"COMPARATIVE EVALUATION OF ANTIFUNGAL EFFICACY AND POTENCY OF SOFT-LINERS INCORPORATED WITH POWDER EXTRACTS OF LAWSONIA INERMIS AND WITHANIA SOMNIFERA ON THE GROWTH OF CANDIDA ALBICANS – AN IN VITRO STUDY."

By

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Submitted to the

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In partial fulfillment of the requirements for the degree of

MASTER OF DENTAL SURGERY

In

PROSTHODONTICS AND CROWN & BRIDGE (BRANCH – I)

Under the Guidance of

Dr. HEMA KANATHILA M.D.S

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2019 - 2022

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Dedicated

To

My family

And

My guide

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

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

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"The hand that rocks the cradle may not rule the world, but it certainly makes it a better place"

I owe every success to them and I humbly acknowledge that everything I am today is because they loved me.

Thank you, one and all

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LIST OF ABBREVIATIONS USED IN THE STUDY

ABBREVATIONS	FULL FORMS
C. albicans	*Candida albicans*
SE	Standard error
SD	Standard Deviation
Mm	Millimeter
mL	Milliliter
µL	Microliter
µg	Microgram
hrs.	Hours
Mins	Minutes
°C	Degree Centigrade
%	Percentage
SPSS	Statistical package for social science
DIZ	Diameter of Inhibition Zone
MDR	Multi-Drug Resistant
mm^3	Cubic millimeter
CFU	Colony Forming Unit
G	Gram

ABSTRACT

STATEMENT OF PROBLEM:

Denture induced candidiasis is mainly associated with infection caused by *Candida* species. Application of topical antifungal agents has become a challenge for geriatric denture- wearers due to their reduced motor activity and loss of memory. Topical and systematic antifungal therapy requires patient compliance. Thus, result or the outcome cannot be determined. Denture soft liners are used in conditioning the exploited mucosa due to improper fitting of the denture. Antifungal medicaments are integrated into the soft liner thereby helping the patients in the treatment of Oral Candidiasis. Continuous use of synthetic antifungal agents has resulted in harmful effects on the patients like liver and kidney toxicity and also have disadvantages like drug resistance. Excessive use of these synthetic drugs has increased the emergence of multi-drug resistance (MDR) strains of micro-organisms. Therefore, the use of natural and herbal drugs have come into the role with less adverse effects. The natural powder extract of *Lawsonia inermis* and *Withania Somnifera* have been proven to be effective against Candida *albicans.*

AIM:

To evaluate and compare the antifungal efficacy and potency of denture soft-liners incorporated with leaves powder extract of Lawsonia inermis linn. (lythraceae) and root powder extract of Withania somnifera dunal. (solanaceae) on the growth of candida albicans.

MATERIALS AND METHODS:

A total of 105 samples were taken and was divided into three groups: to check for the antifungal activity with 35 samples each. Lawsonia inermis and Withania somnifera powder extract were incorporated in soft liner at 1%, 3%, 5%, and 7% concentration and the antifungal efficacy against *Candida albicans* was tested by calculating the diameter of inhibition zone (DIZ) for 1, 7 and 14 days. The antifungal efficacy of control group (soft liner without the powder extract) was also evaluated.

RESULTS:

With the increase in the concentration of Lawsonia inermis and Withania somnifera, antifungal activity increases, but with the increase in day point time, antifungal activity decreased in each group. Both the powder extract showed antifungal efficacy against Candida albicans. Ashwagandha root powder extract exhibited higher zone of inhibition when compared with henna leaves powder extract at all the concentration.

When comparison of four sub groups (1%, 3%, 5% and 7%) were done with the mean zone of antifungal efficacy at 1^{st} day, 7^{th} day and 14^{th} day time points in both the groups by one way ANOVA, statistically significant results were obtained with p- value of 0.0001. Also, when Pair wise comparison was done for four sub groups (1%, 3%, 5% and 7%) with mean zone of antifungal efficacy at 1st day, 7th day and 14th day time points in Group B and Group C by Tukeys multiple posthoc procedures, significant results (p value of 0.0001) were obtained.

CONCLUSIONS

These natural herb extracts have shown to have antifungal activity against *Candida albicans* and can be used as an alternative for the synthetic antifungal drugs. Thus, incorporation of these powder extracts into the denture soft liner could prove to be beneficial to improve oral health status of the geriatric patients with cognitive disturbances, medically compromised conditions and reduced manual dexterity.

KEY WORDS: Denture soft liner, *Candida albicans, Lawsonia inermis, Withania somnifera*, antifungal activity.

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INTRODUCTION:

The edentulous patient wearing complete denture may need refitting of complete denture after some years of service. For relining procedure, denture relining material is frequently used in dental clinics. Denture relining materials are soft and resilient material used in the treatment of patients with denture soreness and also use in the field of "Maxillofacial prosthetics".[1] Their success in handling such instances has been allocated to their intrinsic elasticity and viscoelastic property that allow them to prevent the distribution of pressure over the denture supporting area and also impart a cushioning effect under the load of mastication. Many clinical trials that use resilient liners with denture in edentulous patients show greater comfort, improved speech, reduce pain and soreness, superior ability to chew and masticate, enhanced retention and stability and rise in psychological consolation.[2]

Denture Liners can be interim resilient liners or long-term resilient liners. Tissue conditioners are interim or short-term denture liners which can be used in the treatment of denture soreness. This material temporarily relines ill-fitting prosthesis and facilitate tissue repair without abandoning the use of denture.[3] Plasticizer leaches out quickly over time, causing the material to stiffen and lose its cushioning function within a few days to a week. Hence, ideally After 3-4 days, a tissue conditioner should be replaced.[4]

Although long term resilient liners are used to avert soreness of denture and also advocated as a precautionary measure in edentulous subject with Atwood type IV ridges, patients with thin atrophied mucosa showing less tolerance to stresses, subjects

confronting pain at nerve fiber ending and in patients where prosthesis exhibits less retention leading to recurrent sore spots below the denture.[5]

Depending on the composition, long term denture soft liners have been classified into two groups --- Plasticized acrylic denture liner and Silicon elastomers denture liner. Both can be either self-polymerised (auto-cured) or heat cured. Heat cure silicon elastomeric liners are available as a single paste system whereas auto cured silicon elastomeric liners are available in the form of a two-paste cartridge - base paste and liquid catalyst. Unlike the case of acrylic based liners, plasticizers are not required to produce the softening effect. Also, hydrophobicity is a potential drawback of silicon-based elastomeric liners. Plasticized acrylics liner is available as a polymer and a liquid monomer. The powder usually consists of 'poly ethyl methacrylate' or 'poly butyl methacrylate' together with peroxide initiator. The monomer of self-polymerized acrylic resilient liners comprises '2 ethylhexyl methacrylate', 'tertiary amine' and plasticizer. The monomer of heat-cure acrylic liner is a blend of 'methyl methacrylate' and plasticizer.[6]

In a study by Murata et al, masticatory function was improved in the following order: acrylic based permanent "soft denture liner" > silicone elastomeric permanent denture liner > interim denture liner > denture resin. Thus, plasticized acrylics may best match the demand of a resilient denture liner but they significantly lag behind silicon elastomers in terms of durability due to the presence of leachable plasticizers. Murata et al. also found that plasticizers which are existing in acrylic based denture liner impart flexibility and softness. But as they are not linked with the resin mass led to decline in the surface and mechanical properties.[7]

Mante et al. in his study concluded that the use of surface sealants can prevent the rise in the hardness value of the acrylic-based denture liner for longer time and hence provide durability to the material.[8] Studies have concluded that rugged surface of the liners allows the sticking of the microorganisms and favour fungal growth. Kang et al. found that 'acrylic based denture soft liner' showed a greater adhesion to *Candida albicans* when compared with silicon-based denture liners. *Candida albicans,* primarily adhere to intaglio surface of liner and later give rise to a condition called denture induced stomatitis.[9]

So, there is need to bring about an alteration in the relining material with antifungal agent. Keeping this background in mind a study is planned to evaluate antifungal property acrylic based soft relining material.

NEED FOR THE STUDY:

'Candida albicans' is a common pathognomic opportunistic species of oral microflora not only responsible for symptomless inflammation of underlying mucosa but also responsible for deep tissue infection which can reach the bloodstream.[10] Denture induced stomatitis is nothing but an inflammation of the mucosa below the prosthesis and its prevalence advances with age.[11]

In the last 20 years, a high incidence rate of fungal infection was illustrated not only in people with poor immunity but also in normal healthy population. Microbes are seen mainly in the human oral fissure as it provides the best habitat for its growth. A removable dental acrylic prosthesis with denture liners is the commonest treatment modality in prosthetic specialty.[12]

Acrylic resin materials are considered as a gold standard for the fabrication of dental prosthesis and temporary denture soft liners provide tissue conditioning which will enhance the fit of dentures.[13] However, problem with their inherent rough surface and long-term use of these material will lead to more plaque accumulation and subsequent microbial adhesion and colonisation. These microorganisms can be removed with the help of chemical, mechanical or by using ultraviolet light. But these methods may cause surface degradation and deformation of soft liner.[14]

Denture stomatitis is otherwise familiar as denture sore mouth, prosthetic stomatitis, inflammatory papillary hyperplasia, chronic denture palatitis, chronic atrophic candidiasis, stomatitis prothetica, and denture related stomatitis.[15]

Risk factors like hormonal imbalance, oral contraceptive use, metabolic disorder, sex, age and poor oral hygiene may lead a person to local or systemic

Candida albicans infection. Surface porosity, low saliva ph, carbohydrate rich diet accelerates adhesion and colonization of '*Candida albicans*' which furthermore contribute to denture stomatitis.[16] Several researchers had documented that the cause of denture stomatitis is multifactorial such as lack of oral hygiene status, denture base allergy, existing fungal infection, trauma from occlusion, dietary deficiency and haematological disorders.[17]

Conventional treatment options for denture stomatitis include rigorous oral hygiene and systemic and topical applications of anti-fungal agents. Topical application of anti-fungal agent is not being encouraged as the medication gets washed away by the saliva leaving an insufficient concentration at the site of action and systemic administration requires large doses of drugs which have serious risk of side effects.[18] Although, systemic intake of antifungal medications may be effective against Oro-mucosal lesions, but they have been proven less effective against candida-infested denture fitting surface.[19]

In order to minimize the problem of colonization, many products have been tested such as synthetic antimicrobials, but these drugs have a disadvantage like increased multidrug resistance strains. Recently, more attention has been directed towards the incorporation of novel antimicrobials agents.[20] Thus, the role of a prosthodontist comes into picture as this is a important denture related problem which should be treated carefully for the successful use of the prosthesis. Hence, to obtain better results against candidiasis, active pharmaceutical ingredients like nystatin, amoxicillin and natural antifungal drugs are integrated into the denture soft liners to prevent oral candidiasis.[21] Antifungal drugs used can be categorized into two groups: natural and synthetic origins. Synthetic drugs are Nystatin, Ketoconazole,

Amphotericin B, Miconazole, Fluconazole, Chlorhexidine, Clotrimazole, Itraconazole. Natural agents like origanum oil, Thai herbs, *C.nutans, C. sappan Linn,* lemongrass oil, seed oils like *Ocimum sanctum Linn, Linum usitatissimum.* Many inorganic antifungal agents are also used like silver zeolite, photocatalyst, silver nanoparticles, magnesium oxide.[22] Synthetic drugs have been revealed to have many disadvantages and side effects like rise in the appearance of resistance to different drugs, many systemic complications etc. [23]

So, in this study powder of '*Lawsonia inermis'* and '*Withania somnifera'* was incorporated in soft liner and checked for its antifungal efficacy against the growth of '*Candida albicans'*, which can be beneficial for patients with denture stomatitis. *sonia inermis* leaves are safe without any health effect. Kirkland and Marzin have concluded that *Lawsonia inermis* has no genotoxic consequences and this information was also observed by the study of Yusufetal.[24] In the year 2010, phytopharmaceutical study stated that Henna has 'analgesic, hypoglycemia, hepatoprotective, immuno-stimulant, anti-inflammatory, antibacterial, anti-bacterial, anti-microbial, antimicrobial, antiviral, anti-parasitic, antidermatophytic, tuberculostatic and anticancer properties'.[25] Morever, another studies also concluded that *lawsonia inermis* leaves have antimicrobial and anti-candidial effects.[26]

All of the parts of *Withania somnifera* such as leaves, root and fruit provide potential health benefits for human well-being because of the adequate amount of antioxidants and polyphenols.[27] Studies have suggested that *Withania somnifera* in particular solution can reduce the spread of candidial infection.[28]

Therefore, this study is chosen to evaluate the antifungal effectiveness of soft liner when incorporated with powder extract of herbal plants.

