

Comparative Evaluation of Antifungal Activity of Moringa Oleifera Leaf and Stem Bark Powder Incorporated into Soft Liner-An Invitro Study

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Abstract:

➤ Background and Objectives:

Denture stomatitis caused by *Candida albicans* is a common clinical concern associated with soft denture liners. Conventional antifungal agents such as fluconazole are effective but may lead to resistance or side effects with prolonged use. This study aimed to evaluate and compare the antifungal efficacy of Moringa oleifera leaf powder, stem bark powder, and fluconazole when incorporated into a soft liner material.

➤ Materials and Method:

A total of 40 samples (10 mm x10mm x 2 mm) were fabricated using a soft liner material. These were divided into four groups (n=10 each) as follows: Group A (control – soft liner without additives), Groups B (Moringa leaf powder at 5% w/w), Groups C (Moringa stem bark powder 5% w/w), and Groups D (fluconazole 2% w/w). The antifungal activity of each group was tested using the colony forming unit method against *Candida albicans*. The number of colony forming unit were measured and statistically analyzed using one-way ANOVA followed by post hoc tests.

➤ Results:

All experimental groups exhibited varying degrees of antifungal activity compared to the control group. The group with 5% Moringa leaf powder showed the highest antifungal activity compared with 5% w/w Moringa stem bark among the herbal groups. Fluconazole at 2% exhibited the maximum antifungal effect overall. Moringa stem bark groups demonstrated moderate antifungal activity against *Candida albicans*.

Keywords: Moringa Oleifera; Soft Denture Liner; Antifungal Activity; Candida Albicans; Leaf Powder; Stem Bark Powder; Herbal Antifungal Agent; Denture Stomatitis.

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I. INTRODUCTION

Edentulousness is not a disease in itself, but rather a consequence of underlying pathological conditions. The increasing incidence of edentulousness in recent years has raised concerns about the adequacy of dental care. Despite advancements, the complete denture remains the mainstay for managing edentulous patients. Prosthetic rehabilitation not only restores function but also improves aesthetics and psychological health¹. However, treating elderly edentulous

patients presents challenges beyond denture construction, including complications from continuous denture wear.

Alveolar bone resorption often leads to prosthesis maladaptation. This resorption varies by individual and site, causing discomfort due to functional loss and insufficient support for denture fabrication. A common solution to this issue is relining the denture².

Relining involves resurfacing the intaglio (tissue-facing) surface of a removable dental prosthesis with new base material to improve its fit on the denture-bearing area³.

A soft liner is a viscoelastic material applied to the fitting surface of dentures provide cushioning effect and distribute occlusal forces evenly across the mucosal tissues. These materials are particularly useful for patients with atrophic ridges, bony undercuts, bruxism, oral defects, xerostomia, natural opposing dentition, and post-maxillofacial surgery obturators⁴.

Resilient liners have been used in dentistry for over a century. Early materials included natural rubber, followed by plasticized polyvinyl resins in 1945 and silicone-based materials in 1958, which offered improved comfort and physical properties⁵.

Soft liners are broadly categorized into silicone-based and acrylic-based materials, each available in auto-polymerizing and heat-cured forms. Acrylic liners typically contain polymer powders and liquid methacrylate monomers with plasticizers. Silicone liners, made of dimethylsiloxane polymers, form elastic rubbers suitable for clinical use. An optimal liner thickness of 2.5–3 mm is recommended for shock absorption⁶.

These materials aid in healing inflamed or distorted mucosa and are used in cases of ridge resorption, mucosal soreness, and knife-edge ridges. Studies, such as by Kawano et al⁷, confirm soft liners cushioning effect in reducing functional impact forces during function.

They must be ideally resilient in long-term use, highly adhesion to the denture base, easy to handle, tastelessness, odourless, visually acceptable, having low water absorption, dimensionally stable, and colour retention⁸. Given that they remain in direct contact with oral tissues, biocompatibility, non-toxicity, and resistance to microbial colonization are critical⁹.

However, soft liners are easily colonized by *Candida albicans*, the most common fungal pathogen in humans, especially with poor hygiene. This can cause denture stomatitis as well as other systemic complications such as aspiration pneumonia in vulnerable patients. Therefore, proper prosthesis hygiene protocol and *Candida* control are very important^{10,11}.

Denture stomatitis, also known as denture sore mouth or candidiasis, is an inflammatory condition beneath dentures, mainly caused by *Candida albicans*. Other species such as *C. krusei*, *C. glabrata*, *C. tropicalis*, and *C. parapsilosis* may also be involved. The prevalence ranges from 25% to 66.7%, particularly affecting the elderly¹².

