol{y}$ is Juncus effuses powder percent needed. Then Juncus effuses powder is added the mass of $\boldsymbol{x}$ and adjust the mass to be 3g according to the manufacturer.
and Juncus free samples (n = 4) served as controls group.

A tissue conditioning fluid was then added and mixed for 20 seconds with the Juncus / conditioning agent combination before being poured the powder and liquid of the tissue conditioners were mixed according to the corresponding manufacturer's recommendation using a glass bowl and plastic spatula, and each mixture was transferred into 12-well plates were labeled for each trial. Then, after 3 hours, the mold's top and bottom masts were sealed (Figures 1 and 2).

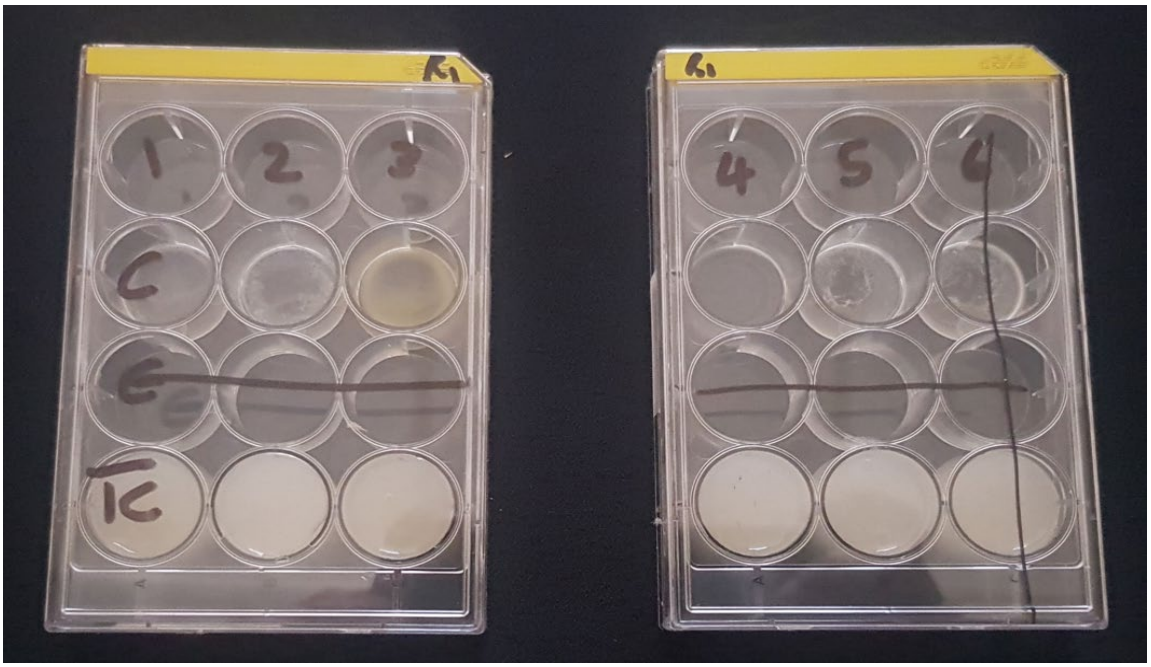



*Figure 1: 12-well plates were labeled for each trial, differentiating between the experimental groups and untreated samples.*

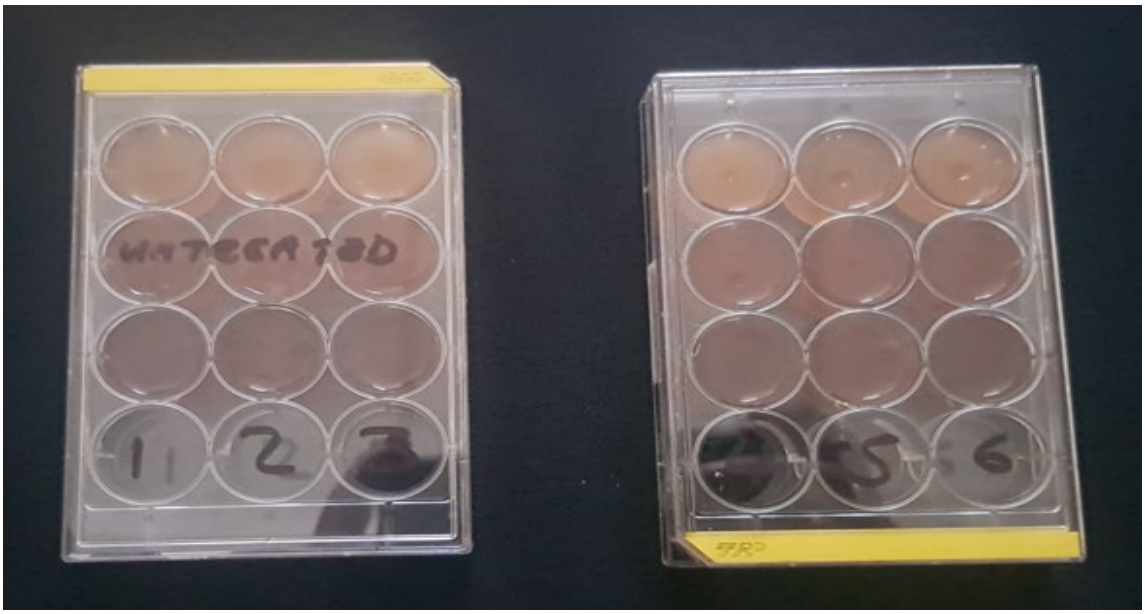



*Figure 2: 12-well plates were labeled for each trial, with Juncus powder that was mixed with Visco-gel tissue conditioner at concentrations of 2.5%, 5.0%, and 10.0% by mass.*

## 3.6.2.Evaluation of Growth Inhibitory Effect

### Procedure: Yeast Culture

1. ***Candida albicans Procurement:***

*Candida albicans* ATCC 10231 (American Type Culture Collection) donated by University of the Western Cape.

2. **Resuscitation and Purification:**

*Candida albicans* was revived from freeze-dried culture and purified in BHI and SDA with chloramphenicol.

3. **Preparation of Pure Culture:**

Before each experiment, a 24-hour pure culture of *Candida albicans* was cultivated in SDA at 37°C.

4. **Adjustment to 0.5 McFarland Standard:**

On the day of the experiment, the 24-hour cultures were standardized to 0.5 McFarland standard by isolating 2 or 3 samples, mixing them in Phosphate Buffered Saline (PBS), and subsequently adjusted to 0.5 McFarland using a densitometer.

### 5. Random Selection of Isolates:

Isolates were randomly chosen for each of the six samples of each group.

### 6. Preparation of Culture Samples:

Two hundred milliliters from each of the six sample adjusted cultures were transferred to labeled Eppendorf tubes for each trial.

### 7. Microbe Treatment:

- Two sets of 12-well plates were labeled for each trial, differentiating between the experimental groups and untreated samples (Fig. 1 and Fig. 2)
- One hundred milliliters of 0.5 McFarland-adjusted Candida (Ca) were pipetted into each well of the experimental set for the designated trial.
- Steps for microbe treatment were repeated across the plates for each culture set.
- One Hundred milliliters of MHB was added to each well, and the treatment proceeded after allowing the culture to acclimatize for approximately 30 seconds.
- Plates were incubated in the shaking incubator at room temperature (37°C) for precisely 24 hours.

### 8. Observation of Effects:

- One hundred microliters from each experiment were transferred to respective wells in 96-well plates, and performed in triplicate to mitigate pipetting errors.
- The 96-well plates were read using spectrophotometry for turbidity.
- Results were recorded in Excel for further analysis.

The initial trial was systematically repeated three times on separate occasions, ensuring the use of fresh isolates for each iteration to uphold rigor and reliability.

### 3.6.3.Evaluation of Mechanical properties

To evaluate the mechanical characteristics of the tissue conditioner containing Juncus, the fluidity and hardness of samples were assessed utilizing techniques accredited in accordance with ISO 10139-1. In total, five specimens were created as previously described for the examination of fluidity, penetration, and penetration ratio.

| ITEM | IOS standard | DATA |
|---|---|---|
| **Flow** | 25-75 mm | 50-70 mm |
| **Penetration** | 1.8 mm for ≤ 120 minutes,<br>≥ 0.18 mm for 7 days. | 1-1.5 mm≤ 120 minutes,<br>2-1.5 mm for 7 days. |
| **Penetration ratio** | ≤ 5.0 | ≈ 1 |

*Table 1: show the data of flow, Penetration and Penetration ratio compares to ISO 10139-1 standard*

#### 3.6.3.1. Evaluation of the penetration and penetration proportion

- A needle penetration examination was conducted 120 minutes after the materials were mixed. The depth of penetration was gauged using a dial 1.5 seconds after the penetration. Three assessments were carried out at diverse points on the specimen, and the average of these three values constituted the penetration measurement (A) (Fig.3). An identical procedure was repeated after seven days, and the resulting average was designated as penetration (B) (Fig.4).