HYPOTHESIS:

NULL HYPOTHESIS:

- "There will be no change in the antifungal efficacy and potency of denture soft-liners incorporated with powder extract of '*Lawsonia inermis'* and '*Withania somnifera'* on the growth of *Candida albicans.*"

ALTERNATIVE HYPOTHESIS:

- "There will be change in the antifungal efficacy and potency of denture soft-liners incorporated with powder extract of '*Lawsonia inermis'* and '*Withania somnifera'* on the growth of *Candida albicans*."

AIMS AND OBJECTIVES:

AIM OF THE STUDY:

- "To evaluate and compare the antifungal efficacy and potency of denture soft-liners incorporated with leaves powder extract of '*Lawsonia inermis linn.'* (lythraceae) and root powder extract of '*Withania somnifera dunal*.' (solanaceae) on the growth of *Candida albicans.*"

OBJECTIVES:

- "To evaluate antifungal efficacy and potency of soft-liners incorporated with powder extract of *Lawsonia inermis* (Henna) leaves at different concentrations on first, seventh, and fourteenth day against the growth of *Candida albicans.*"
- "To evaluate antifungal efficacy and potency of soft-liners incorporated with powder extract of *Withania somnifera* (Ashwagandha) root at different concentrations on first, seventh, and fourteenth day against the growth of *Candida albicans.*"
- "To compare the antifungal efficacy and potency of soft-liners incorporated with powder extract of *Lawsonia inermis* (Henna) leaves and *Withania somnifera* (Ashwagandha) root at different concentrations on first, seventh, and fourteenth day against the growth of *Candida albicans.*"

REVIEW OF THE LITERATURE:

1. **Falah-Tafti A et al (2010)** conducted an in-vitro study to investigate the antifungal activity of nystatin and fluconazole in combination with tissue conditioner against Candida albicans. They concluded that tissue conditioner with 10% fluconazole or 1% to 10% nystatin can thoroughly restrict the bond and colonization of *Candida albicans*.[29]
2. **Hema Kanathila et al (2011)** conducted an in-vitro investigation to see if magnesium oxide has antifungal action when paired with two tissue conditioners (Viscogel and GC Soft) to limit Candida albicans development. Magnesium oxide integrated in tissue conditioners were shown to be an effective alternative against Candida albicans development in the study. When compared to Viscogel with magnesium oxide, GC "soft denture liner" with magnesium oxide produced better results.[30]
3. **Srivatstava A et al (2013)** conducted an in-vitro study to examine the antifungal activity and properties of a tissue conditioner (visco gel) by integrating origanum oil. They concluded that, origanum oil can be used as a antifungal agent in tissue conditioner to decrease the bonding of *Candida albicans* by not remarkably affecting its bond strength to heat-polymerized acrylic resin.[31]
4. **Singla S1 et al (2013)** conducted in-vitro research to test the fungicidal activities of *Punica granatum* peel extract and *Henna* leaves (Lawsonia inermis) in various solvents, such as ethanol, methanol, and chloroform, at varying concentrations, such as 25%, 50%, and 75%, against pure Candida albicans colonies. His research found that extracts of Punica granatum peel and

Lawsonia inermis leaves inhibit Candida albicans in considerable zones, and hence may be utilised as a cost-effective option for treating candidiasis.[32]

5. **Pokpong Amornvit et al (2014)** conducted an in-vitro study to check the inhibitory efficacy of *lemon grass* when integrated with COE-COMFORT tissue conditioner opposing *Candida albicans.* They concluded that oil of *lemon grass oil* have evidently exhibited an impressive anti-Candida effect.[33]
6. **Mansourian A et al (2014)** conducted an in-vitro study to assess the antifungal activity of herbal extract of *Syzygium aromaticum* and *Punica granatum* and also nystatin on development of *Candida albicans*. They concluded that both the herbal extract showed higher antifungal efficacy than nystatin with a zone of inhibition of 29.62mm for *Syzygium aromaticum* and 28.48mm for *Punica granatum.*[34]
7. **Shyamapada Mandal and Manisha Mandak (2015)** published a review on *Coriander (Coriandrum sativum) oil* in which they concluded that Coriandrum sativum essential oil exhibited antifungal property and can be used as a treatment for oral disorders such as denture candidiasis.[35]
8. **Koteswara Rao Pachva et al (2015)** conducted an in-vitro study to examine whether the denture soft liners integrated with *tea tree oil* would prevent the development of *Candida albicans.* He concluded that the CFU/mm2 for samples without the oil increased and for samples with tea tree oil CFU decreased after 1, 30 and 60 days. The disks with the oil were successful in preventing the maturation of C. albicans till 60 days following water storage. Thus, they concluded that integrating tea tree oil into soft liner remarkably decreased the maturation of C. albicans therefore suggested that tea tree oil is an effective antifungal agent for denture stomatitis.[36]

9. **Amal Nawasrah (2016)** conducted an in-vitro investigation to assess the impact of henna against *Candida albicans* adhered to acrylic resin block as a potential alternative strategy for preventing denture-related stomatitis. They used varying concentrations of henna powder in the denture acrylic polymer, with henna:polymer ratios of 1 percent, 2.5 percent, 5%, 7.5 percent, and 10%, respectively. His study concluded that the difference in the number of live *Candida albicans* were significant with the increase in the concentration of henna powder.[37]
10. **Fereshteh Javadian et al (2016)** conducted an in-vitro study in which the extracts of 'Withania somnifera' were tested for antifungal efficacy against Candida albicans. According to the findings, the minimum inhibitory concentration (MIC) of ashwagandha necessary to suppress Candida albicans growth is 50 ppm to 250 ppm.[38]
11. **Aparna H Gopal Krishna et al (2016)** conducted an in vitro study to examine the antifungal efficacy of *Ocimum sanctum* and *Centratherum anthelminticum seed oils* against different candidal strains and evaluated the synergistic activity of the test oil. This study concluded that both the seed oils had a good antifungal activity and by increasing the oil concentration, Centratherum anthelminticum seed oil exhibited more antifungal activity when compared with Ocimum sativum oil. Maximum ZOI was seen with Centratherum anthelminticum of 75.7mm for *Candida albicans* and less with Candida dubliniensis with a DIZ of 45.7mm.[39]
12. **Madeira PL et al (2016)** conducted an in-vitro study to check the activity of *Lemongrass Extract* (LGE) on '*Candida albicans biofilms'*, 'Human cells viability' and on 'denture surface'. The MIC of LGE needed to inhibit C.

albicans growth was 0.625 mg/mL. The existence of LGE during biofilm growth resulted in a decrease of cell counts, which built the MIC adequate enough to 90% of cells. The exposure of Lemongrass extract after biofilm progression also had remarkable antifungal effect at all concentrations. It concluded that effective minimization in C. albicans was seen when the dentures were immersed in LGE.[40]

13. **Sushma Krishnamurthy et al (2016)** conducted an in-vitro study to analyze the retentivity, colonization and invasion of the 4 denture liners specifically Ufi Gel Hard C, Molloplast B, GC Soft Liner and Permaflex by *Candida albicans*. The study exhibited higher confinement of C. albicans on rough than on smooth surfaces. When comparing between the smooth surfaces, it was seen that Molloplast B exhibited higher retention and GC Soft Liner exhibited lowest confinement of C. albicans. But when comparing between the rough surfaces, the difference in the retention of C. albicans was not remarkably significant. Permaflex and Molloplast B exhibited a mean inhibition zone of 14.80±3.8mm and 16.9±4.8mm respectively thus concluding that smoother surfaces retained very few cells than irregular surfaces and denture lining materials have no anti-fungal properties.[41]
14. **F. M. Lima et al (2016)** conducted an in-vitro study to check the MIC, solubility and sorption of denture soft liner with antifungal. The analysis found that when comparing the Chlorhexidine to the control group at time interval of fourteen days, there was a rise in water sorption in the Chlorhexidine group. For all time intervals, the solubility increased with the incorporation of Chlorhexidine and Ketoconazole when compared with the control group. They concluded that, the inclusion of Nystatin and Ketoconazole in both Trusoft and

Softone and inclusion of Chlorhexidine in Trusoft had no influence on Water sorption after 2 weeks. The solubility of these 2 materials wasn't affected by the inclusion nystatin for upto 14 days.[42]

15. **Iqbal Z et al (2016)** conducted a review to look into the present state of information on integration of antifungals into the denture soft tissue lining materials for a better treatment of denture induced stomatitis. The Google-Scholar, PubMed/MEDLINE and ISI web science databases were used in the study. Study reported that integration of antifungal medications into tissue conditioners is effectual with very less effects on the properties of the conditioners. Thus, they stated that incorporation of various antifungal medicaments into available conditioners can be suggested for treating denture induced stomatitis.[43]
16. **Seshagiri Muttagi et al (2017)** conducted an in-vitro study to analyze the surface roughness, antifungal property, wettability, glucose sorption and weight change when incorporating seed oils into soft liners. They concluded that incorporating *Linum usitatissimum, Ocimum sanctum Linn* and *Centratherum anthelminticum* seed oils into soft liner remarkably decreased the progression of *Candida albicans*, decreased surface roughness, showed better wettability and reduced the absorption of glucose.[44]
17. **Barua DR et al (2017)** conducted a study by integrating extract of neem leaf and 3 antimicrobial agents (nystatin, ketoconazole and chlorohexidine diacetate) into tissue conditioner and to compare their antifungal efficacy against *S. mutans* and *Candida albicans*. The study resulted that extract of Neem leaf revealed to be an effective antimicrobial alternative.[45]