Etiological factors include fungal infection, mechanical trauma, poor hygiene, continuous denture wear, xerostomia, medications, and nutritional deficiencies. Management typically involves improved hygiene, denture adjustment

with tissue conditioners, and topical antifungal application upon fungal confirmation¹³.

Although short-term liners help with denture adaptation during stomatitis treatment, they can also support *Candida* colonization. In vitro studies suggest that prolonged use may encourage fungal biofilm formation, as salivary pellicles aid adhesion and penetration into the liner material¹⁴.

Douglas et al¹⁵ (1973) proposed incorporating antifungal agents into tissue conditioners, offering prolonged drug action, cost-effectiveness, and enhanced healing. Applying antifungals directly to the mucosa is often impractical due to compliance issues, particularly among institutionalized patients. Hence, embedding antifungal agents into soft liners provides a controlled, sustained release with easier administration.

For example, topical antifungal agents include topical treatments like amphotericin B and nystatin, while systemic azoles are fluconazole, this latter preferred for broad-spectrum efficacy, low toxicity, and good tolerance¹⁶.

Phytomedicine using plant derived products for treatment has come under the attention owing to emerging drug resistance and demand for natural alternatives. *Moringa oleifera* or "tree of life," has exhibited antifungal and anti-inflammatory properties. Its inclusion into denture liner could provide a biocompatible, functional approach to treating denture-related candidiasis¹⁷.

Moringa species include *M. oleifera*, *M. arborea*, *M. rivae*, and others. Phytochemical analysis of *M. oleifera* has identified antimicrobial bioactives such as alkaloids, flavonoids, saponins, tannins, and phenols¹⁸. Traditionally, its bark has been used to treat ailments like ulcers, toothache, and hypertension¹⁹.

These properties indicate the potential of *Moringa oleifera* for use as an antifungal agent, especially against *Candida albicans*. The current in vitro study evaluates the antifungal activity of both *Moringa oleifera* leaf, stem bark powders and fluconazole when incorporated into a soft liner, aiming to offer sustainable, patient-friendly alternatives in prosthetic care²⁰.

Conventional antifungals like fluconazole face rising resistance and recurrence rates, creating a need for plant-based, biocompatible substitutes¹⁶.

Various applications of *Moringa oleifera* have been reported such as antimicrobial, anti-cancer, anti-inflammatory and antioxidant therapies^{18,19,20}. Presently in the literature, there are limited studies conducted to incorporate plant based extract in soft liner to determine anti-fungal efficacy.

This study seeks to assess and compare the antifungal potential of *Moringa oleifera* leaf and bark extracts versus fluconazole, providing insight into novel therapies for denture-induced candidiasis in clinical prosthodontics.

Therefore, this study endeavors to bridge the existing gap by comparatively evaluating the antifungal efficacy of Moringa oleifera leaf and stem bark powders incorporated into a soft denture liner, using fluconazole as the reference standard. The findings may contribute to the development of safe, biocompatible, and plant-derived alternatives for managing denture-induced candidiasis, ultimately enhancing patient comfort and treatment outcomes in prosthodontic care.

II. MATERIALS AND METHODS

➤ Fabrication of Softliner Samples

For the purpose of the study a total number of 40 samples were prepared.

To fabricate the soft liner samples, a standard wax block with dimensions of 10 mm × 10 mm × 2 mm was prepared. This standard block was intended for use in evaluating antifungal efficacy.

- (Control group) To investigate the antifungal action of Moringa oleifera leaf powder, Stem bark powder and fluconazole powder on candida albicans when incorporated in soft tissue liners and compare it with soft liner.

- (Group A) Ten softliners samples (10 mm x 10mm x 2mm) (GC SOFT liner, GC Co., Ltd, Tokyo, Japan) were prepared as per the soft-liner manufacturer's instructions.
- (Group B) Ten softliners samples (10 mm x 10mm x 2mm) incorporated with Moringa oleifera leaf powder 5% w/w were prepared. (Moringa oleifera leaf powder is added to the softliner powder and mixed to get uniform mix then liquid is added and manipulated according to manufacturer's instructions to prepare the samples.)).
- (Group C) Ten softliners samples (10 mm x 10mm x 2mm) incorporated with Moringa oleifera stem bark powder 5% w/w will be prepared. (Moringa oleifera stem bark powder is added to the softliner powder and mixed to get uniform mix and then liquid is added and manipulated according to manufacturer's instructions to prepare the samples.).
- (Group D) Ten softliners samples (10 mm x 10mm x 2mm) incorporated fluconazole powder 2% w/w will be prepared. (Fluconazole powder is added to the softliner powder and mixed to get uniform mix and then liquid is added and manipulated according to manufactures instruction to prepare the samples.)