The penetration ratio, denoted by σ, was computed using the formula: $\sigma = A/B$.

In this equation, σ represents the penetration ratio, A signifies the penetration after 120 minutes, and B represents the penetration after 7 days.

*Figure 3: Three assessments were carried out at diverse points on the specimen after 120 minutes(A)*

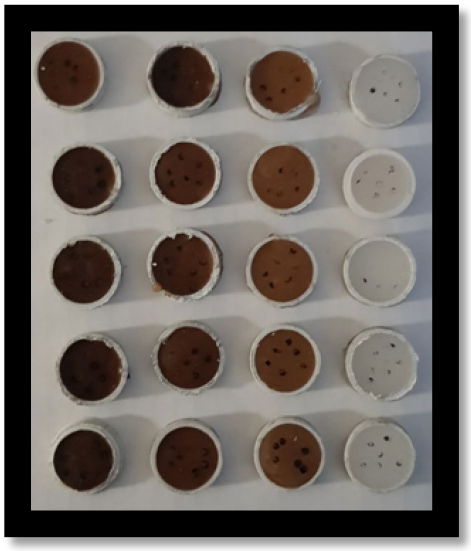


*Figure 4: Three assessments were carried out at diverse points on the specimen after 7 days(B).*

### 3.6.3.2. Evaluation of the fluidity

A 2-milliliter of etch of the five sample (5 sample of the tissue conditioner as controlled group, 5 samples of tissue conditioner containing 2.5 % of the juncus powder, tissue conditioner containing

5 % of the juncus powder and tissue conditioner containing 10 % of the juncus powder), prepared as described earlier, was positioned on a glass surface. Within 30 seconds, another glass surface (weighing 100 grams) was delicately positioned on the sample. Subsequently, after 120 seconds, a load of 1000 grams was introduced onto the upper glass surface, resulting in a total load of 1100 grams on the specimen. Following a 60-second application of the load, the maximum and minimum width of the compressed specimen was determined by drawing three lines and measure by ruler, and the average value of these measurements was calculated to represent the fluidity of the specimen.

## 3.8 Data analysis

Continuous data are presented as means and standard deviations in our documentation.

All tests are deemed statistically significant at $p < 0.05$.

Group differences were scrutinized using ANOVA or a linear model considering the four groups involved.

In adherence to a double-blind protocol, the statistician received data labeled with letters instead of names. This approach ensured that the statistician remained unaware of which sample produced specific results during the study.

## 3.9 Data management and disposal

The gathered data was securely stored on the researcher's laptop, protected by a password. Access to this data was granted to the researcher, supervisors, and the designated statisticians. Following the conclusion of the research, all data was permanently removed from the researcher's laptop. Any data exported to statistical software programs, like Excel or SPSS, was archived on the UWC institutional research data repository, Kipapu.

## 3.10 Ethical considerations

The researcher affirmed that there was no conflict of interest in this research endeavor.

Ethical approval for conducting the research was obtained from the University of the Western Cape BMREC (Appendix 2).

Full transparency was maintained as the researcher disclosed self-funding as the source of financial support. Any prospective sponsorship, particularly logistical assistance, was clearly documented in written form.

It was emphasized that any sponsorships received in support of this research project would not exert any influence on the impartial reporting of study findings.

To ensure objectivity, data management adopted a blind approach. Each sample was randomly assigned a number from 1 to 100 by an independent operator, and the information was recorded in an Excel sheet, ensuring blinding of the specimens to the primary operator. To further enhance impartiality, double blinding of the data was achieved by assigning random letters to each group before submission to the statistician. This meticulous process minimized the risk of bias in the study's outcomes.

# CHAPTER 4

# RESULTS AND STATISTICS

This chapter is organized to present the findings clearly and systematically, beginning with an overview of the growth inhibitory effect against *Candida albicans*. Subsequently, the mechanical properties, including fluidity, penetration, and penetration proportion, were delineated. The statistical analyses conducted to discern patterns and significance within the data are reported.

## 4.1. Growth inhibitory effect of Juncus powder-mixed tissue conditioner

The number of colony counts per plate were initially converted to colony-forming units per milliliter (CFU/mL) using the formula: CFU/mL = Number of colonies × dilution factor/amount plated. Subsequently, for ease of data management, the CFU/mL values were log-transformed, for instance: Ln (31,400 CFU/mL) = 10.4 (Table 2).

| LN (Colony forming units/mL) | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Candida | 10.4 | 10.1 | 10.1 | 9.3 | 9.3 | 9.3 | 9.5 | 9.4 | 10.1 | 10.1 | 9.6 | 9.6 | 9.5 | 9.1 | 10.1 | 10.1 | 9.8 | 9.5 |
| TC | 6.6 | 6.2 | 6.2 | 8.3 | 7.5 | 8.5 | 6.1 | 5.5 | 6.4 | 6.1 | 6.4 | 6.1 | 5.2 | 4.4 | 4.1 | 5.3 | 3.7 | 6.4 |
| TC/E 2.5% | 6.2 | 7.4 | 6.6 | 9.0 | 7.4 | 7.2 | 6.2 | 5.3 | 4.6 | 4.9 | 4.8 | 5.3 | 3.7 | 4.4 | 4.1 | 3.7 | 3.7 | 4.1 |
| TC/E 5% | 6.2 | 6.2 | 7.1 | 7.4 | 6.6 | 7.4 | 4.9 | 5.8 | 5.3 | 5.3 | 5.4 | 5.4 | 4.6 | 4.1 | 4.1 | 4.6 | 3.7 | 4.1 |
| TC/E 10% | 7.8 | 6.1 | 6.2 | 6.2 | 6.2 | 7.8 | 6.3 | 5.5 | 5.5 | 5.8 | 5.7 | 5.9 | 4.6 | 4.1 | 3.0 | 4.1 | 4.9 | 4.1 |

*Table 2: LN (Colony forming units/mL)*

*TC tissue conditioner, TC/E refers to tissue conditioners mixed with E Juncus extract powder in 2.5%, 5% and 10% where a total of 18 samples of each group were observed*

Upon comparison of mean values across the four experimental groups, the addition of the Juncus plant extract to the tissue conditioner demonstrated a positive effect in reducing the Candida

ablicans load. Specifically, Ln (CFU/mL) values were 6.05 for Tissue Conditioner (TC), and 5.48, 5.46, and 5.55 for TC/E2.5%, TC/E5%, and TC/E10%, respectively (Fig. 5).

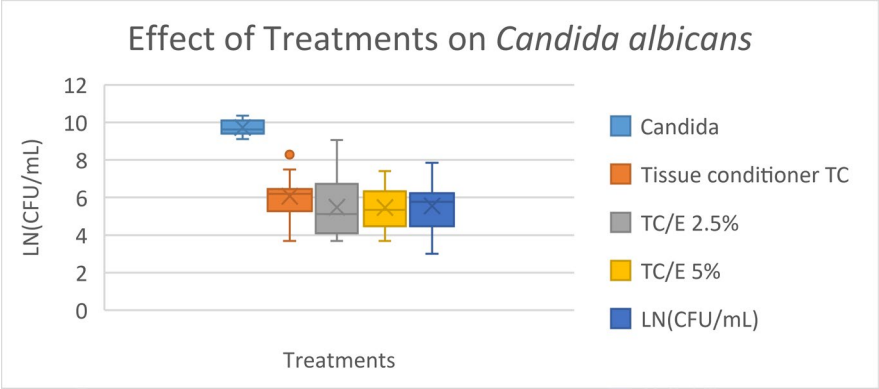



*Figure 5 Box Plot Overview of different Treatment Effects on Candida albicans*

*The figure illustrates a box plot summarizing the impact of different treatments on Candida albicans, represented in logarithmic-transformed CFU/mL, with n = 18 for each treatment.*

## Analysis of Variance (ANOVA) and Post-hoc Tests

The ANOVA yielded a highly significant p-value of 1.48E-19 (Fcrit = 2.48, df = 89), indicating substantial differences among the five experimental groups groups. Subsequently, a pairwise Bonferroni Post-Hoc test (α = 0.001) was conducted to discern specific significant differences.