18. **Alaa Dakhil Yasser et al (2017)** conducted an in-vitro investigation to determine the influence of zirconium nanoparticles in heat cure acrylic soft lining material on Candida albicans development, as well as the shear bond strength after zirconium nanoparticles were addedAccording to this study, the application of zirconium nanoparticles increased the antifungal efficacy of denture lining material against Candida albicans, as well as the shear bond strength of the material.[46]
19. **Machado Goncalves et al (2018)** conducted an in-vitro study to assess the consequence of *Terminalia catappa Linn* extract opposing *Candida albicans* seen on denture acrylic. They found that dipping the denture in Terminalia catappa Linn extract decreased the number of Candida albicans biofilms that formed on the acrylic surface of the denture.[47]
20. **Kumar SM et al (2018)** conducted an in-vitro study to evaluate the mechanical properties and antifungal efficacy of "soft denture liner"s opposing *Candida albicans* after the integration of Garlic and Neem powder extract. The outcome of the investigation was that both Neem and Garlic has an antifungal effect against *Candida albicans*. They concluded that, to decrease the bonding of C. albicans, herbal antifungal like neem and garlic can be added without affecting the physical properties of the acrylic resin.[48]
21. **Aseel Riyadh Abdulwahhab (2018)** conducted an in-vitro experiment to see how well Aloe vera powder combined with heat cure acrylic "soft denture liner" polymer affected Candida albicans adhesion, shear bond strength, and tear strength. According to the findings, mixing aloe vera powder with heat-cure denture soft-liner powder helps to provide an anti-candidal effect in the soft liner, as well as improving tear strength and shear bond strength.[49]

22. **Chandana Kalita et al (2018)** conducted an in vivo study to estimate the antifungal activity of *Tulsi, Neem* and *Pochotia* using agar well diffusion method. They came to a conclusion that Pochotia and neem revealed better antifungal activity against *Candida albicans* when compared with another agent.[50]
23. **Zeinab Khorram et al (2019)** evaluated the anti candidial effect of *Cuminum cyminum oil* and *Foeniculum vulgare oil* and compared it with nystatin against Candida. They concluded that Nystatin (zone of inhibition 23mm) had shown the highest antifungal efficacy followed by *Cuminum cyminum* (14mm) and then by *Foeniculum vulgare* (5mm).[51]
24. **Gayathri Krishnamoorthy et al (2019)** conducted a study to test the antifungal efficacy and tensile strength of tissue conditioner when comprised with *Cocos nucifera oil* and concluded that 10% w/w *Cocos nucifera* when integrated into Viscogel tissue conditioner decreased the colonization of Candida and also increased tensile strength of the tissue conditioner.[52]
25. **Kumar PS et al (2020)** conducted an in-vitro trial to check the efficacy of three antifungal agents (Azadirachta indica, Melaleuca alternifolia oil and Cocos nucifera oil) when incorporated in a soft relining material at minimum inhibitory concentration and concluded that antimycotic activity of M. alternifolia, C. nucifera, and A. indica mixed with the Visco-gel tissue conditioner can be used as an alternative therapy for denture stomatitis.[53]
26. **P. Anushya1 et al (2020)** published a detailed review on "Role of herbal medicine in dental health" and stated that the main advantages of using herbal medicine are easy availability, cost-effectiveness, increased time period and low toxicity. He added that the Preclinical and clinical trials are needed to gauge

biocompatibility and safety before Herbal medicine are often recommended conclusively for oral care.[54]

27. **AZ Godil et al (2021)** conducted an in-vitro study to investigate the activity of incorporated antifungal agents like Fluconazole and Ocimum sanctum oil (Tulsi) in the denture soft liners to reduce the risks of Candida albicans. They concluded that this approach allows the prolonged drug release in the oral cavity which simultaneously treats the injured denture bearing tissues and also the infection, biofilms of candida without compromising on their physical properties.[55]
28. **Khaled Hosny et al (2021)** conducted an invitro study to produce an oregano essential oil-based nanoemulsion (OEO-SNEDD) that would have antibacterial and antifungal effects against oral microbiota. This study concluded that nanoemulsion essential oil can provide good protection against oral microbial infections like candida albicans and S. mutans.[56]
29. **Myung-Jin Lee et al. (2021)** conducted an invitro study to investigate the antifungal efficacy and surface characteristic of soft denture liner linked with herbal Cnidium officinale extracts and concluded that material containing the CO extract exhibited antimicrobial efficacy against C. albicans and exhibited no significant difference between the surface characterization of the experimental and control group.[57]

MATERIAL AND METHODOLOGY:

SOURCE OF DATA:

This in-vitro study was conducted in-

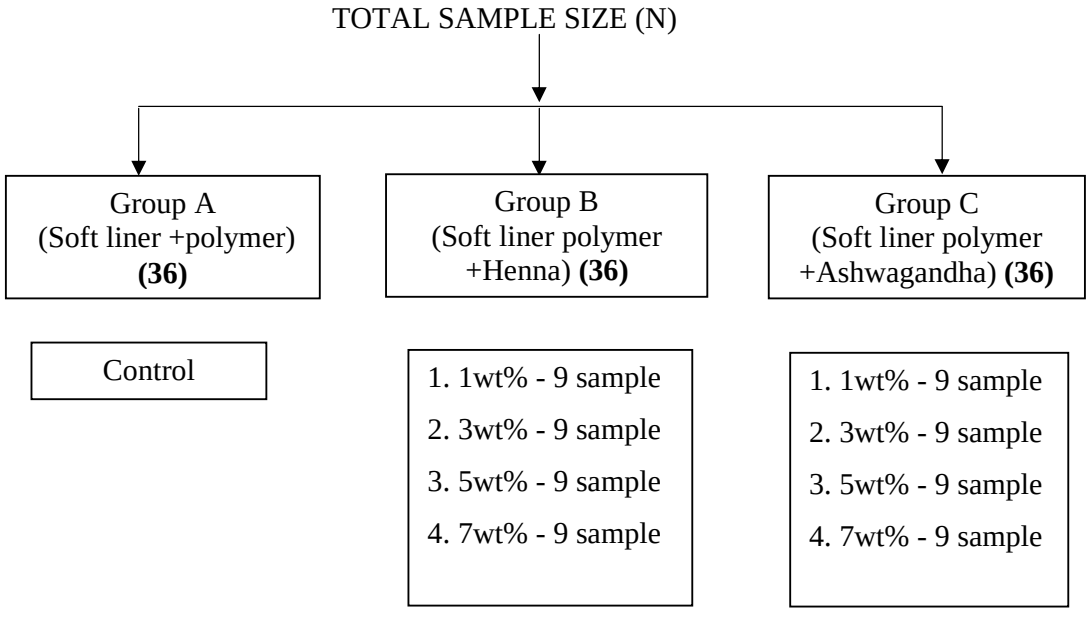
- "The Department of Prosthodontics, Crown and Bridge, KAHER KLE V.K Institute of Dental Sciences, Belagavi."
- "Dr. Prabhakar Kore Basic Science Research Centre, KLE University, Belagavi."
- "National Institute of Traditional Medicine, Belagavi (ICMR)."

SAMPLE SIZE:

Table 1: Sample size.

Sample size was obtained by the formula:

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



Total of 108 samples were taken to assess the antifungal efficacy. These samples were divided into 3 groups. They are-

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

Group B: *Lawsonia inermis leaf powder* incorporated in soft liner.

Group C: *Withania somnifera* root powder incorporated in soft liner.

Each of these groups were further divided into 4 subgroups of concentration 1%, 3%, 5% and 7% concentration with 9 samples each.

GC soft liner (100gm of softliner polymer and 100ml of softliner monomer)

Table 2: Sub-Group (Group A, B and C) concentration.

Group A (GC Soft Liner)

Group B (Soft liner polymer + Leaves powder extract of Lawsonia inermis)

SUBGROUP CONC.	POLYMER	HENNA POWDER	MIXTURE POWDER
1. 1wt%	99GM	1GM	100GM
2. 3wt%	97GM	3GM	100GM
3. 5wt%	95GM	5GM	100GM
4. 7wt%	93GM	7GM	100GM

Group C (Soft liner polymer + Root powder extract of Withania somnifera)

SUBGROUP CONC.	POLYMER	ASHWAGANDHA POWDER	MIXTURE POWDER
1. 1wt%	99GM	1GM	100GM
2. 3wt%	97GM	3GM	100GM
3. 5wt%	95GM	5GM	100GM
4. 7wt%	93GM	7GM	100GM

INCLUSION CRITERIA:

- Authenticated plants from ICMR.
- Powder fineness within 180µm.

EXCLUSION CRITERIA:

- Powder fineness more than 180µm.

MATERIALS USED IN THE STUDY:

Table 3: Material used in the study.

MATERIALS	DESCRIPTION	MANUFACTURER
Lawsonia inermis leaf powder	**-**	**Authenticated by ICMR**
Withania somnifera root powder	**-**	**Authenticated by ICMR**
GC Soft liner	**1609031**	**GC Corp. Tokyo, Japan**
Sabouraud dextrose agar	**LOT D18JI4200- TR- 13C**	**Hi media, Mumbai**
***Candida albicans* strain**	**90028**	**MTCC No. 2091**
Petri plates	**PW011**	**Hi- Media**

MATERIALS AND ARMAMENTARIUM:

MATERIALS:

- Pulverised dried fine powder of *Lawsonia inermis* (Henna) leaves.
- Pulverised dried fine powder of *Withania somnifera* (Ashwagandha) root.
- GC soft liner (polymer and monomer).
- Sabouraud dextrose agar.

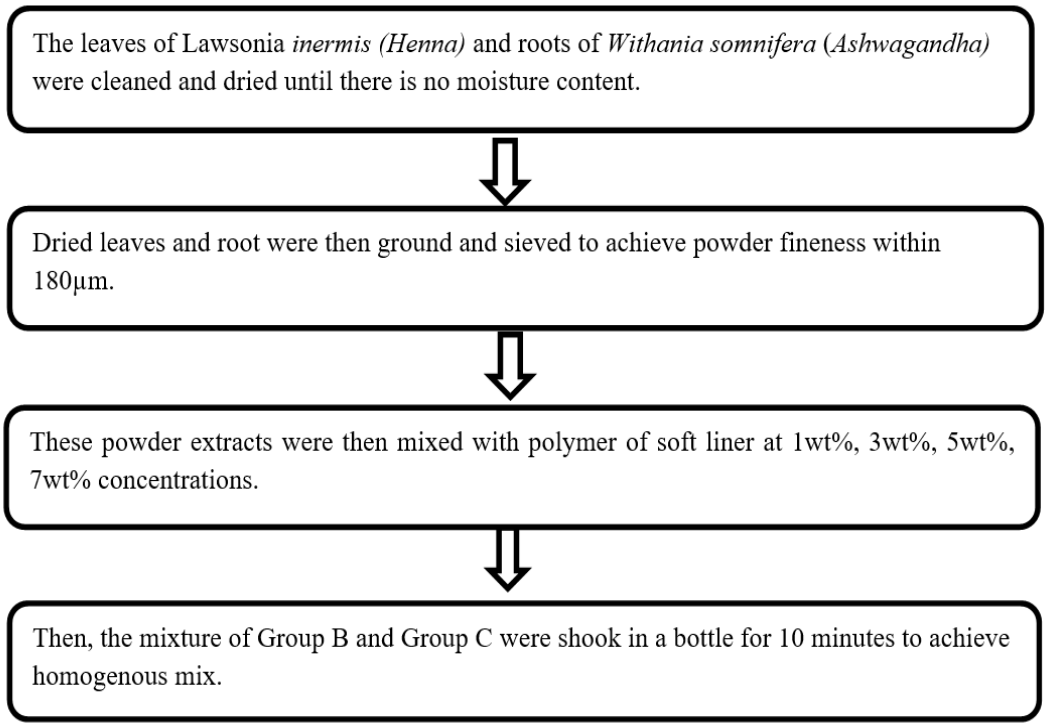
ARMAMENTARIUM:

- Leaf grinder.
- Bacteriological Incubator- Biotechnics India (BTI-25).
- Digital analytic balance: UniBloc (AUW220D) and Kern and Sohn GmbH- (240-3N).
- Petri dish and swab stick.
- Eppendorf microtiter pipette (1000 and 100 µl)
- Mixing jar and spatula- Prime Dental Products Pvt Ltd.
- Sterile cork borer.
- Dapen dish.
- Metallic scale.