➤ Grouping of Test Samples:

Table 1 Grouping of Test Samples

TEST PERFORMED	Control group Soft liner	Moringa oleifera leaf powder With soft liner	Moringa oleifera stem bark powder with soft liner	Fluconazole powder With Softliner
ANTIFUNGAL ACTIVITY	Group A N=10	Group B N=10	Group C N=10	Group D N=10

➤ Fabrication of Mould

For the purpose of the study a total number of 40 samples were prepared.

To fabricate the soft-liner samples, a standard wax block with dimensions of 10 mm × 10 mm × 2 mm was prepared. This standard block was intended for use in evaluating antifungal efficacy.

A model wax block (10 mm × 10 mm × 2 mm) (Diagram 1) was created using modelling wax. To ensure standardization across all samples, a mold was fabricated from this model. For duplication, the wax pattern was positioned on a glass slab, with the 10 mm × 2 mm surface facing the glass. It was then boxed with boxing wax, leaving

equal space around all sides. Light body polyvinyl siloxane impression material was injected into the boxed area using a dispensing gun to completely cover the wax block model.

To duplicate the wax samples, the die stone casing was held firmly together and molten wax was poured into the mold space. Once the wax had completely set, the wax block was carefully removed without applying pressure. Using this standardized mold, a total of 40 wax samples were fabricated. The dimensions of all wax samples were verified using a vernier caliper to ensure consistency.

A total of 40 blocks of dimensions 10mm x 10 mm x 2mm as described in ADA specification No.12 will be utilized for antifungal efficacy.

The prepared wax samples were then invested in dental plaster. Petroleum jelly was applied to the flask. Dental plaster was mixed with water in a rubber bowl according to the manufacturer's instructions. The mixed dental plaster was poured into the base of the flask and 3 wax blocks were placed on the surface of the dental plaster and were allowed to set. Once the plaster was set, a cold mold separating medium was applied on the dental plaster and counter flashing was done. A layer of dental stone was brushed upon the samples and the rest of the space was filled with a pour of dental plaster and clamped. Once the dental plaster was completely set, the flask was kept in boiling water for 10 minutes for dewaxing in the dewaxing unit. After 10 minutes the flask was removed, opened and the residual wax was removed by pouring hot water and brush. The test samples were then randomly distributed among different groups.

After the flask had cooled, could mould seal was applied to both the flasked and counter-flasked Then softliner resin was mixed with monomer liquid in a ceramic jar according to the powder-liquid ratio mentioned by the manufacturer. The flasks were packed with the material in liquid consistency. Then, pressed on a hydraulic bench press. Trial packing was done to remove the flash and finally clamps were tightened. Flasks were kept for curing for 15 minutes and left for drying.

The flasks were then opened and samples were retrieved carefully without applying any undue force. The dimensions of each sample were measured again with a vernier caliper to verify the original dimension.