## Pairwise Comparisons: Untreated *Candida albicans* vs. *experimental groups*

The Bonferroni Post-Hoc analysis revealed significant differences between untreated *Candida albicans* (negative control) and all other experimental groups. The differences were noteworthy for Tissue Conditioner (p = 1.10E-10), TC/E2.5% (p = 4.44E-10), TC/E5% (p = 7.69E-13), and TC/E10% (p = 8.64E-12), with average Ln (CFU/mL) values of 6.1, 5.5, 5.5, and 5.6, respectively (Figures. 6 &7).

*Figure 6: Candida albicans colony after 24 hours untreated specimen (negative control)*

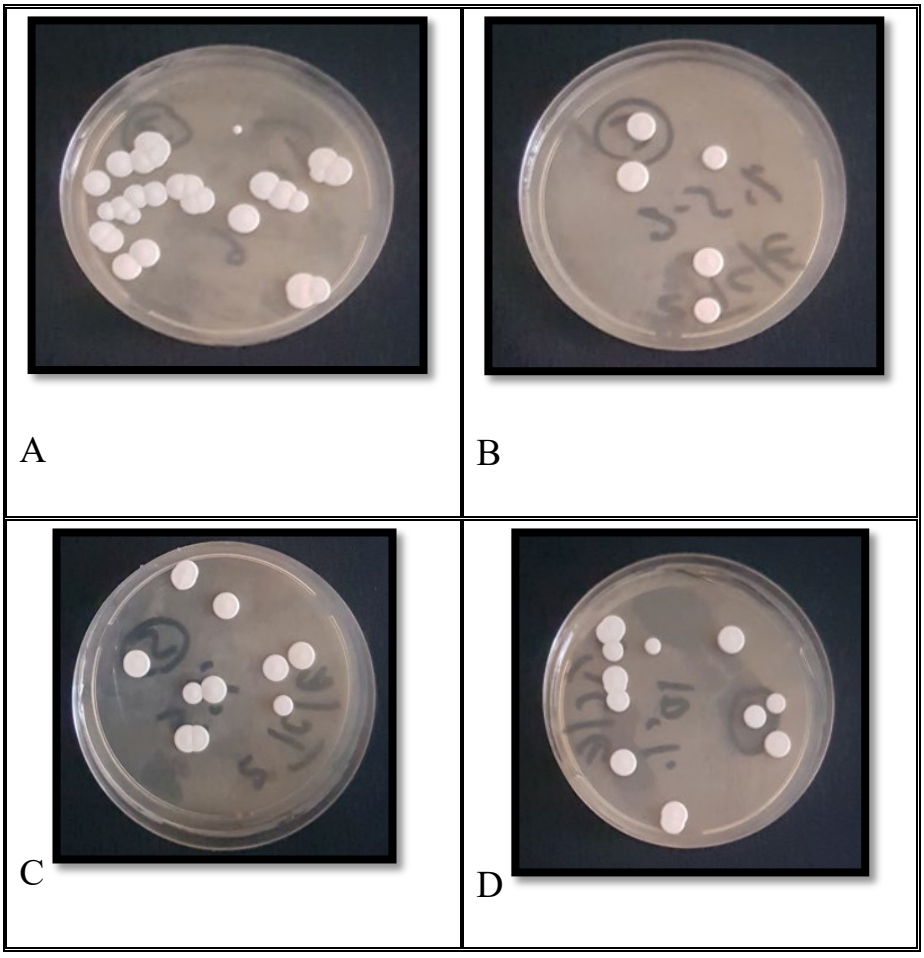



*Figure 7: Candida albicans colony after 24 hours, A; TC without plant extract, B; TC with 2.5%* Juncus powder*, C; TC with 5%* Juncus powder *and D; TC with 10%* Juncus powder

### Pairwise Interactions Between experimental groups

Pairwise interactions among Tissue Conditioner, TC/E2.5%, TC/E5%, and TC/E10% revealed no significant differences. Tissue conditioner vs TC/E2,5% (P-value 1,19E-01), Tissue conditioner vs TC/5% (P-value 7,73E-02), Tissue conditioner vs TC/E10% (P-value 1,22E-01), TC/E2,5% vs TC/E 5% (P-value 4,81E-01), TC/E2,5% vs TC/10% (P-value 4,41E-01). Notably, the interaction between Tissue Conditioner vs. TC/5% at p = 0.08 may be considered the closer to significant value.

## 4.2. Flow Properties Evaluation:

The study assessed the flow properties of different specimens 5 sample for etch experimental groups, including the Tissue Conditioner (TC), TC/E 2.5%, TC/E 5%, and TC/E 10%. The flow measurements were conducted by drawing three lines the maximum width, minimum width and average width in mm of each specimen. The measurements, presented in millimeters (Fig. 8), are summarized as follows in (Table 3).

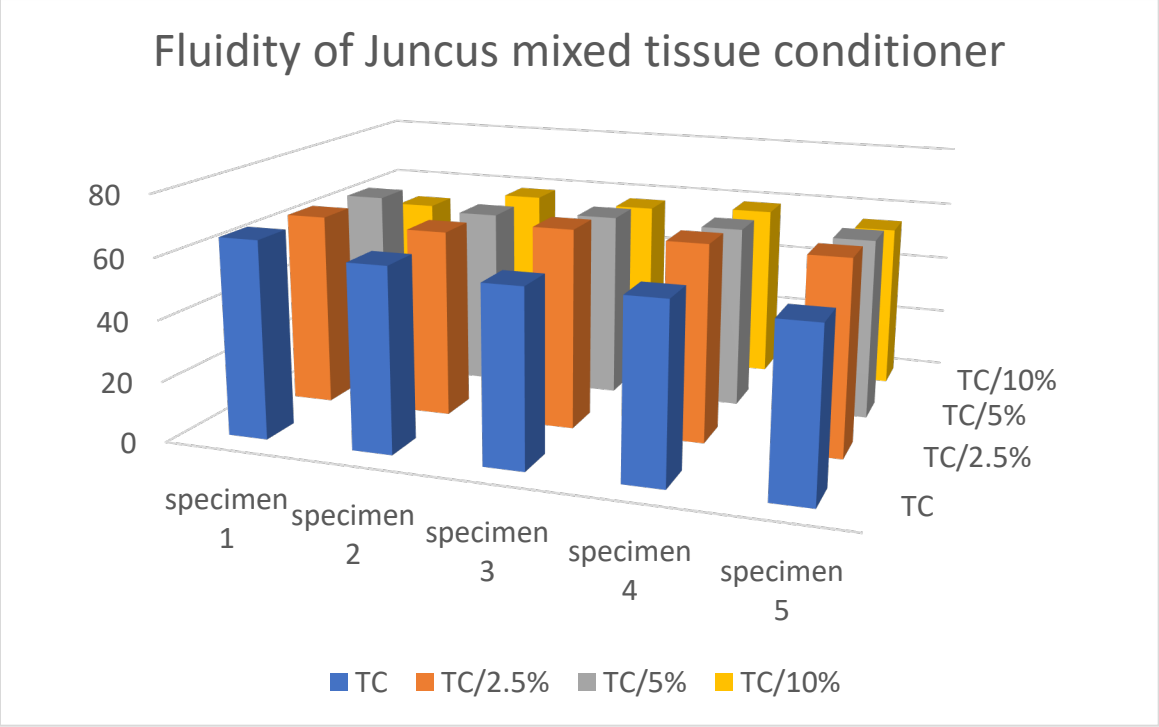
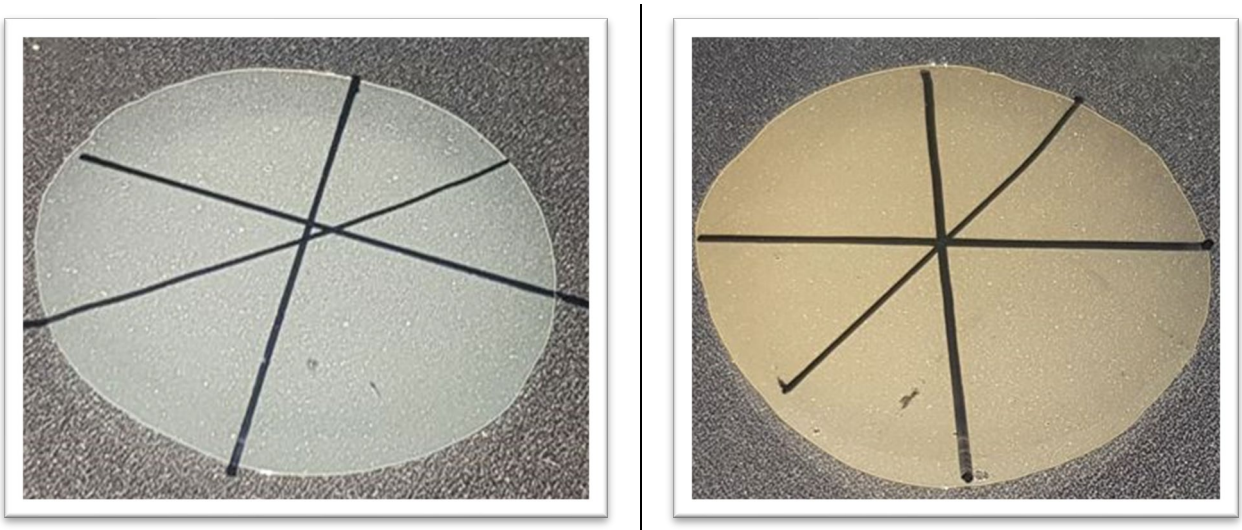