DETAILS OF THE PROCEDURES WERE CONDUCTED DURING THE RESEARCH:

- **PREPARATION OF POWDER EXTRACT OF HENNA AND ASHWAGANDHA:**
  - For preparation of powder of *Lawsonia inermis (Henna)* and *Withania somnifera (Ashwagandha),* the plants were collected from Ayurvedic college and were authenticated by the botanist from 'National Institute of Traditional Medicine, Belagavi'. Then the leaves and roots were cleaned and dried until there is no moisture content.
  - Dried leaves and roots were then ground into fine powder of size 180µm by using No. 180 sieve having 0.180mm size of aperture. Powder was sieved to achieve even powder size. Later powder extracts of *L.inermis* and *W.somnifera* were mixed with soft liner polymer at 1wt%, 3wt%, 5wt%, 7wt% concentration.
  - Then, Group B (leaves powder extract of L.inermis + soft liner polymer) and Group C (root powder extract of W. sonmifera + soft liner polymer) mixture bottle were shook for 10 minutes to achieve homogenous mix.
  - Group A (GC soft liner polymer) act as control in this study.
- **TO CHECK FOR THE ANTIFUNGAL PROPERTY OF THESE POWDER IN DENTURE SOFT LINER:**
  - *Candida albicans* was obtained from KLE's "Dr. Prabhakar kore basic science research center, Belagavi."

- Using Sabouraud dextrose agar on 90mm diameter petri plates, antifungal susceptibility was assessed using the well diffusion technique. The inoculum was then smeared onto the culture plates. A sterile cork borer was used to punch a 6mm diameter and 5mm depth well once the inoculum has dried.
- Then, Group B and Group C mixture were mixed separately with monomer of soft-liner according to manufacturer instructions and be placed into the punched wells on Sabouraud dextrose agar. Group C (Control Group) was mixed same as that for Group A and Group B. Then agar plates were incubated at 37˚±1˚C for fourteen days. The diameter of inhibition zone (DIZ) will be measured with metallic scale after first, seventh, and fourteenth day.



TO CHECK FOR THE ANTIFUNGAL PROPERTY OF HENNA AND ASHWAGANDHA POWDER EXTRACT IN DENTURE SOFT LINERS:

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

⇩

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

⇩

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

⇩

Later, Wells was punched (6mm diameter, 5mm depth) with sterile cork borer.

⇩

Polymer of Group B and Group C of particular concentration was mixed separately with monomer of soft liner according to manufacturer instruction and was then placed into the punched wells. Group A (GC Soft Liner) polymer was

⇩

Agar plates was then incubated at 37˚±1℃ for fourteen days.

⇩

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

Figure 1: Dry Leaves of Lawsonia inermis and roots of Withania somnifera.

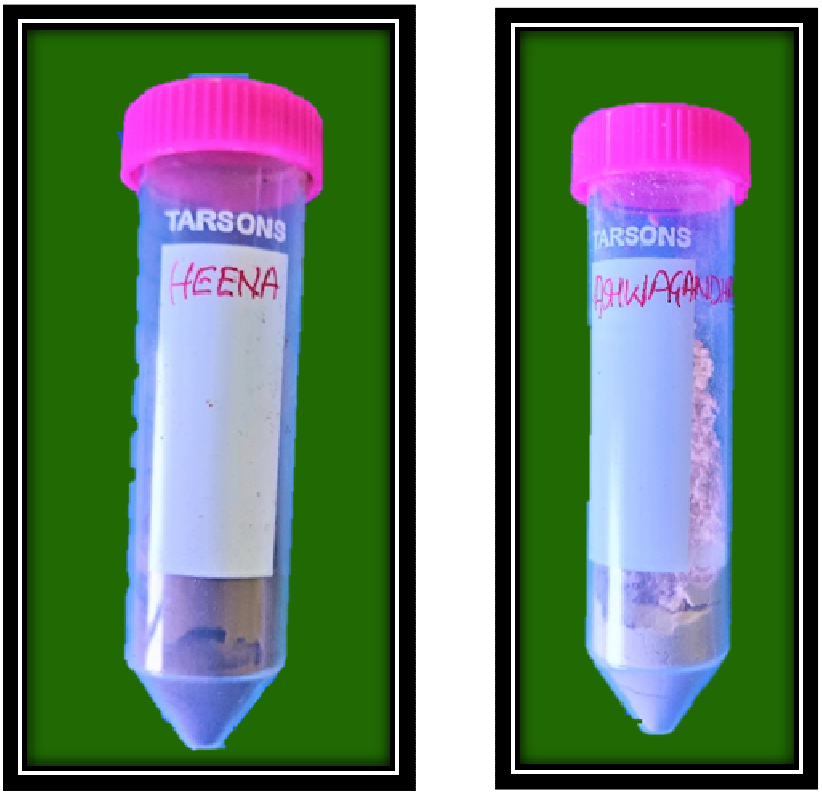


Figure 2: Powder extract of Lawsonia inermis and Withania somnifera.

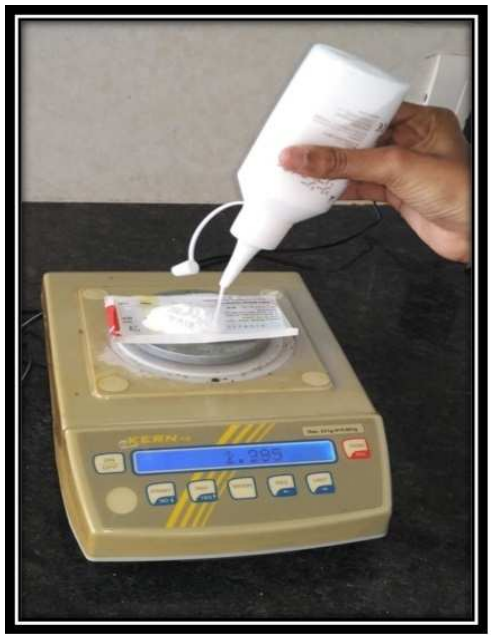


Figure 3: Measurement of soft-liner polymer according to manufacturer instruction.

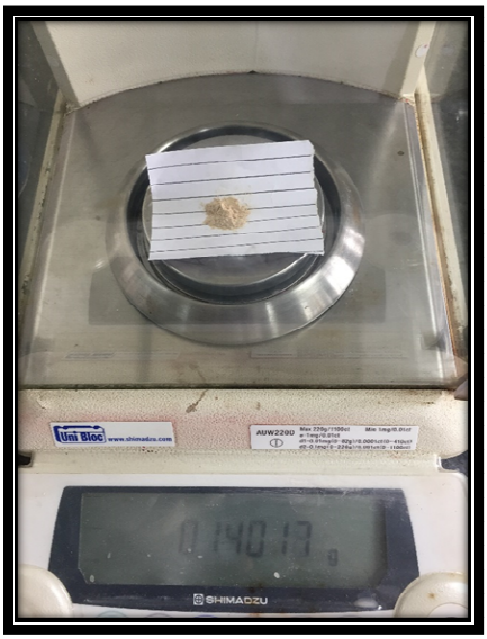


Figure 4: Measurement of powder extract to incorporated into soft liner polymer.

Figure 5: Various Concentration (1%,3%,5% and 7%) of Lawsonia inermis integrated with soft liner polymer.

Figure 6: Various Concentration (1%,3%,5% and 7%) of Withania somnifera integrated with soft liner polymer.

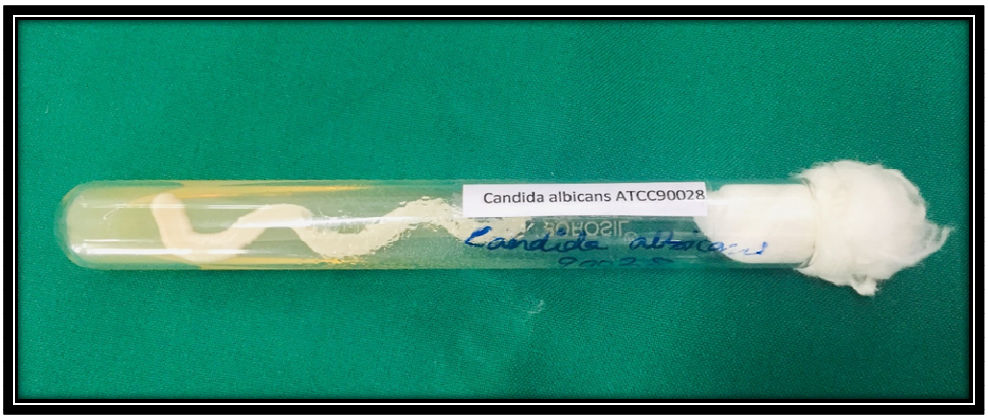


Figure 7: ATCC strain of Candida

Figure 8: Candida albicans inoculum.

Figure 9: Streaking of Candida albicans inoculum on the Sabouraud dextrose agar.

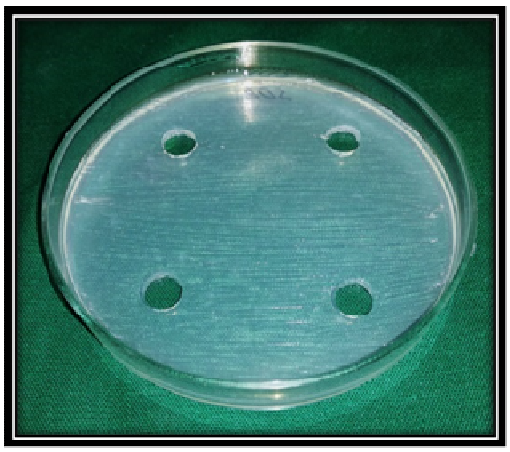


Figure 10: Wells of 6mm diameter and 5mm depth punched.

Figure no. 11: Herbal powder extract integrated with soft liner polymer mixed with monomer in a glass jar.