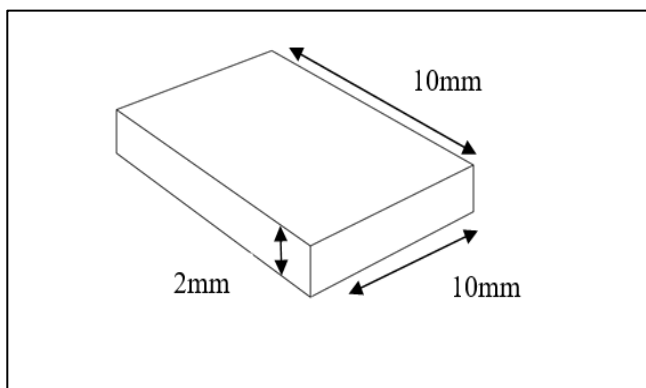


Fig 1 Diagrammatic Presentation of the Sample Dimensions

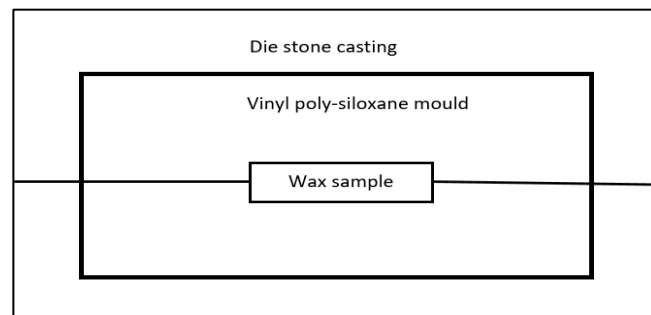


Fig 2 Diagrammatic Representation of Duplicating Mould

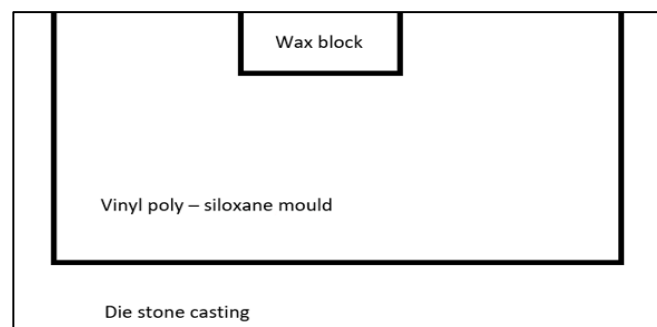


Fig 3 Diagrammatic Representation of Duplicating Mould (Sectional view).

III. RESULTS

The present in vitro study was conducted to evaluate and compare the antifungal effect of *Moringa oleifera* leaf powder, *Moringa oleifera* stem bark powder, and fluconazole incorporated in soft liner against *Candida albicans*. A total of 40 samples were fabricated and equally divided into four groups (n=10). The obtained values of all the 40 samples were subjected to the following statistical test for analysis:

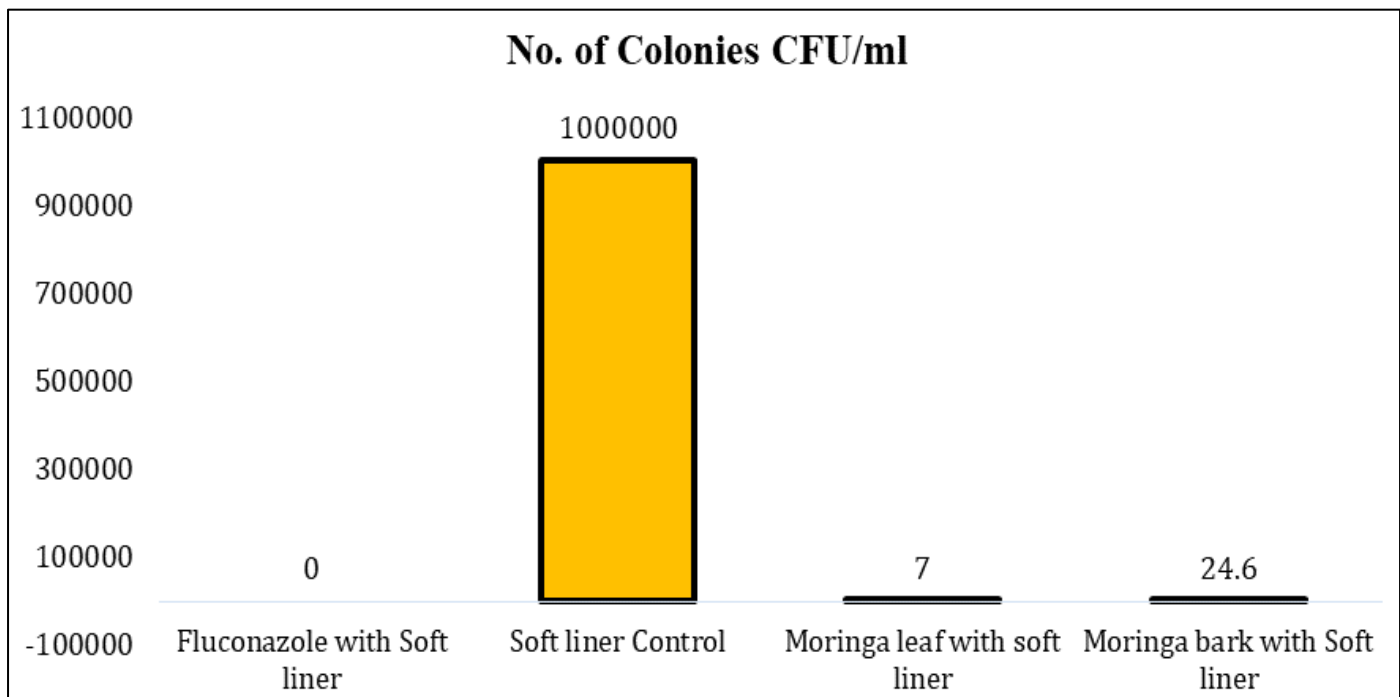
➤ Statistical Analysis Observations of the Study

The present in vitro study was conducted to evaluate and compare the antifungal effect of *Moringa oleifera* leaf powder, *Moringa oleifera* stem bark powder, and fluconazole, each incorporated into soft liner material, against *Candida albicans*.