*Figure 8 Fluidity of Juncus mixed tissue conditioner of the different mass % and the control group*

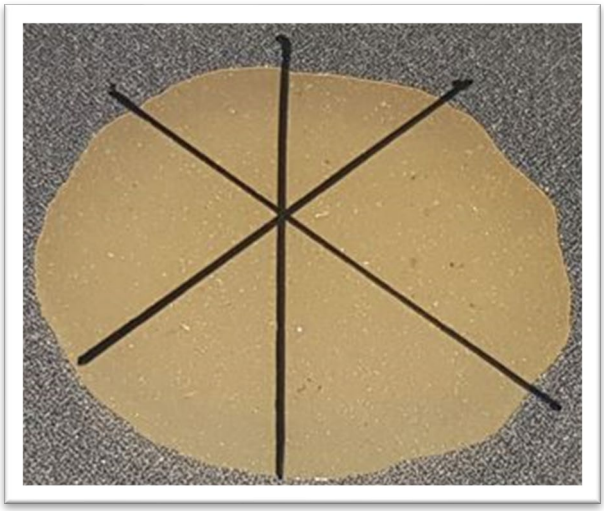
| Specimen | highest | lowest | average |
|---|---|---|---|
| Tissue Conditioner (TC) | 67 mm | 52 mm | 59.5 mm |
| TC/E 2.5% | 70 mm | 60 mm | 65 mm |
| TC/E 5% | 64 mm | 59 mm | 61.5 mm |
| TC/E 10% | 50 mm | 59 mm | 54.5 mm |

*Table 3: shows the highest, lowest, and average values by mm of the different concentrations.*

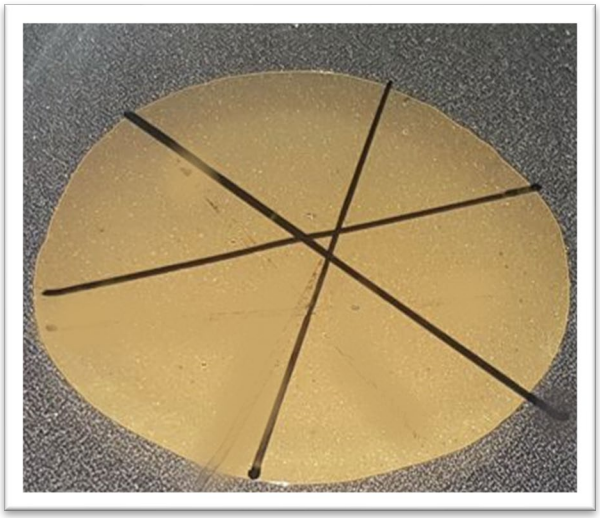
The flow properties of the specimens were measured across three lines drawn across the width of the specimens to capture any variations in fluidity. The Tissue Conditioner (TC) demonstrated consistent flow measurements across the three lines, with average values of 59.07 mm. In comparison, the TC/E 2.5%, TC/E 5%, and TC/E 10% exhibited varying flow characteristics (Fig. 9).



a b



C



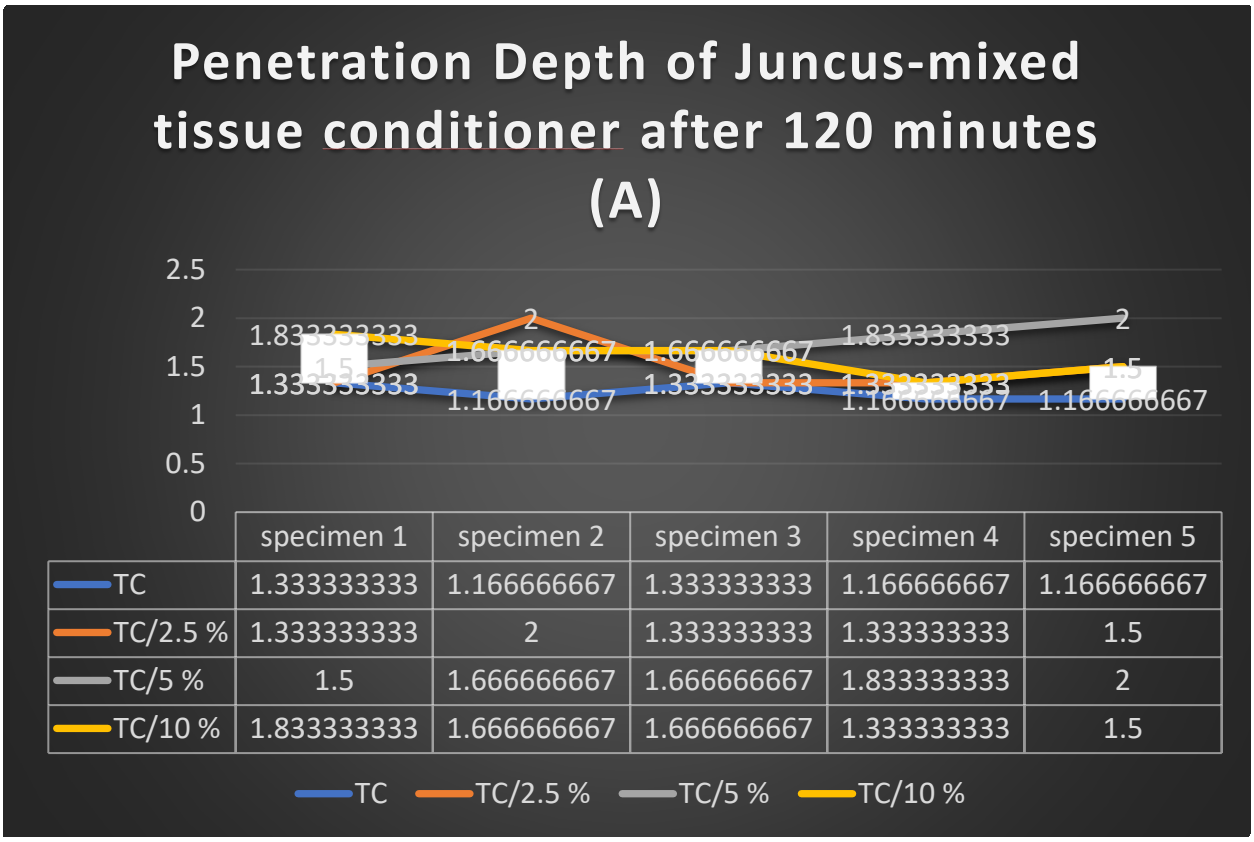
d

*Figure 9: a; shows the measurement of the TC, b; 2.5%, c; 5% and d; 10% of E*

Notably, the TC/E 2.5% showed an increase in flowability compared to the TC, with an average of 65 mm. Conversely, the TC/E 5% and TC/E 10% displayed a reduction in flow properties, with averages of 61.5 mm and 54.5 mm, respectively.