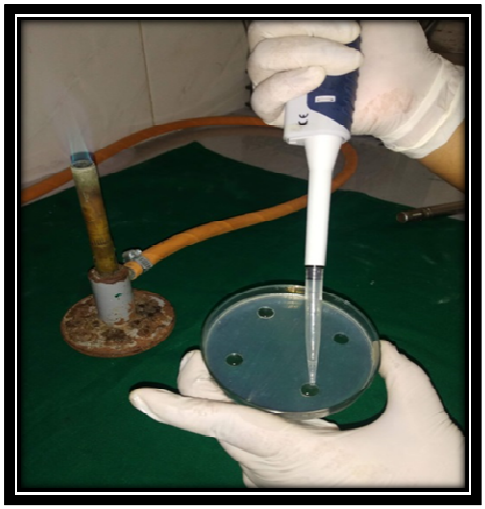


Figure no. 12: Punched wells filled with the mixture resin at different concentrations.

Figure no 13: Petri plates placed in an incubator at 37^0C.

Control 'Group A'

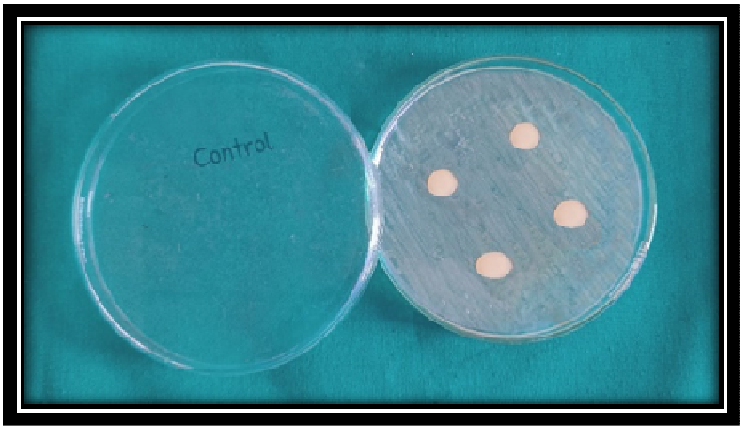


Figure 14: Control group day

Figure 15: Control group day

Figure 16: Control group day

Comparison of Group B and Group C samples at 1% concentration at 1st, 7th and 14th day:

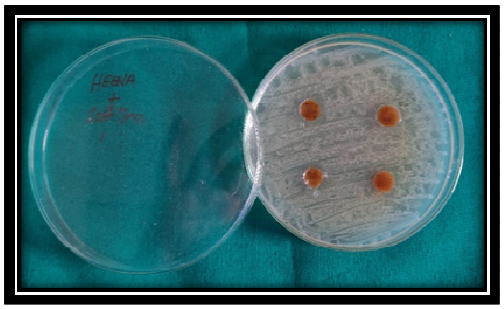


Figure 17: Soft-liner + 1% Henna: 1st Day

Figure 18: Soft-liner + 1% Ashwagandha: 1st Day.

Figure 19: Soft-liner + 1% Henna:7th Day.

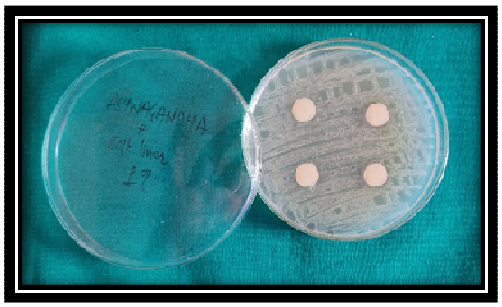


Figure 20: Soft-liner + 1% Ashwagandha: 7th Day.

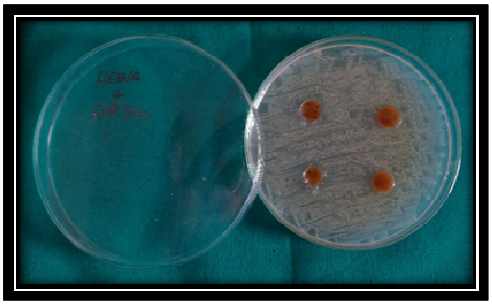


Figure 21: Soft-liner + 1% Henna: 14th Day

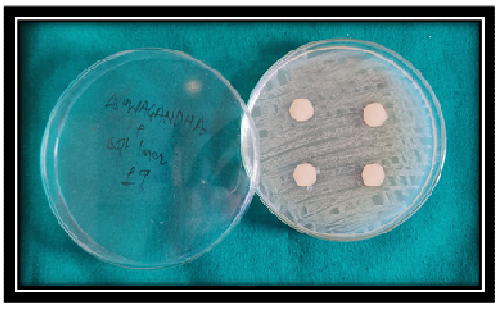


Figure 22: Soft-liner + 1% Ashwagandha: 14th Day

Comparison of Group B and Group C samples at 3% concentration at 1st, 7th and 14th day:

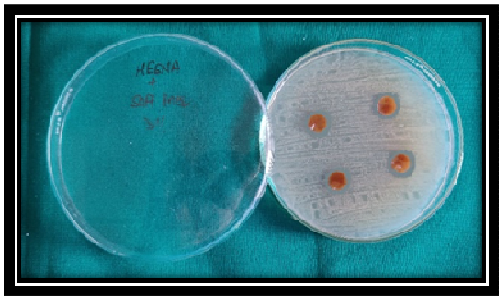


Figure 23: Soft-liner + 3% Henna:1st Day.

Figure 24: Soft-liner + 3% Ashwagandha: 1st Day.

Figure 25: Soft-liner + 3% Henna: 7th Day

Figure 26: Soft-liner + 3% Ashwagandha: 7th Day.

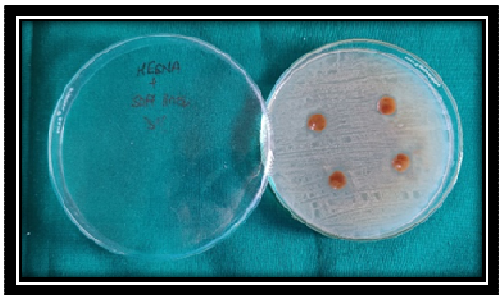


Figure 27: Soft-liner + 3% Henna:14th Day.

Figure 28: Soft-liner + 3% Ashwagandha: 14th Day.

Comparison of Group B and Group C samples at 5% concentration at 1st, 7th and 14th day:

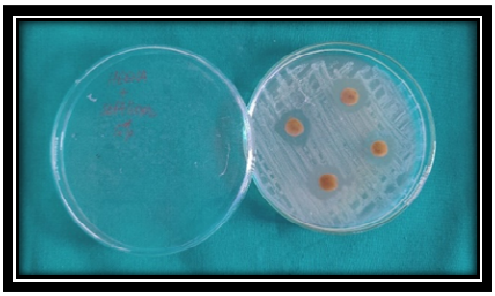


Figure 29: Soft-liner + 5% Henna:1st Day.

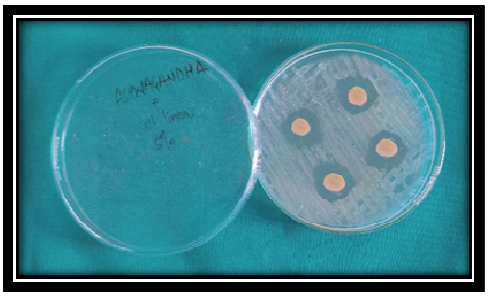


Figure 30: Soft-liner + 5% Ashwagandha: 1st Day.

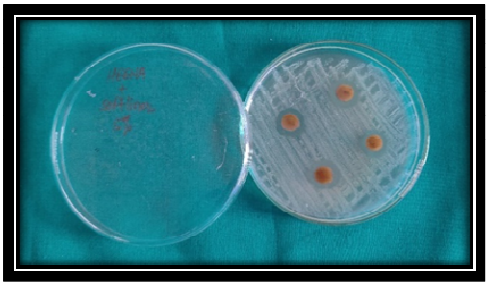


Figure 31: Soft-liner + 5% Henna: 7th Day.

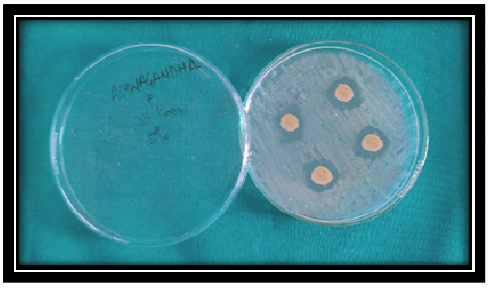


Figure 32: Soft-liner + 5% Ashwagandha: 7th Day.

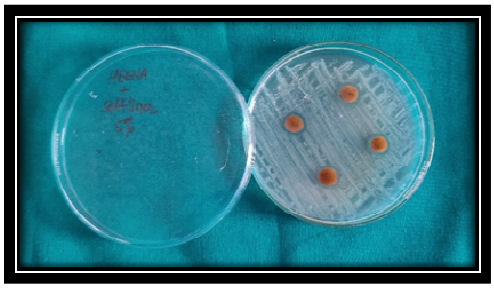


Figure 33: Soft-liner + 5% Henna: 14th Day.

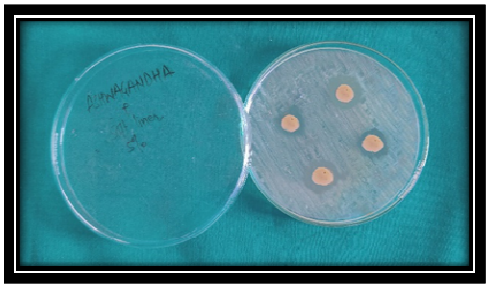


Figure 34: Soft-liner + 5% Ashwagandha: 14th Day.

Comparison of Group B and Group C samples at 7% concentration at 1st, 7th and 14th day:

Figure 35: Soft-liner + 7% Henna: 1st Day.

Figure 36: Soft-liner + 7% Ashwagandha: 1st Day.

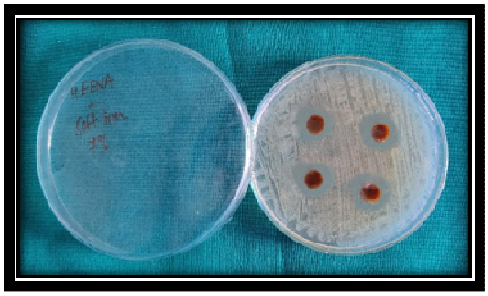


Figure 37: Soft-liner + 7% Henna: 7th Day.

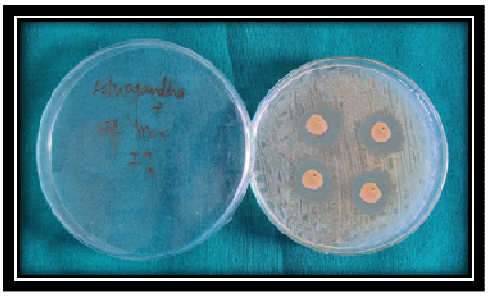


Figure 38: Soft-liner + 7% Ashwagandha: 7th Day.

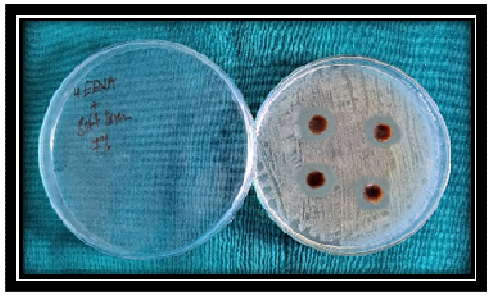


Figure 39: Soft-liner + 7% Henna: 14th Day.