A total of 40 samples were fabricated and evenly divided into four groups (n=10 per group). The samples were subjected to candidal adhesion testing using colony-forming unit. (CFU/ml) analysis. The obtained results were tabulated, and mean and standard deviation values were calculated for each group.

Table 2 Showing Comparison of No. of CFU/ml Among Control Group (Soft Liner) and Experimental Groups (Fluconazole, Moringa Leaf and Moringa Bark Powder).

Kruskal Wallis ANOVA				
No. of Colonies CFU/ml				
	N	Mean	Std. Deviation	p
2% w/w Fluconazole with Soft liner	10	0.00	0.000	<0.001
Soft liner Control	10	1000000.00	0.000	
5% w/w Moringa leaf with soft liner	10	7.00	5.033	
5% w/w Moringa bark with Soft liner	10	24.60	7.876	



Graph 1 Illustrates the mean number of *Candida* colonies (CFU/ml) across the following groups: Control Group (Soft liner only), experimental group (Moringa leaf powder incorporated into soft liner, Moringa stem bark powder incorporated into soft liner, Fluconazole incorporated into soft liner). The Fluconazole group showed complete inhibition of fungal growth (0 CFU/ml), demonstrating the highest antifungal efficacy. In contrast, the Control group exhibited the highest colony count (1,000,000 CFU/ml). Among the Moringa groups, the Moringa leaf group showed better antifungal activity (mean = 7.00 CFU/ml) compared to the Moringa bark group which had a mean of 24.60 CFU/ml.

IV. DISCUSSION

Treatment of edentulous individuals with complete dentures not only rehabilitates them functionally, but also esthetically and psychologically. Prosthetic rehabilitation of the aged and handicapped has been a great concern; the difficulties that arise may not be attributed to denture construction but also to associated problems with continuous denture wearing. The most commonly used material for fabricating removable partial and complete dentures is acrylic resin^{1,2}. In some patients with atrophic ridge, bony undercuts, bruxism, congenital or acquired oral defects, xerostomia, denture opposing natural teeth, traumatic ulceration and for obturators after maxillofacial surgery denture soft lining materials are used by coating over permanent or semi-permanent bases.^{1-3,5,9}

Direct soft lining material have been widely used, because they are economical, easy to manipulate and comfortable to the patients, but they have some disadvantages such as water sorption and surface roughness.⁵

The risk of in-growth of microorganisms is commonly seen in persons whose denture hygiene is poor.^{3,4} The adherence of microorganisms to polymers, such as denture acrylic resin and soft lining materials, is the first step in colonization and development of infection.^{5,9,13,17} The soft liner denture materials act as a reservoir for different microorganisms because of their surface texture and the physical/chemical affinity.^{5,10}

Gruber et al⁵⁹ in 1966 have indicated that tissue conditioners promoted or supported candida growth and/or colonization in vivo or in vitro. Furthermore, Allison RT et al²² in 1973 have clearly shown that *Candida albicans* deeply penetrated soft-lining materials. These materials supported mycelial growth, which is widely known as one of the most important virulence factors of this fungus.

Candida albicans, a dimorphic fungus and normal commensal of the human oral cavity, can transition into a pathogenic state under favorable conditions, such as the presence of a warm, moist, and nutrient-rich environment like a denture base. Soft liners, with their increased water absorption and lack of antimicrobial defenses, become ideal substrates for *Candida* adhesion, proliferation, and biofilm formation. Once established, the biofilms are highly resistant to routine denture cleansing methods and antifungal agents, making management of denture stomatitis more complex. Moreover, persistent candidal colonization can result in inflammatory responses, burning sensations, erythema, and discomfort, ultimately affecting the patient's oral health-related quality of life. A commonly occurring condition in denture wearers is denture stomatitis secondary to Candidal infection. *Candida* among all fungal infection acts as main reason for oral mycosis and plays an important role in denture stomatitis.^{4,5,7,10,12,14,}

The conventional treatment strategy for denture stomatitis often includes topical or systemic antifungal medications such as nystatin, miconazole, and fluconazole. However, long-term use of systemic antifungals may lead to

undesirable side effects such as hepatotoxicity, gastrointestinal issues, and drug resistance. Additionally, topical formulations, although safer, tend to have short intraoral retention times and require patient compliance for multiple daily applications. These limitations have sparked interest in modifying denture base materials and soft liners with antimicrobial agents, allowing for localized and sustained antifungal action directly at the site of infection.