## 4.3. Penetration Results and Penetration Ratio:

The penetration data for Test (A) at 120 minutes (Table 4) and Test (B) at 7 days is presented below (Table 5). These results are essential in understanding the behavior of Juncus-mixed specimens, particularly Tissue Conditioner TC, and its variations (TC/E 2.5%, TC/E 5%, and TC/E 10%) in terms of penetration depth. The penetration ratio was calculated as the average penetration at 120 minutes divided by the average penetration at 7 days (Fig.10), (Fig.11).




| | specimen 1 | specimen 2 | specimen 3 | specimen 4 | specimen 5 |
|---|---|---|---|---|---|
| TC | 1.333333333 | 1.166666667 | 1.333333333 | 1.166666667 | 1.166666667 |
| TC/2.5 % | 1.333333333 | 2 | 1.333333333 | 1.333333333 | 1.5 |
| TC/5 % | 1.5 | 1.666666667 | 1.666666667 | 1.833333333 | 2 |
| TC/10 % | 1.833333333 | 1.666666667 | 1.666666667 | 1.333333333 | 1.5 |

*Figure 10: a; shows Penetration Depth of Juncus-mixed tissue conditioner after 120 minutes (A) for TC, 2.5%, 5%, and 10% concentration. Depth of penetration into Juncus-mixed tissue conditioner. The standard range recommended by ISO 10139-1 is 1.8 mm or less for 120 minutes.*

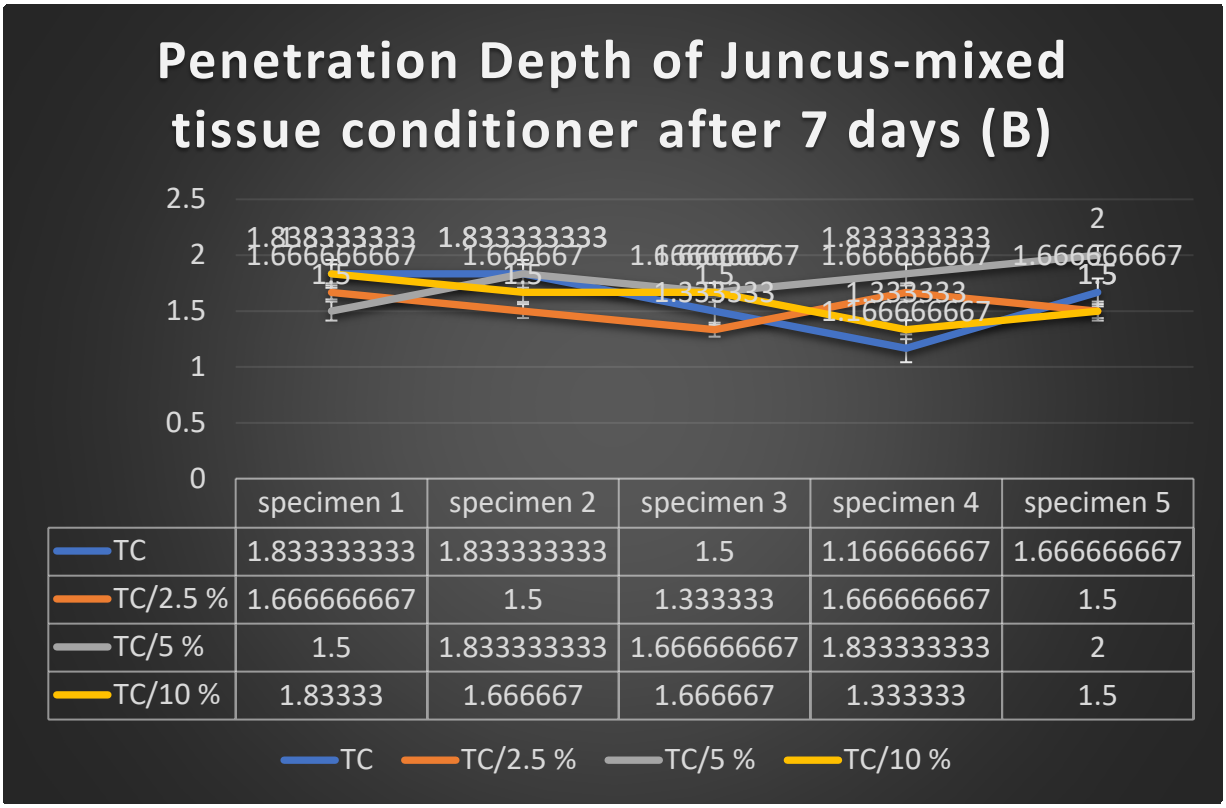



*Figure 11: shows Penetration Depth of Juncus-mixed tissue conditioner after 7 days (B) for TC, 2.5%, 5%, and 10% concentration. The standard range recommended by ISO 10139-1 is 0.18 mm or more for 7 days.*

## Test (A) 120 Minutes:

| Specimen | Highest Penetration value | Lowest Penetration value | The most frequently repeated reading |
|---|---|---|---|
| Tissue Conditioner TC | 1.5 | 1 | 1 |
| TC/E 2.5% | 2 | 1 | 1 |
| TC/E 5% | 2 | 1 | 1.5 |
| TC/E 10% | 2.5 | 1 | 1.5 |

*Table 4: shows the highest, lowest, and most frequently repeated readings by mm of the different concentrations after 120 minutes.*

## Test (B) 7 Days:

| Specimen | Highest Penetration value | Lowest Penetration value | The most frequently repeated reading |
|---|---|---|---|
| Tissue Conditioner TC | 2 | 1 | 1.5 |
| TC/E 2.5% | 2 | 1 | 2 |
| TC/E 5% | 2 | 1 | 1.5 |
| TC/E 10% | 2 | 1 | 1.5 |

*Table 5: shows the highest, lowest, and most frequently repeated readings by mm of the different concentrations after 7 days.*

## Penetration Ratio:

The Penetration Ratio (PR) was calculated as the average penetration at 120 minutes divided by the average penetration at 7 days (Table 6).

| Specimen | Penetration Ratio |
|---|---|
| Tissue Conditioner TC | 0.770833 |
| TC/E 2.5% | 0.978261 |
| TC/E 5% | 1 |
| TC/E 10% | 1 |

*Table 6: shows penetration ratios of the experimental groups*

These results demonstrate that the mechanical properties of all Juncus-mixed specimens were within the regulated ranges specified by ISO 10139-1. In terms of penetration, the average depth varied between specimens and over time (Fig.12).

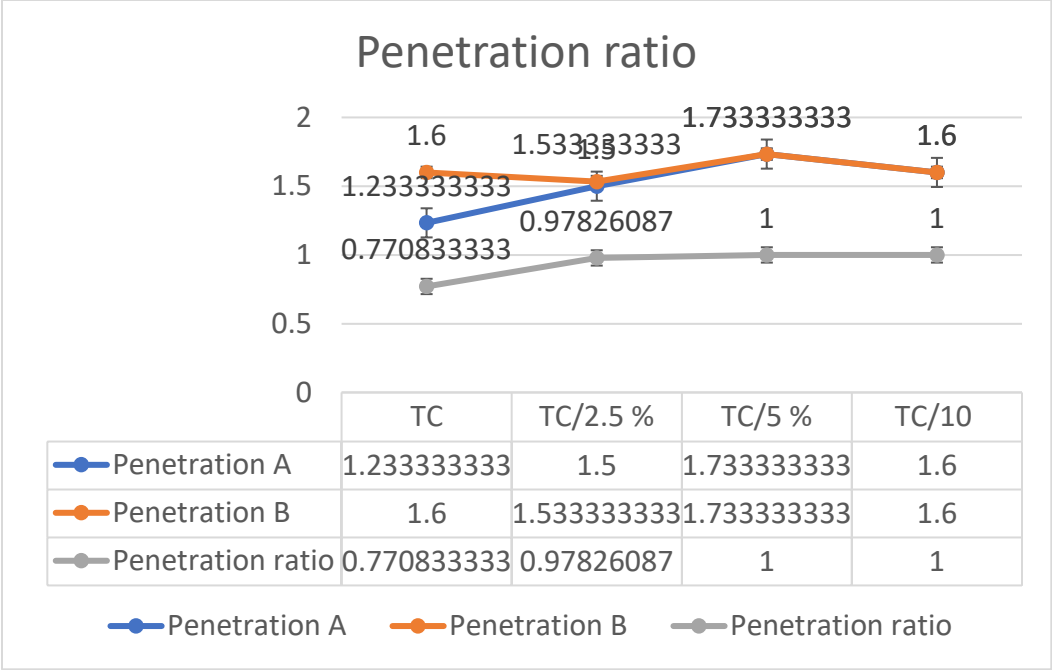



*Figure 12: Penetration ratio =value from A after 120 min/value from B after 7 days.*

*Penetration ratio of Juncus-mixed tissue conditioner. The standard range recommended by ISO 10139-1 is* $\leq 5$.

# CHAPTER 5

# DISCUSSION

To the best of our knowledge, Yoshihito Naito et al is the only researcher who has examined the impact of incorporating Juncus powder into tissue conditioner in a single study.

Tissue conditioner has recently acquired appeal as a temporary lining material for dentures by treating the alveolar mucosa beneath them before taking an impression. However, studies have shown that the rough surface of tissue conditioner functions as a reservoir for pathogenic bacteria, which may have an influence on denture wearers' general health. McGhie et al. found a relationship between streptococci colonization in the oral cavity and the development of bacterial endocarditis (McGhie et al., 1977).