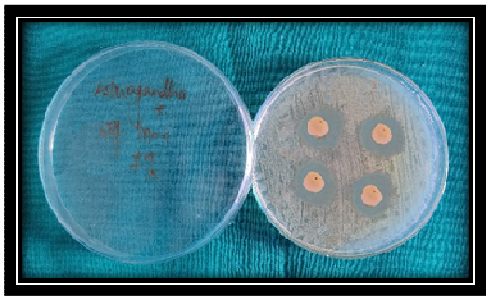


Figure 40: Soft-liner + 7% Ashwagandha: 14th Day.

RESULTS

The goal of this study was to assess and compare the antifungal activity of a denture soft liner mixed with Lawsonia inermis leaf powder and Withania somnifera root powder.

Statistical Analysis

The data from the study was entered into a Microsoft Excel spreadsheet, and the statistical analysis was performed using SPSS version 20 software. To explain the data's underlying structure, descriptive statistics were used.

Independent t test was used for comparison of two groups with mean zone of inhibition in millimetre (mm) at different time intervals and pair wise comparison of 4 subgroups (1%, 3%, 5%. 7%) with mean zone of inhibition scores at different time points by Tukey's multiple post hoc procedures. Dependent t test was used for comparison of 1^{st} day, 7^{th} day's and 14^{th} day's time points with mean zone of inhibition scores in two study groups. Comparison of different times (1^{st}, 7^{th}, 14^{th} day) in each sub group of Group B and Group C were done by using paired t test.

- **Antifungal activity test**

Antifungal activity was evaluated for *Lawsonia Inermis* groups and *Withania sominfera* group for four different concentration 1%, 3%, 5% and 7% at three different time intervals i.e., on 1^{st} day, 7^{th} day and 14^{th} day. Mean, standard deviation and standard error were calculated. (Table 4 and 5)

Table 4: Summary of Zone of antifungal efficacy on 1st day, 7th day and 14th day time points in four sub groups (1%, 3%, 5% and 7%) in Group B

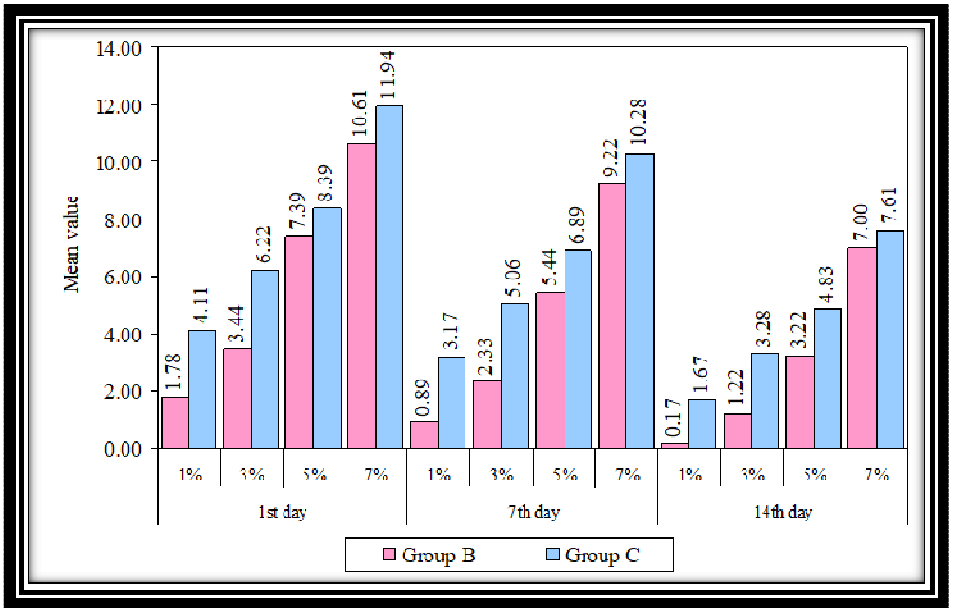
Times	Sub groups	Mean	Std. Deviation	Std. Error
1st day	1%	1.78	0.36	0.12
	3%	3.44	0.53	0.18
	5%	7.39	0.42	0.14
	7%	10.61	0.42	0.14
7th day	1%	0.89	0.22	0.07
	3%	2.33	0.35	0.12
	5%	5.44	0.46	0.15
	7%	9.22	0.26	0.09
14th day	1%	0.17	0.25	0.08
	3%	1.22	0.51	0.17
	5%	3.22	0.36	0.12
	7%	7.00	0.35	0.12

Table 5: Summary of Zone of antifungal efficacy on 1st day, 7th day and 14th day time points in four sub groups (1%, 3%, 5% and 7%) in Group C.

Times	Sub groups	Mean	Std. Deviation	Std. Error
1st day	1%	4.11	0.42	0.14
	3%	6.22	0.36	0.12
	5%	8.39	0.33	0.11
	7%	11.94	0.63	0.21
7th day	1%	3.17	0.25	0.08
	3%	5.06	0.39	0.13
	5%	6.89	0.42	0.14
	7%	10.28	0.44	0.15
14th day	1%	1.67	0.25	0.08
	3%	3.28	0.26	0.09
	5%	4.83	0.35	0.12
	7%	7.61	0.42	0.14

The mean value (DIZ) for the Lawsonia inermis groups at 1% concentration for 1st day, 7th day and 14th day were 1.78, 0.89, 0.17 (0 means complete Candida growth) respectively. The mean value (DIZ) for *Withania somnifera* group at 1% concentration for 1st day, 7th day and 14th day were 4.11, 3.17 and 1.67 respectively. The mean value (DIZ) for the Lawsonia inermis groups at 3% concentration for 1st day, 7th day and 14th day were 3.44, 2.33 and 1.22. respectively. The mean value (DIZ) for *Withania somnifera* group at 3% the concentration for 1st day, 7th day and

14th day were 6.22, 5.06 and 3.28 respectively. The mean value (DIZ) for the Lawsonia inermis groups at 5% concentration for 1st day, 7th day and 14th day were 7.39, 5.44 and 3.22 respectively. The mean value (DIZ) for *Withania somnifera* group at 5% concentration for 1st day, 7th day and 14th day were 8.39, 6.89 and 4.83 respectively. The mean value (DIZ) for the Lawsonia inermis groups at 7% concentration for 1st day, 7th day and 14th day were 10.61, 9.22 and 7.00 respectively. The mean value (DIZ) for *Withania somnifera* group at 7% concentration for 1st day, 7th day and 14th day were 11.94, 10.28 and 7.61 respectively. (Graph 1)



Graph 1: Comparison of Group B and Group C in four sub groups (1%, 3%, 5% and 7%) with mean zone of antifungal efficacy at 1st day, 7th day and 14th day time points.

Thus, *Withania somnifera* Group C exhibited more antifungal activity as compared to the *Lawsonia inermis* Group B. Control Group A had complete growth of Candida and therefore it was not considered in the analyses.

Table 6: Comparison of four sub groups (1%, 3%, 5% and 7%) with mean zone of antifungal efficacy at 1st day, 7th day and 14th day time points in Group B by one way ANOVA.

Times	Sources of variation	Sum of Squares	df	Mean Square	F-value	p-value
1st day	Between groups	426.5830	3	142.1940	751.4130	0.0001*
	Within groups	6.0560	32	0.1890		
	Total	432.6390	35			
7th day	Between groups	368.3060	3	122.7690	1071.4340	0.0001*
	Within groups	3.6670	32	0.1150		
	Total	371.9720	35			
14th day	Between groups	244.7990	3	81.6000	566.2810	0.0001*
	Within groups	4.6110	32	0.1440		
	Total	249.4100	35			

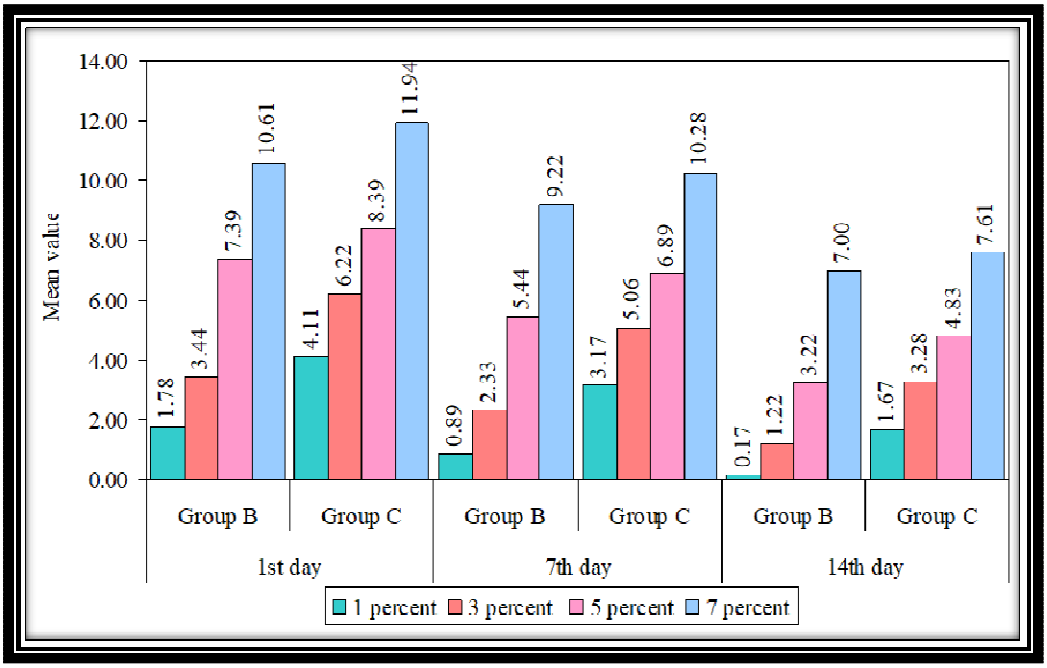
*$p<0.05$

Table 7: Comparison of four sub groups (1%, 3%, 5% and 7%) with mean zone of antifungal efficacy at 1st day, 7th day and 14th day time points in Group C by one way ANOVA.

Times	Sources of variation	Sum of Squares	df	Mean Square	F-value	p-value
1st day	Between groups	301.9440	3	100.6480	491.2990	0.0001*
	Within groups	6.5560	32	0.2050		
	Total	308.5000	35			
7th day	Between groups	247.7430	3	82.5810	566.2700	0.0001*
	Within groups	4.6670	32	0.1460		
	Total	252.4100	35			
14th day	Between groups	172.9650	3	57.6550	535.6340	0.0001*
	Within groups	3.4440	32	0.1080		
	Total	176.4100	35			

*$p<0.05$

When comparison of four sub groups (1%, 3%, 5% and 7%) were done with the mean zone of antifungal efficacy at 1st day, 7th day and 14th day time points in both Group B and Group C by one way ANOVA, statistically significant results were obtained with p- value of 0.0001. (Table 6 and 7) With the increase in the concentration of Lawsonia inermis and Withania somnifera, antifungal activity increases, but with the increase in day point time, antifungal activity decreased in each group. (Graph 2)



Graph 2: Comparison of four sub groups (1%, 3%, 5% and 7%) with mean zone of antifungal efficacy at 1st day, 7th day and 14th day time points in Group B and Group C.