Fluconazole, a triazole antifungal agent, has emerged as a gold standard due to its potent fungistatic and fungicidal activity against *Candida albicans*, good bioavailability, and minimal side effects. When incorporated into denture materials, fluconazole has shown promising results in preventing fungal colonization and maintaining drug release over extended periods.

On the other hand, there has been a rising interest in plant-based antifungal agents, particularly those with a broad antimicrobial spectrum and favorable biocompatibility profiles. One such promising natural agent is *Moringa oleifera*, commonly known as the drumstick tree. Traditionally used in various cultures for its nutritional and medicinal properties, *Moringa* has demonstrated antibacterial, antiviral, antifungal, and antioxidant effects, attributed to its rich phytochemical content including flavonoids, alkaloids, tannins, terpenoids, and saponins. Various parts of the plant such as leaves, seeds, bark, and flowers have been evaluated for their antimicrobial efficacy, with the leaf extract often showing superior bioactivity due to a higher concentration of bioactive compounds.

The rationale for selecting *Moringa* in this study stems from its natural origin, low toxicity, affordability, and potential as an alternative antifungal agent, particularly in populations with limited access to conventional medications. Moreover, incorporating *Moringa* powder directly into a soft liner could potentially enable localized drug delivery, reduce fungal load, and minimize systemic side effects thus addressing both the microbial and functional limitations of existing liner materials.

The present study was thus conducted with the objective of comparing the antifungal efficacy of fluconazole, *Moringa oleifera* leaf powder, and *Moringa oleifera* stem bark powder when each was incorporated into a commercially available soft liner material, and their effects were tested in vitro against *Candida albicans*. The colony-forming units (CFU/mL) obtained from each group were counted to evaluate and compare the ability of these materials to inhibit fungal colonization or fungal adherence. The data obtained not only confirmed the antifungal potential of the test agents but also offered valuable insights into the relative effectiveness of synthetic and natural antifungal compounds when embedded within dental prosthetic materials.

The results confirm that fluconazole-incorporated liners exhibited complete inhibition of *Candida albicans* (0 CFU/mL), followed by *Moringa* leaf (mean CFU 7.00) and *Moringa* bark (mean CFU 24.60). The control group showed the highest fungal growth (1,000,000 CFU/mL), confirming

the susceptibility of unmodified soft liners to fungal adhesion and colonization.

Rogers et al²⁵ in 1986 highlighted that fluconazole exhibited 20 times greater in vivo efficacy against *C. albicans* compared to ketoconazole in systemic infections. Falah-Tafti et al³² in 2010 demonstrated that 10% fluconazole incorporated into tissue conditioners was sufficient to completely inhibit *Candida* colonization. Chopde et al³³ in 2012 compared fluconazole, miconazole, and nystatin, concluding that fluconazole consistently provided the highest inhibition zones when mixed with soft liners. Dorocka et al³⁰ in 2007 similarly reported fluconazole's efficacy against 88.7% of *C. albicans* clinical isolates, reinforcing its use as a gold standard in antifungal delivery via denture liners.

Moringa oleifera, a plant known for its nutritional and medicinal properties, has gained interest in recent years for its antimicrobial potential. In this study, *Moringa* leaf powder demonstrated greater antifungal activity than stem bark powder. This finding aligns with Rehman et al³¹ in 2008, who found that ethyl acetate extracts of *Moringa* bark exhibited moderate antifungal activity against *C. albicans*, though less potent than other plant parts. Ahmadu et al⁴⁶ in 2021 reported strong antifungal efficacy of methanolic leaf extracts of *Moringa* against *Botrytis cinerea*, highlighting its phytochemical strength. Santos et al⁴⁹ evaluated the antifungal properties of *Moringa* seed lectins and found effective MICs and MFCs against *Candida* strains, including *C. albicans*.

Isitua et al³⁹ confirmed the antifungal action of *Moringa* leaf extracts against a broad spectrum of fungi, including *Candida albicans*, noting stronger potency in ethanolic preparations. Gani et al⁵³ demonstrated that *Moringa* leaf extracts induced metabolic stress in *C. albicans*, inhibiting its growth and biofilm-forming capacity across 24, 48, and 72-hour intervals. Sulistyani et al⁵⁵ determined the minimum inhibitory concentration (MIC) for *Moringa* leaf extract to be 6.25%, with biofilm inhibition observed at even lower concentrations of 3.13%, making it effective at sub-lethal levels.