Furthermore, Nikawa et al found a significant incidence of Candida albicans in the alveolar mucosa of patients with denture stomatitis. This emphasizes the potential health hazards linked with the usage of tissue conditioner due to microbial growth on the rough surface (Nikawa et al., 1995)

The results of this study shed light on the potential of a Juncus-containing tissue conditioner in inhibiting the growth of *Candida albicans* and influencing the mechanical properties of the material. The discussion encompasses the implications of these findings in the context of denture-related conditions, the integration of natural substances in dental materials, and the prospects for further research and clinical applications.

## 5.1. Growth Inhibitory Effect

The incorporation of metallic components into tissue conditioners has sparked interest due to its possible antifungal activities and influence on mechanical qualities. Homsiang et al. investigated zinc oxide nanoparticles (ZnOnps) and found that introducing 15 wt% ZnOnps into tissue conditioner has antifungal effects against Candida albicans while preserving penetration depth and tensile bond strength (Woraporn et al., 2021).

Mousavi et al. studied silver, zinc oxide, and chitosan nanoparticles and discovered that their combination in tissue conditioners prevented fungal and bacterial development (Seyyed et al.,

2020). Tonprasong et al. tested tissue conditioner with a surface pre-reacted glass-ionomer (S-PRG) nanofiller and discovered reduced C. albicans adherence, indicating a suitable alternative soft lining material (Watcharapong et al., 2021).

However, the use of metallic components in tissue conditioners has some restrictions and downsides. One drawback is the concentration-dependent variability of microbial growth inhibition. For example, in a study utilizing silver (Ag), zinc oxide (ZnO), and chitosan nanoparticles, growth suppression of Candida albicans occurred at a concentration of 2.5%, while inhibition of Streptococcus mutans, Enterococcus faecalis, and Pseudomonas aeruginosa required a concentration of 5% (Seyyed et al., 2020).

Furthermore, the efficacy of metallic components against fungus and bacteria can vary. The same study found that a mixture of Ag, ZnO, and chitosan nanoparticles inhibited fungal growth more efficiently than bacterial growth (Seyyed et al., 2020).

Furthermore, the addition of metallic components may affect the physical and mechanical properties of tissue conditioners. For example, whereas zinc oxide nanoparticles (ZnOnps) did not reduce penetration depth or tensile bond strength, the antifungal impact was only evident for 14 days (Woraporn et al., 2021).

It is critical to understand that these restrictions and drawbacks differ depending on the individual metallic component and its concentration in the tissue conditioner. More study is needed to fully understand the ramifications of employing metallic components as antifungal agents in tissue conditioners and to improve their use in prosthodontic treatment.

In the present study, the addition of Juncus to the tissue conditioner significantly reduced the growth of Candida albicans in all concentration 2.5%, 5% and 10% in an vitro setting. The plant extract displayed antifungal capabilities, correlating with prior studies by Naito, *et al*. confirming Juncus effuses' antimicrobial potential without effecting the physical properties flow and penetration of the tissue conditioner within the range of ISO standards, also another superior advantage of the planet derivatives antifungal components is the eliminations of the metallic toxicity (Naito *et al*., 2018).

Tissue conditioners accompanied with plant-derived components show promising antifungal and mechanical properties. Chitosan nanoparticles (CSNPs) have been combined with essential oils

(EO) such as oregano oil, lemongrass, and Ocimum basilicum essential oil (OBEO) to create tissue conditioners. These ingredients have demonstrated significant antifungal effectiveness against Candida albicans, the major cause of denture stomatitis (Hina et al., 2020; Aiemeeza et al., 2022).

Integrating these plant-derived materials into tissue conditioners has not resulted in significant changes to the material's color, surface roughness, or hardness (Maryam et al., 2021; Nevin et al 2022).

Furthermore, the tissue conditioners have been shown to release essential oils consistently throughout time. These results suggest that tissue conditioners containing plant-derived components have the potential to successfully treat denture stomatitis by inhibiting fungus development while retaining mechanical characteristics. However, more research is needed to determine the long-term efficacy and therapeutic viability of these treatments.

This finding is particularly promising in the context of denture stomatitis, where candida infections are a common etiological factor (Renner et al., 1979). The significant differences observed between the Juncus-containing tissue conditioner and the control group underscore the potential clinical relevance of this approach.

The demonstrated growth inhibitory effect of Juncus-containing tissue conditioner against *Candida albicans* holds significant clinical promise for denture wearers, particularly those susceptible to denture stomatitis. By effectively reducing *Candida albicans* growth and addressing the reservoir potential of tissue conditioners, this approach offers a tangible and practical solution to enhance the oral health and overall well-being of individuals relying on dentures, emphasizing its potential for widespread clinical application in managing denture-related fungal infections.

According to Morita (2006), these findings showed that tissue conditioners containing Juncus powder reduced candida development and subsequent biofilm formation on material surfaces, reducing denture stomatitis and oral candidiasis which is compatible with the present study. However, further research is warranted to explore the optimal concentration of Juncus and its long-term efficacy.

These findings signify a robust statistical foundation supporting the efficacy of the addition of Juncus to TC in reducing *Candida albicans* load.

## 5.2. Mechanical Properties

According to Saitoh *et al*., (2010), it is extremely difficult to assess and analyze its mechanical qualities of modified tissue conditioners because many researchers utilize different methodologies (Hong *et al*., 2010).

To analyze the mechanical properties of Juncus-mixed specimens, we used procedures certified according to the ISO 10139-1 2018 standard to assess changes in fluidity and hardness. Both attributes of all tested mixes were found to be within the ISO 10139-1 standard values which is compatible with the only study made by Yoshihito et al 2018. Mechanical aspects, including flow properties and penetration characteristics, were evaluated to provide insight into the behavior of the Juncus-containing tissue conditioner.

The increase in flowability seen in TC/E 2.5% demonstrates that the addition of Juncus can affect the fluidity of the substance. Higher Juncus concentrations (TC/E 5% and TC/E 10%) had a declining influence on fluidity. The observed increase in fluidity at the lowest concentration (TC/E 2.5%) and decrease at higher concentrations (TC/E 5% and TC/E 10%) in the Juncus-containing tissue conditioner could be attributed to several factors related to the interaction between the plant extract and the material.

Concentration-dependent effects; The material characteristics may vary depending on the concentration of Juncus. At a lower concentration (2.5%), certain components of the plant extract may improve the fluidity of the tissue conditioner. However, at greater concentrations (5% and 10%), the introduction of other Juncus components may alter the material's constitution, resulting in decreased fluidity.

Interaction with the base material; The addition of Juncus to the tissue conditioner may alter the interactions between the plant extract and the base material. At lower concentrations, the interaction may increase fluidity, whereas at larger concentrations, it may interfere with polymerization or cross-linking of the material, resulting in decreased fluidity. Plant extracts are complicated combinations of different chemicals. Different components of the Juncus extract may have varying effects on the material characteristics. At lower quantities, beneficial components may predominate, resulting in greater fluidity, at higher concentrations, other components may become more influential, resulting in decreased fluidity.

Ideal formulation; Achieving the required material qualities requires determining the ideal concentration of the additive Juncus powder in order to balance its positive and negative effects. The concentration-dependent change in fluidity suggests a delicate balance, with surpassing a specific concentration having a negative impact on the material's flow qualities.

The plant extract may influence the polymerization and cross-linking activities of the tissue conditioner. At lower doses, it may enhance these activities, resulting in increased fluidity. At greater doses, however, interaction with these mechanisms may cause decreased fluidity.

These variances show the significance of carefully adjusting natural substance concentrations to produce the desired material properties.

Understanding the specific mechanisms at play would require more detailed investigations into the chemical and physical interactions between Juncus components and the tissue conditioner matrix. Further research could explore the individual components of Juncus, their concentrations, and their effects on the polymerization and mechanical properties of the tissue conditioner.

The observed penetration behaviors, especially the settling effect in Tissue Conditioner offer considerations for refining the formulation to enhance stability.