When Pair wise comparison was done for four sub groups (1%, 3%, 5% and 7%) with mean zone of antifungal efficacy at 1st day, 7th day and 14th day time points in Group B and Group C by Tukeys multiple posthoc procedures, significant results (p value of 0.0001) were obtained. Higher the concentration of test sample, higher the zone of antifungal efficacy was noted. (Table 8 and 9).

Table 8: Pair wise comparison of four sub groups (1%, 3%, 5% and 7%) with mean zone of antifungal efficacy at 1st day, 7th day and 14th day time points in Group B by Tukeys multiple posthoc procedures.

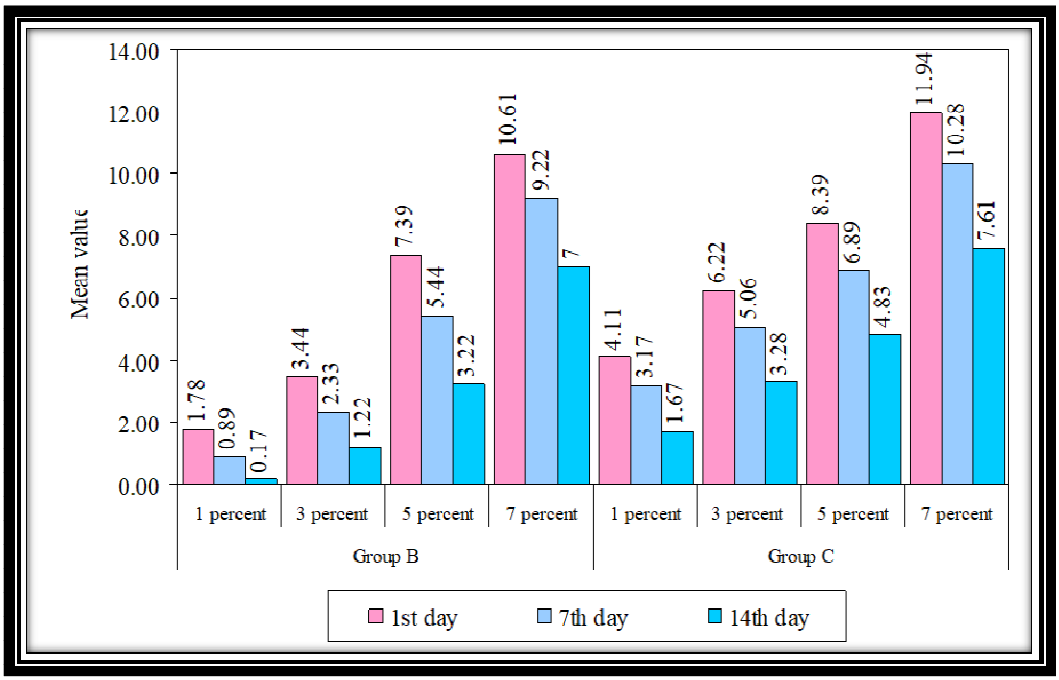
Times	Subgroups	Mean Difference (I-J)	Std. Error	p-value
1st day	1% vs 3%	-1.6667	0.2051	0.0001*
	1% vs 5%	-5.6111	0.2051	0.0001*
	1% vs 7%	-8.8333	0.2051	0.0001*
	3% vs 5%	-3.9444	0.2051	0.0001*
	3% vs 7%	-7.1667	0.2051	0.0001*
	5% vs 7%	-3.2222	0.2051	0.0001*
7th day	1% vs 3%	-1.4444	0.1596	0.0001*
	1% vs 5%	-4.5556	0.1596	0.0001*
	1% vs 7%	-8.3333	0.1596	0.0001*
	3% vs 5%	-3.1111	0.1596	0.0001*
	3% vs 7%	-6.8889	0.1596	0.0001*
	5% vs 7%	-3.7778	0.1596	0.0001*
14th day	1% vs 3%	-1.0556	0.1790	0.0001*
	1% vs 5%	-3.0556	0.1790	0.0001*
	1% vs 7%	-6.8333	0.1790	0.0001*
	3% vs 5%	-2.0000	0.1790	0.0001*
	3% vs 7%	-5.7778	0.1790	0.0000
	5% vs 7%	-3.7778	0.1790	0.0000

*$p<0.05$

Table 9: Pair wise comparison of four sub groups (1%, 3%, 5% and 7%) with mean zone of antifungal efficacy at 1st day, 7th day and 14th day time points in Group C by Tukeys multiple posthoc procedures.

Times	Subgroups	Mean Difference (I-J)	Std. Error	p-value
1st day	1% vs 3%	-2.1111	0.2134	0.0001*
	1% vs 5%	-4.2778	0.2134	0.0001*
	1% vs 7%	-7.8333	0.2134	0.0001*
	3% vs 5%	-2.1667	0.2134	0.0001*
	3% vs 7%	-5.7222	0.2134	0.0001*
	5% vs 7%	-3.5556	0.2134	0.0001*
7th day	1% vs 3%	-1.8889	0.1800	0.0001*
	1% vs 5%	-3.7222	0.1800	0.0001*
	1% vs 7%	-7.1111	0.1800	0.0001*
	3% vs 5%	-1.8333	0.1800	0.0001*
	3% vs 7%	-5.2222	0.1800	0.0001*
	5% vs 7%	-3.3889	0.1800	0.0001*
14th day	1% vs 3%	-1.6111	0.1547	0.0001*
	1% vs 5%	-3.1667	0.1547	0.0001*
	1% vs 7%	-5.9444	0.1547	0.0001*
	3% vs 5%	-1.5556	0.1547	0.0001*
	3% vs 7%	-4.3333	0.1547	0.0000
	5% vs 7%	-2.7778	0.1547	0.0000

*$p<0.05$



Graph 3: Comparison of different treatment times in each sub group of Group B and Group C.

Antifungal activity of both Group B and C were indirectly proportional to the time period. With the progressing time period, the antifungal activity was found to be less effective when compared with the last time period (Graph 3). Although antifungal efficacy was noted in both the group at progressive time interval, higher concentrations of these herbal antifungal agent showed more efficacy. Also, the efficacy noted on the 1st day was statistically significant when compared with 7th and 14th day.

DISCUSSION:

Candidiasis has become a threatful infection mainly in immunocompromised patients, older patients and in patients under medications. A rise in systemic and oral fungal infection has been outlined in the last few years.[58] The treatment of Candida infections is currently challenging owing to the inadequate number of available drugs, increased resistance to these drugs, high costs, and toxicity. Denture induced candidiasis has been the most repeatedly occurring oral mucosal infection which is mostly concerned with the use of a removable dental prosthesis and are seen in almost 11% to 70% of all the denture wearers. A rise in the fungal infection has led to a greater need of antifungal drugs. But the use of synthetic drugs over the time has resulted to have many harmful side effects which in need led to the application of herbal medicines in various fields.[59]

To avoid the emergence of fungal resistance, novel alternative treatments that are better than standard antifungal drugs are necessary. Few studies have assessed the anticandidal activity of herbal extracts. So, incorporating these herbal powder extracts in denture base or relining material may show the positive result.[60]

Denture soft liner liners are generally used for conditioning the denture supporting mucosa which is inflamed by ill-fitting dentures. They lower the stress generated by denture and act as a cushion. But in return they provide hub for intraoral microorganism because of increased surface roughness when compared with denture surface. It is found that, denture soft liners do not exhibit any antifungal activity. Conventional treatment option for denture stomatitis includes topical application of commercially available antifungal agents. These commercially available drugs have

side effects like rise in appearance of resistance. Many studies have incorporated herbal extract into denture soft liner and concluded improved efficacy against the fungal growth with no side effect like resistance development. Therefore, incorporation of antifungal medicament into the denture soft liners are done to treat denture induced candidiasis.[61]

In this study, herbal medicines like *Lawsonia inermis (Henna)* leaf and Withania somnifera (Ashwagandha) root powder extract have been incorporated into the denture soft liner and was tested against the *Candida albicans* growth for its antifungal efficacy till 14 days. Incorporation of these powders into the resilient soft lining material resulted to have an antifungal effect and thus rejecting the null hypothesis of the study.

Thus, the focus on modifying the "soft denture liner" made of poly methyl methacrylate (PPMA) was either by modifying the surface or via a herbal modification. Our research dealt with the second type of modification, i.e., using Henna and Ashwagandha powder extract which is added to the acrylic polymer and then mixed with the monomer. The results of the study showed that powder of Henna and Ashwagandha integrated into soft liner, both found to have an antifungal activity but Ashwagandha root powder exhibited to have more effective inhibition of the *Candida* when compared with henna leaf powder.

The minimum inhibitory concentration (MIC) is the amount of an agent that leads to inhibition of visible growth of a microorganism. According to M Patel et al., the minimum amount of Lawsone mouthwash necessary to suppress Candida albicans multiplication was 0.4 g/L..[62] The study of. Abirami Sasi et al., concluded that the

MIC and MBC/MFC of lawsone extracts were 0.039-0.625 mg/ml and 0.039-1.25 mg/ml, respectively.[63]

"Henna possesses analgesic, hypoglycemic, hepatoprotective, immunostimulant, anti-inflammatory, antibacterial, antimicrobial, antiviral, antiparasitic, antidermatophytic, antioxidant, antifertility, tuberculostatic, and anticancer effects, according to a phytopharmacological study published in 2010".[64] Other pharmacological characteristics of henna include anticancer, anthelmintic, antioxidant, immunomodulatory, burn wound healing, UV protection, and antibacterial. Henna is a plant used to colour hair, skin, and nails. This plant has been shown to have antibacterial and antifungal properties in the literature.[65] Henna leaves showed the biggest zone of inhibition (mean 20 mm), followed by pomegranate peels (mean 13.3 mm), and seeds (mean 3.75mm) according to a study by Singla et al. They also highlighted that the usage of medicinal plants against candida might be a feasible alternative to other antifungal drugs since they provide a low-cost and effective module for controlling Candida species-related oral infections.[32]

The anticandidal activities of L. inermis (henna) in paste form were assessed using the agar diffusion technique in a research study by Demet YT et al. Henna demonstrated the most potent antifungal action (20mm inhibition zone) against 68 (35.4%) clinical Candida isolates, whereas 73 (38.0%) isolates had moderate activity (5-15mm inhibition zone). Henna paste resistance was found in 51 (26.5%) isolates (No inhibition zone).[66] When we employed henna leaves extract powder, we found that it had comparable anti-Candidal growth inhibitory properties.