Comparative analysis of *Moringa* leaf and bark in this study also reflects previous findings. Nweke et al³⁷ reported that *Moringa* leaf extracts were more potent than stem bark extracts due to higher concentrations of flavonoids, tannins, and alkaloids. Al-Khalasi et al⁵² echoed similar conclusions, observing the highest antifungal activity from *Moringa peregrina* leaf extract when compared with seed and root extracts, attributed to its phytochemical density.

Patel et al³⁶ highlighted that *Moringa* leaf extracts contain bioactive agents such as terpenoids, glycosides, and saponins, which offer broad antifungal effects though their action against *C. albicans* was modest in aqueous extracts. Ningsih A W et al⁴⁸ found that ethanol extracts showed weak but measurable zones of inhibition against *C. albicans*, whereas decoctions showed no antifungal effect, emphasizing the importance of extraction method in therapeutic efficacy.

Plant-based antifungal agents have also shown promise when incorporated into soft liners. Khan M A et al³⁸ found that thyme essential oil and *Nigella sativa* demonstrated antifungal effects against *C. albicans* adhered to soft liners. Rawat P et al⁴² found that fluconazole-incorporated Viscogel showed the highest antifungal activity, but natural agents preserved the material's viscoelasticity better over seven days. Bueno et al⁴¹ observed that adding antifungals such as ketoconazole or nystatin to temporary soft liners significantly reduced *Candida* biofilm without compromising mechanical properties.

The underlying cause of denture stomatitis is well supported in literature. Davenport et al²¹ showed that microbial colonization on dentures is the root cause, and interventions should be directed toward the denture base. Allison RT et al²² visually confirmed colonization by *Candida albicans* hyphae on soft liner surfaces. Samaranayake et al²³ LP in 1980 emphasized that surface roughness and intraoral factors influence *Candida* adhesion. Wright et al²⁴ and Okita et al²⁶ both reported higher *C. albicans* colonization in soft liners compared to conventional acrylic bases.

The significance of modifying soft liners to enhance resistance to fungal adhesion has been reinforced by Chow et al²⁸, who successfully incorporated antifungals into various tissue conditioners. Krishnamurthy S et al⁴⁰ emphasized that smoother surfaces, possibly resulting from antifungal incorporation, tend to harbor fewer fungal cells. Khadka et al⁴³ and Gauch et al⁴⁴ further confirmed that *C. albicans* is the predominant species responsible for denture-associated candidiasis and remains highly susceptible to antifungal agents like fluconazole.

Recent studies also emphasize the innovation of using Moringa-based nanoparticles. Mondal A et al⁵⁰ in 2022 demonstrated that silver and gold nanoparticles synthesized using Moringa leaf, bark, and flower possessed excellent antifungal and insecticidal properties. Mahmoud S et al⁵¹ in 2022 reported that Moringa oil had comparable antifungal effects to tea tree oil, outperforming grape seed oil against *Candida albicans*.

Thus, from the present study and corroborating literature, it is evident that while fluconazole remains the most potent antifungal agent, Moringa oleifera, particularly the leaf extract, demonstrates considerable antifungal efficacy. Its natural origin, low toxicity, cost-effectiveness, and ease of incorporation into dental materials make it an attractive adjunct or alternative for managing fungal infections associated with denture use.

However, since this was an in vitro study, real-world oral conditions such as salivary flow, pH changes, and mechanical wear were not simulated. Therefore, further in vivo studies and long-term assessments are essential to evaluate mechanical integrity, release kinetics, and clinical effectiveness of Moringa oleifera-based antifungal liners.



Fig 4 Samples for Antifungal Testing (A) Softliner (Control), (B) Soft Liner Incorporated with Moringa Oleifera Leaf, (C) Soft Liner Incorporated with Moringa Oleifera Bark and (D) softliner Incorporated with Fluconazole.

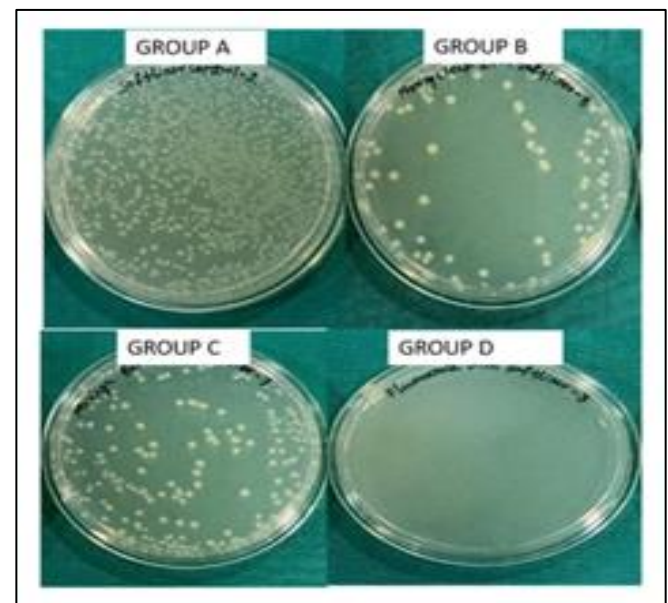


Fig 5 Culturing of Candida Albicans Obtained from Incubation Samples for Colony Forming Unit