Tissue Conditioner (TC) exhibited a decrease in penetration from 120 minutes to 7 days, suggesting a potential settling or stabilization effect. On the other hand, TC/E 2.5%, TC/E 5%, and TC/E 10% displayed different penetration behaviors, indicating potential influences of the plant extract on material properties. The Penetration Ratio further emphasizes the temporal variations in penetration, providing insights into the material's stability and potential applications in dental contexts. The observed penetration behaviors should be considered for optimizing the formulation and ensuring the desired material properties

## 5.3. Clinical Implications and Future Directions

The findings of this study have potential implications for clinical dentistry. The antifungal properties of Juncus suggest its potential as a natural and effective component in tissue conditioners, especially for patients prone to candida-associated stomatitis. Moreover, the influence on material properties opens avenues for personalized denture fabrication, considering

individual patient needs and preferences. However, the study is an *in-vitro* exploration, and further clinical trials are necessary to validate these findings in real-world scenarios.

The exploration of natural substances like Juncus also aligns with the growing interest in holistic and sustainable healthcare practices. This approach may resonate with patients seeking alternatives to conventional antifungal medications. Integrating ancient wisdom, as seen in the use of Juncus effuses, with modern dental science, presents a fascinating intersection that warrants further investigation.

## 5.4. Limitations

While this study contributes valuable insights, it is not without limitations. The *in-vitro* nature of the study limits the translation of findings to clinical settings. The concentration of Juncus was based on preliminary investigations, and further research is needed to determine the optimal concentration for clinical efficacy and safety. Additionally, the mechanical properties evaluated in this study are essential, the study's shortcomings include the difficulty of comparing and analyzing the mechanical properties of the tissue conditioner due to the different methods utilized by the researchers. Furthermore, as the concentration of Juncus powder increases, the fluidity of the Juncus-mixed tissue conditioner decreases, thus limiting its use. Furthermore, while the addition of Juncus powder decreased Candida albicans growth, the particular mechanisms of this inhibitory effect were not fully understood in the study, which could be viewed as a drawback.

# CHAPTER 6

# CONCLUSION

In conclusion, the integration of Juncus Effuses into tissue conditioners shows promising potential for addressing candida-associated denture stomatitis. The present study reveals a significant growth inhibitory effect, highlighting the substance's impact on mechanical properties and its potential clinical relevance. Specifically, the findings suggest that a 2.5% concentration of Juncus exhibits antifungal inhibitory properties whilst maintaining mechanical properties of the TC material to within ISO standards.

This study marks a preliminary exploration, and further research is imperative to refine the formulation, assess long-term efficacy, and validate the findings in clinical settings. The dynamic interplay between traditional and indigenous knowledge, as exemplified by Juncus and modern dental science paves the way for innovative and patient-centered approaches in dentistry.

As the field of dental research evolves, the integration of natural substances and holistic healthcare practices holds promise for providing patients with safer, more effective, and personalized solutions.

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# APPENDIX 1:

t-Test: Two-Sample Assuming Unequal Variances

| | *Candida* | *Tissue conditioner TC* |
|---|---|---|
| Mean | 9.721744 | 6.053043 |
| Variance | 0.145578 | 1.630571 |
| Observations | 18 | 18 |
| Hypothesized Mean Difference | 0 | |
| df | 20 | |
| t Stat | 11.67909 | |
| P(T<=t) one-tail | 1.1E-10 | |
| t Critical one-tail | 1.724718 | |
| P(T<=t) two-tail | 2.19E-10 | |
| t Critical two-tail | 2.085963 | |

t-Test: Two-Sample Assuming Unequal Variances

| | *Candida* | *TC/E 2.5%* |
|---|---|---|
| Mean | 9.721744 | 5.481078 |
| Variance | 0.145578 | 2.458466 |
| Observations | 18 | 18 |
| Hypothesized Mean Difference | 0 | |
| df | 19 | |
| t Stat | 11.14926 | |
| P(T<=t) one-tail | 4.44E-10 | |
| t Critical one-tail | 1.729133 | |
| P(T<=t) two-tail | 8.88E-10 | |
| t Critical two-tail | 2.093024 | |

t-Test: Two-Sample Assuming Unequal Variances

| | *Candida* | *TC/E 5%* |
|---|---|---|
| Mean | 9.721744 | 5.458957 |
| Variance | 0.145578 | 1.367143 |
| Observations | 18 | 18 |
| Hypothesized Mean Difference | 0 | |
| df | 21 | |
| t Stat | 14.7045 | |
| P(T<=t) one-tail | 7.89E-13 | |
| t Critical one-tail | 1.720743 | |
| P(T<=t) two-tail | 1.58E-12 | |
| t Critical two-tail | 2.079614 | |

t-Test: Two-Sample Assuming Unequal Variances

| | *Candida* | *TC/E 10%* |
|---|---|---|
| Mean | 9.721744 | 5.551768 |
| Variance | 0.145578 | 1.580413 |
| Observations | 18 | 18 |
| Hypothesized Mean Difference | 0 | |
| df | 20 | |
| t Stat | 13.46637 | |
| P(T<=t) one-tail | 8.64E-12 | |
| t Critical one-tail | 1.724718 | |
| P(T<=t) two-tail | 1.73E-11 | |
| t Critical two-tail | 2.085963 | |

t-Test: Two-Sample Assuming Unequal Variances

| | *Tissue conditioner TC* | *TC/E 2.5%* |
|---|---|---|
| Mean | 6.053043 | 5.481078 |
| Variance | 1.630571 | 2.458466 |
| Observations | 18 | 18 |
| Hypothesized Mean Difference | 0 | |
| df | 33 | |
| t Stat | 1.200037 | |
| P(T<=t) one-tail | 0.119335 | |
| t Critical one-tail | 1.69236 | |
| P(T<=t) two-tail | 0.238669 | |
| t Critical two-tail | 2.034515 | |

t-Test: Two-Sample Assuming Unequal Variances

| | *Tissue conditioner TC* | *TC/E 5%* |
|---|---|---|
| Mean | 6.053043 | 5.458957 |
| Variance | 1.630571 | 1.367143 |
| Observations | 18 | 18 |
| Hypothesized Mean Difference | 0 | |
| df | 34 | |
| t Stat | 1.455761 | |
| P(T<=t) one-tail | 0.077316 | |
| t Critical one-tail | 1.690924 | |
| P(T<=t) two-tail | 0.154631 | |
| t Critical two-tail | 2.032245 | |

t-Test: Two-Sample Assuming Unequal Variances

| | *Tissue conditioner TC* | *TC/E 10%* |
|---|---|---|
| Mean | 6.053043 | 5.551768 |
| Variance | 1.630571 | 1.580413 |
| Observations | 18 | 18 |
| Hypothesized Mean Difference | 0 | |
| df | 34 | |
| t Stat | 1.186843 | |
| P(T<=t) one-tail | 0.121758 | |

| | |
|---|---|
| t Critical one-tail | 1.690924 |
| P(T<=t) two-tail | 0.243516 |
| t Critical two-tail | 2.032245 |

t-Test: Two-Sample Assuming Unequal Variances

| | *TC/E 2.5%* | *TC/E 5%* |
|---|---|---|
| Mean | 5.481078 | 5.458957 |
| Variance | 2.458466 | 1.367143 |
| Observations | 18 | 18 |
| Hypothesized Mean Difference | 0 | |
| df | 31 | |
| t Stat | 0.047984 | |
| P(T<=t) one-tail | 0.481019 | |
| t Critical one-tail | 1.695519 | |
| P(T<=t) two-tail | 0.962037 | |
| t Critical two-tail | 2.039513 | |

t-Test: Two-Sample Assuming Unequal Variances

| | *TC/E 2.5%* | *TC/E 10%* |
|---|---|---|
| Mean | 5.481078 | 5.551768 |
| Variance | 2.458466 | 1.580413 |
| Observations | 18 | 18 |
| Hypothesized Mean Difference | 0 | |
| df | 32 | |
| t Stat | -0.14923 | |
| P(T<=t) one-tail | 0.441154 | |
| t Critical one-tail | 1.693889 | |
| P(T<=t) two-tail | 0.882308 | |
| t Critical two-tail | 2.036933 | |

t-Test: Two-Sample Assuming Unequal Variances

| | *TC/E 5%* | *TC/E 10%* |
|---|---|---|
| Mean | 5.458957 | 5.551768 |
| Variance | 1.367143 | 1.580413 |
| Observations | 18 | 18 |
| Hypothesized Mean Difference | 0 | |

| | |
|---|---|
| df | 34 |
| t Stat | -0.22935 |
| P(T<=t) one-tail | 0.409985 |
| t Critical one-tail | 1.690924 |
| P(T<=t) two-tail | 0.81997 |
| t Critical two-tail | 2.032245 |