The antifungal activities of henna against Candida albicans were studied by Amal Nawasrah et al. He added several concentrations of Yamani henna powder to the polymer i.e., 1%, 2.5 percent, 5%, 7.5 percent, and 10%. With a p-value of 0.0001, he found that the difference in live Candida between the control and test groups was statistically significant. Variations in live Candida were also significant whether the powder concentration was 7.5 % or 10%, when compared to the control group, with p-values of 0.0001 and 0.001, respectively.[37]

Ashwagandha (Withania somnifera Dunal) is widely utilised in Indian herbal medicines and nutraceuticals. It's an annual herb that grows wild in dry, arid soil and is well-known for immunomodulation and antiaging in Ayurveda, according to the ancient Indian school of plant medicine. Additionally, Withania somnifera has anti-inflammatory, anti-tumor, anti-radio sensitizing, and analgesic properties.[28]

Although adding higher concentrations of these antifungal agents to the 'denture relining material' may increase antifungal activity, there are chances of deterioration of other material property (physical and mechanical) by increasing concentration. Amal Nawasrah et al. found that as the concentration of these drugs increased, the surface roughness values increased (p 0.01) while the hardness value decreased (p 0.0001). As a result, group concentration was limited in the current investigation, with just 1%, 3%, 5%, and 7% being used.[37]

The main goal of the study was to evaluate and check the antifungal efficacy of henna and ashwagandha for longer duration. Leachable antifungals like essential oil or water soluble synthetic antifungal drugs might not last for longer time period, but these herbal powder extracts will serve the purpose as these get embedded within the polymeric matrix of soft liner, thus contributing to a longer duration of antifungal

activity. Also, the finer particle size of these powder extracts will not impact the surface properties of the soft-liner coating.[67]

In our research, henna with a concentration of more than 5% and Ashwagandha with a concentration of more than 3% showed considerable antifungal efficacy till the 14th day. Both of these natural antifungal medicines can be used to treat fungal infections caused by Candida albicans for short term requirement.

SCOPE OF STUDY

"The antifungal activity of an acrylic based denture soft liner integrated with *lawsonia inermis* and *withania somnifera* powder extract was studied and compared in this study."

Further research is suggested to evaluate exact mechanism of action of antifungal activity of '*lawsonia inermis'* and '*withania somnifera'* against *Candida albicans.*

Further investigations are required to assess the stability, cytotoxicity and durability of the *lawsonia inermis (Henna leaves)* and *withania somnifera (Ashwagandha root)* powder extract.

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

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

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

LIMITATIONS OF THE STUDY

- Since this is an in-vitro study, application of the results in clinical conditions might yield different result.
- Diameter of inhibition zone of *Lawsonia inermis* and *Withania somnifera* powder extract was evaluated against only 1 strain of *Candida albicans*. The prosthetic biofilm is complex, formed not only by fungi but also by bacteria, favoring the adhesion of fungal cells to the internal prosthesis surfaces by co-aggregation.
- Only one acrylic type of interim resilient liner was evaluated in this study. Different liners might yield different results.
- Incorporation of the *henna and Ashwagandha powder* caused discoloration of the soft liner which might gave a slight unaesthetic result. However, the discoloration was slight and can be masked with the acrylic resin denture surface.

CLINICAL IMPLICATION

As denture soft liner can be used as a vehicle to deliver drugs, incorporation of antifungal drugs can be considered as a promising medicament against denture stomatitis to improve oral health status of the geriatric patients with cognitive disturbances, medically compromised conditions and reduced manual dexterity. Because of an increase in emergence of drug resistant microbes, herbal antifungal agents might prove beneficial. As these herbal plant extracts have no side-effects, it can be clinically useful.

CONCLUSION:

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

- The powder extract of Lawsonia inermis (Henna) with soft liner showed antifungal efficacy at higher concentration (7%) when compared with lower concentrations (3%,5%) till 14^{th} day of the research study. The first day antifungal test revealed antifungal activity at 3%,5% and 7% concentrations. Antifungal activity decreases with the time at all concentrations. 1% concentration has not shown any efficacy against candida albicans.
- The powder extract of Withania somnifera (Ashwagandha) with soft liner has shown antifungal efficacy against the growth of candida albicans at all concentrations (1%,3%,5%,7%). Concentration more than 5 % has shown antifungal activity till 14^{th} day of the research study. Antifungal activity decreases at all concentrations with the progressing time period of the study.
- Ashwagandha root powder extract was superior to henna leaf extract at all concentrations along with the time period mentioned in the study. The antifungal activity of these powder extracts was shown to be enhanced at higher concentrations in both the test groups. When compared to the lower concentrations, the 7% concentration of these herbal antifungal agents with soft liner exhibited extraordinary effectiveness till 14 days in both the groups.

SUMMARY:

The purpose of this in-vitro study was to test and compare the antifungal activity of a denture soft liner infused with Lawsonia inermis (Henna) powder extract and Withania somnifera powder extract (Ashwagandha).

The powder extract of henna and ashwagandha was prepared by grinding the leaves and roots. To obtained even size of particle, powder was sieved. A total of 105 samples were used in this study. 35 for control group, 35 for henna group and 35 for Ashwagandha group. Test was done to evaluate for the antifungal activity using well diffusion method. Concentration selected for each test group was 1%, 3%, 5% and 7%. Each sample were evaluated after 24hr, 7 days and 14 days. Each group was subdivided according to the time interval of evaluation. Thus, each subgroup comprised of 9 samples.

The resultant data was charted and subjected to statistical analysis using SPSS software version 20. For antifungal activity: Two-way ANOVA was used for comparison of two groups with mean zone of inhibition in mm (antifungal activity) scores at different time intervals and pair wise comparison of two groups with mean zone of inhibition (mm) scores at different time points by Tukey's multiple posthoc procedures. The dependent t test was used to compare the mean zone of inhibition (mm) values in two research groups at 1 day, 7 days, and 14 days.

According to the results obtained, both the powder extract showed antifungal efficacy against *Candida albicans*. Ashwagandha root powder extract group exhibited highest zone of inhibition when compared with henna leaves powder extract group at all the concentrations.

Since the denture soft liner are intended to be used for shorter duration of time, incorporation of these powder extract is not contraindicated. Thus, incorporation of henna leaves powder and ashwagandha root powder into the denture soft liner can prove to be beneficial to improve oral health status of the geriatric patients with cognitive disturbances, medically compromised conditions and reduced manual dexterity.

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ANNEXURE I

ETHICAL CLEARANCE

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CERTIFICATE

This is to Certify that the synopsis titled

COMPARATIVE EVALUATION OF ANTIFUNGAL EFFICACY OF SOFT LINERS INCORPORATED WITH POWDER EXTRACTS OF LAWSONIA INERMIS AND WITHANIA SOMNIFERA ON THE GROWTH OF CANDIDA ALBICANS : AN IN-VITRO STUDY

Submitted by Dr. RAHUL . S . JAISWAL *P. G. Student / Staff, Guided by* DR. HEMA KANATHILA *from Department of* PROSTHODONTICS & CROWN & BRIDGE *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date :

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

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

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ANNEXURE II

AUTHENTICATION FORM

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Department of Health Research,
Ministry of Health & Family Welfare, Govt. of India

Date: 18-07-2019

AUTHENTICATION

This is to authenticate that the plants submitted by Dr. Rahul Jaiswal, Post Graduate Student, Dept. of Prosthodontics, KLE's VK Institute of Dental Sciences, Belagavi are identified as ***Withania somnifera* Dunal. (Solanaceae)** and ***Lawsonia inermis* L. (Lythraceae)**. The voucher specimens of the same have been deposited in our herbaria with accession numbers RMRC-1426 and RMRC-1427 respectivly.

Harsha Hegde
Scientist 'D'

ANNEXURE III

Table 10: Diameter of Inhibition Zone (mm) (antifungal activity) of *Lawsonia Inermis* powder extract at the concentration of 1% after 1, 7 and 14 days.

SAMPLE NO.	1 DAY	7 DAYS	14 DAYS
1.	2	1	0
2.	1	1	0
3.	2	0.5	0
4.	2	1	0.5
5.	2	1	0.5
6.	1.5	0.5	0
7.	2	1	0
8.	1.5	1	0.5
9.	2	1	0

ANNEXURE IV

Table 11: Diameter of inhibition zone (mm) (antifungal activity) of *Withania somnifera* powder extract at 1% concentration after 1, 7 and 14 days.

SAMPLE NO.	1 DAY	7 DAYS	14 DAYS
1.	3.5	3	1.5
2.	4.5	3	1.5
3.	4	3.5	2
4.	3.5	3	1.5
5.	4.5	3.5	2
6.	4.5	3	1.5
7.	4	3	1.5
8.	4	3.5	2
9.	4.5	3	1.5

ANNEXURE V

Table 12: Diameter of inhibition zone (mm) (antifungal activity) of *Lawsonia inermis* powder extract at the concentration of 3% after 1, 7 and 14 days.

SAMPLE NO.	1 DAY	7 DAYS	14 DAYS
1.	3	2	1.5
2.	4	2.5	0.5
3.	3	2.5	1.5
4.	4	2.5	0.5
5.	4	2	1.5
6.	3	2	1
7.	3	2.5	1.5
8.	4	2	1
9.	3	3	2

ANNEXURE VI

Table 13: Diameter of inhibition zone (mm) (antifungal activity) of *Withania somnifera* powder extract at the concentration of 3% after 1, 7 and 14 days.

SAMPLE NO.	1 DAY	7 DAYS	14 DAYS
1.	CI	34.2	20.2
2.	CI	36	18
3.	CI	36.5	18.2
4.	CI	34.7	20.1
5.	CI	34	18
6.	CI	32	16
7.	CI	36.2	16.5
8.	CI	32.5	18
9.	CI	34	20.5

ANNEXURE VII

Table 14: Diameter of inhibition zone (mm) (antifungal activity) of *Lawsonia inermis* powder extract at the concentration of 5% after 1, 7 and 14 days.

SAMPLE NO.	1 DAY	7 DAYS	14 DAYS
1.	7	5	3.5
2.	8	6	3
3.	7	5	3.5
4.	7.5	6	3.5
5.	8	6	3.5
6.	7	5	3
7.	7.5	5.5	3.5
8.	7	5	2.5
9.	7.5	5.5	3

ANNEXURE VIII

Table 15: Diameter of inhibition zone (mm) (antifungal activity) of *Withania somnifera powder extract* at the concentration of 5% after 1, 7 and 14 days.

SAMPLE NO.	1 DAY	7 DAYS	14 DAYS
1.	8	6.5	5
2.	8.5	7	4.5
3.	8.5	6.5	4.5
4.	8.5	7.5	5
5.	8	6.5	4.5
6.	8.5	7	5
7.	9	6.5	4.5
8.	8.5	7.5	5
9.	8	7	5.5

ANNEXURE IX

Table 16: Diameter of inhibition zone (mm) (antifungal activity) of *Lawsonia inermis powder extract* at the concentration of 7% after 1, 7 and 14 days.

SAMPLE NO.	1 DAY	7 DAYS	14 DAYS
1.	11	9	7
2.	10.5	9.5	7
3.	11	9	6.5
4.	11	9.5	7
5.	11	9.5	6.5
6.	10	9	7
7.	10	9.5	6.5
8.	10.5	9	7
9.	10.5	9	6.5

ANNEXURE X

Table 17: Diameter of inhibition zone (mm) (antifungal activity) of *Withania somnifera powder extract* at the concentration of 7% after 1, 7 and 14 days.

SAMPLE NO.	1 DAY	7 DAYS	14 DAYS
1.	11	10.5	8
2.	11.5	9.5	8
3.	12.5	10.5	7.5
4.	12	10	8
5.	12.5	10.5	8
6.	12	10	7
7.	11.5	10.5	7.5
8.	13	11	7
9.	11.5	10	7.5