V. CONCLUSION

The present in vitro study was carried out to comparatively evaluate the antifungal activity of 5% w/w Moringa oleifera leaf, 5% w/w stem bark powder, and 2% w/w fluconazole, when incorporated into a soft liner material. Based on the analysis of results obtained and within the limitations of this study, the following conclusions were drawn:

- Incorporation of antifungal agents into the soft liner significantly reduced *Candida albicans* colony-forming units (CFUs) compared with the control group (soft liner without antifungal agent).
- Among all tested groups, fluconazole incorporated soft liner exhibited the highest antifungal activity against *Candida albicans*, showing the maximum reduction in CFUs.

- Moringa oleifera leaf powder incorporated into the soft liner also demonstrated effective antifungal activity, though slightly less than fluconazole.
- Moringa oleifera stem bark powder showed a significant antifungal effect, but was less potent compared to both fluconazole and Moringa oleifera leaf powder.
- The antifungal efficacy followed the order: Fluconazole > Moringa oleifera Leaf Powder > Moringa oleifera Stem Bark Powder > Control group.

From the above observations, it can be inferred that incorporation of Moringa oleifera particularly the leaf powder incorporated into soft liner materials offers a promising natural alternative with notable antifungal properties against *Candida albicans*. However, further research is required to assess the long-term mechanical, physical, and clinical performance of these modified soft liners through in vivo studies.

VI. SUMMARY

Soft liners are frequently used in prosthodontics to improve the fit and comfort of removable dentures, particularly in patients with compromised mucosa. However, their porous and hydrophilic nature makes them highly susceptible to colonization by *Candida albicans*, leading to denture-induced stomatitis. While antifungal agents like fluconazole are effective, the emergence of drug resistance and potential systemic side effects has prompted interest in natural alternatives with fewer adverse effects.

Moringa oleifera, a plant known for its wide range of pharmacological properties including antifungal, antibacterial, and antioxidant activities offers potential as a natural antifungal agent when incorporated into dental materials.

This in vitro study aimed to evaluate and compare the antifungal efficacy of Moringa oleifera leaf powder, stem bark powder, and fluconazole when incorporated into a tissue conditioner (soft liner) against *Candida albicans*. A total of 40 standardized soft liner samples (10 mm × 10 mm × 2 mm) were fabricated and divided into four groups (n=10):

- Group A: Soft liner only (Control)
- Group B: Soft liner with 5% w/w Moringa oleifera leaf Powder
- Group C: Soft liner with 5% w/w Moringa oleifera stem Bark Powder
- Group D: Soft liner with 2% w/w fluconazole

All groups were exposed to a standardized inoculum of *Candida albicans* (1.7×10^7 CFU/mL), and antifungal activity was assessed by colony-forming unit (CFU/mL) enumeration after 48 hours of incubation. Statistical analysis was performed using the Kruskal–Wallis test and Mann–Whitney U test.

The results showed a statistically significant difference in antifungal efficacy among the groups ($p < 0.001$). Group D

(fluconazole) exhibited complete inhibition of fungal activity (0 CFU/mL). Group B (Moringa leaf showed substantial antifungal activity with a mean CFU of 7.00, while Group C (Moringa bark) demonstrated a moderate effect with a mean CFU of 24.60. Group A (control) showed the highest fungal growth (1,000,000 CFU/mL), confirming the susceptibility of soft liners to fungal colonization.

The antifungal efficacy followed the order: Fluconazole > Moringa leaf > Moringa bark > Control group.

Within the limitations of this in vitro study, it can be concluded that both Moringa oleifera leaf and stem bark powders significantly reduce *Candida albicans* adherence when incorporated into soft liners. Among the two, the leaf powder was more effective. Although fluconazole remains the most potent antifungal agent, Moringa oleifera particularly its leaf form shows promising potential as a natural and biocompatible alternative in managing denture-related fungal infections.

Further in vivo investigations are recommended to validate the long-term clinical applicability, mechanical compatibility, and biocompatibility of these natural antifungal-modified soft liners.

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