| Interactions | P-value | | Means | Reference | Comparison |
|---|---|---|---|---|---|
| Candida vs Tissue conditioner | 1.1E-10 | Sig diff | | 9.7 | 6.1 |
| Candida vs TC/E2,5% | 4.44E-10 | Sig diff | | | 5.5 |
| Candida vs TC/E5% | 7.89E-13 | Sig diff | | | 5.5 |
| Candida vs TC/E10% | 8.64E-12 | Sig diff | | | 5.6 |
| Tissue conditioner vs TC/E2,5% | 0.119335 | no sig dif | | 6.1 | 5.5 |
| Tissue conditioner vs TC/5% | 0.077316 | no sig dif | | | 5.5 |
| Tissue conditioner vs TC/E10% | 0.121758 | no sig dif | | | 5.6 |
| TC/E2,5% vs TC/E 5% | 0.481019 | no sig dif | | 5.5 | 5.5 |
| TC/E2,5% vs TC/10% | 0.441154 | no sig dif | | | 5.6 |
| TC/ 5% vs TC/E 10% | 0.409985 | no sig dif | | 5.5 | 5.6 |

| Type of specimen | specimen 1 | specimen 2 | specimen 3 | specimen 4 | specimen 5 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Tissue conditioner TC line 1 | 67 | 61 | 59 | 59 | 56 | 65 | 60.33333 | 57.66667 | 57.66667 | 54.66667 |
| line 2 | 65 | 61 | 58 | 57 | 52 | 295.3333 | | | | |
| line 3 | 63 | 59 | 56 | 57 | 56 | 59.06667 | | | | |
| | 195 | 181 | 173 | 173 | 164 | | | | | |
| TC/E 2.5% line 1 | 64 | 65 | 70 | 68 | 65 | 64 | 62 | 66 | 64.66667 | 63.66667 |
| line 2 | 65 | 60 | 68 | 62 | 63 | 320.3333 | | | | |
| line 3 | 63 | 61 | 60 | 64 | 63 | 64.06667 | | | | |
| | 192 | 186 | 198 | 194 | 191 | | | | | |
| TC/E 5% line 1 | 64 | 60 | 63 | 61 | 61 | 63 | 59.66667 | 61.66667 | 60.66667 | 60 |
| line 2 | 63 | 60 | 62 | 61 | 60 | 305 | | | | |
| line 3 | 62 | 59 | 60 | 60 | 59 | 61 | | | | |
| | 189 | 179 | 185 | 182 | 180 | | | | | |
| TC/E 10% line 1 | 50 | 59 | 59 | 59 | 57 | 52.33333 | 58.33333 | 57 | 58.66667 | 55 |
| line 2 | 50 | 59 | 55 | 58 | 55 | 281.3333 | | | | |
| line 3 | 57 | 57 | 57 | 59 | 53 | 56.26667 | | | | |
| | **157** | **175** | **171** | **176** | **165** | | | | | |

TEST (A) 120 MIN

| Type of specimen | specimen 1 | specimen 2 | specimen 3 | specimen 4 | specimen 5 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tissue conditioner TC penetration piont 1 | 1.5 | 1.5 | 1.5 | 1 | 1 | 1.33333 | 1.16667 | 1.33333 | 1.16667 | 1.16667 | | |
| penetration piont 2 | 1 | 1 | 1 | 1.5 | 1 | 6.16667 | | | | | | |
| penetration piont 3 | 1.5 | 1 | 1.5 | 1 | 1.5 | 1.23333 | | | | | 0.77083 | |
| | 4 | 3.5 | 4 | 3.5 | 3.5 | | | | | | | |
| TC/E 2.5% penetration piont 1 | 1.5 | 2 | 1 | 1 | 1 | 1.33333 | 2 | 1.33333 | 1.33333 | 1.5 | | |
| penetration piont 2 | 1.5 | 2.5 | 1 | 2 | 1.5 | 7.5 | | | | | | |
| penetration piont 3 | 1 | 1.5 | 2 | 1 | 2 | 1.5 | | | | | 0.97826 | |
| | 4 | 6 | 4 | 4 | 4.5 | | | | | | | |
| TC/E 5% penetration piont 1 | 1.5 | 1 | 1 | 2 | 2 | 1.5 | 1.66667 | 1.66667 | 1.83333 | 2 | | |
| penetration piont 2 | 1.5 | 2 | 2 | 1.5 | 2.5 | 8.66667 | | | | | | |
| penetration piont 3 | 1.5 | 2 | 2 | 2 | 1.5 | 1.73333 | | | | | 1 | |
| | 4.5 | 5 | 5 | 5.5 | 6 | | | | | | | |
| TC/E 10% penetration piont 1 | 2 | 1.5 | 2.5 | 1 | 1.5 | 1.83333 | 1.66667 | 1.66667 | 1.33333 | 1.5 | | |
| penetration piont 2 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 8 | | | | | | |
| penetration piont 3 | 2 | 2 | 1 | 1.5 | 1.5 | 1.6 | | | | | 1 | |
| | 5.5 | 5 | 5 | 4 | 4.5 | | | | | | | |

TEST (B) 7 day

| Type of specimen | specimen 1 | specimen 2 | specimen 3 | specimen 4 | specimen 5 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Tissue conditioner TC penetration piont 1 | 1.5 | 1.5 | 1.5 | 1 | 2 | 1.83333 | 1.83333 | 1.5 | 1.16667 | 1.66667 |
| penetration piont 2 | 2 | 2 | 1.5 | 1.5 | 1.5 | 8 | | | | |
| penetration piont 3 | 2 | 2 | 1.5 | 1 | 1.5 | 1.6 | | | | |
| | 5.5 | 5.5 | 4.5 | 3.5 | 5 | | | | | |
| TC/E 2.5% penetration piont 1 | 1.5 | 1 | 2 | 1 | 1 | 1.66667 | 1.5 | 1.33333 | 1.66667 | 1.5 |
| penetration piont 2 | 1.5 | 2 | 1 | 2 | 1.5 | 7.66667 | | | | |
| penetration piont 3 | 2 | 1.5 | 1 | 2 | 2 | 1.53333 | | | | |
| | 5 | 4.5 | 4 | 5 | 4.5 | | | | | |
| TC/E 5% penetration piont 1 | 1.5 | 1.5 | 1.5 | 2 | 2 | 1.5 | 1.83333 | 1.5 | 1.83333 | 2 |
| penetration piont 2 | 1.5 | 2 | 2 | 1.5 | 2.5 | 8.66667 | | | | |
| penetration piont 3 | 1.5 | 2 | 1 | 2 | 1.5 | 1.73333 | | | | |
| | 4.5 | 5.5 | 4.5 | 5.5 | 6 | | | | | |
| TC/E 10% penetration piont 1 | 1 | 1.5 | 1.5 | 1.5 | 1.5 | 1.83333 | 1.66667 | 1.66667 | 1.33333 | 1.5 |
| penetration piont 2 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 8 | | | | |
| penetration piont 3 | 2 | 1 | 1.5 | 1.5 | 1.5 | 1.6 | | | | |
| | 4.5 | 4 | 4.5 | 4.5 | 4.5 | | | | | |

# APPENDIX 2: BMREC APPROVAL LETTER

UNIVERSITY *of the* WESTERN CAPE

60 YEARS *of hope, action & knowledge*

04 April 2022

Dr R Ahmed
Restorative Dentistry
**Faculty of Dentistry**

**Ethics Reference Number:** BM22/2/2

**Project Title:** Antifungal and Mechanical Properties of Tissue Conditioner Containing Plant-Derived Component.

**Approval Period:** **02 April 2022 – 02 April 2025**

I hereby certify that the Biomedical Science Research Ethics Committee of the University of the Western Cape approved the scientific methodology and ethics of the above-mentioned research project and the requested amendment to the project.

Any further amendments, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.

**Please remember to submit a progress report annually by 30 November for the duration of the project.**

For permission to conduct research using student and/or staff data or to distribute research surveys/questionnaires please apply via:
https://sites.google.com/uwc.ac.za/permissionresearch/home

*The permission letter must then be submitted to BMREC for record keeping purposes.*

The Committee must be informed of any serious adverse event and/or termination of the study.

*Ms Patricia Josias*
*Research Ethics Committee Officer*
*University of the Western Cape*

*NHREC Registration Number: BMREC-130416-050*

10th January 2024

## GRAMMARIAN'S CERTIFICATE

This is to certify that the undersigned has reviewed and gone through all the pages of the mini-thesis entitled: "**Antifungal and Mechanical Properties of Tissue Conditioner Containing Plant-Derived Component: An *In Vitro* Study" by Dr RM Ahmed**, as against the set of structural rules that govern the composition of sentences, phrases, and words in the English language.

Signed:

Ms RA Basson (Research Psychology)
Email: renedabasson@gmail.com
Cell: 0769332281