"TO EVALUATE THE ANTI CANDIDA EFFECT OF CLOTRIMAZOLE AND MORINDA CITRIFOLIA (NONI) ON THE PREVELANCE OF ORAL CANDIDA IN DENTURE WEARERS AN IN VIVO STUDY".

BY

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Dissertation

Submitted to
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In

PROSTHODONTICS AND CROWN & BRIDGE
(BRANCH – I)

UNDER THE GUIDANCE OF

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2019 - 2022

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Name: Dr. S. B. Javali

Dedicated

To

My family

And

My guide

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"Feeling gratitude and not expressing it is like wrapping a present and not giving it".

A sense of triumph is very much justified at this stage of completion of my dissertation, even more so is a sense of gratitude to all my peers, mentors and well-wishers.

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I gladly utilize this opportunity to express my deep sense of gratitude and indebtedness to all my TEACHERS.

"The task of the excellence 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 sir and guide ***DR. RAMESH P. NAYAKAR****MDS, Professor, 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. His unlimited patience, meticulous supervision at every step and everlasting zeal for perfection has not only enabled me to complete the dissertation, but has also helped me tremendously during the postgraduate programme.*

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The word "THANKYOU" seem shallow at best for my dear mother ***Mrs. MAYURI R JOSHI,*** *my dear father* ***Mr. RAVINDRA M JOSHI*** *, my dear husband* ***Dr. SHASHANK BABEL****, my sister* ***MISS FORAM R JOSHI*** *and my family for supporting me at my best and at my worst.*

"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

Date: ***Dr. DIVYA R JOSHI***

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LIST OF ABBREVIATIONS USED IN THE STUDY

BOD	Biological oxygen demand
CO_2	Carbon Dioxide
CFU	Colony forming Units
^oC	Degree Celsius
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethylsulfoxide
Group A	Clotrimazole
Group B	Morinda Citrifolia
MIC	Minimum inhibitory concentration
ML	Milliliter
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
OD	Optical density
PBS	Phosphate Buffer Saline
SD	Standard Deviation
%	Percentage

ABSTRACT

STATEMENT OF PROBLEM

Candida species are major human fungal pathogen that cause both the mucosal and other skin infections. Fungal colonization of mucosal surface was considered as major risk factor for development of candida infection. The predisposing local factor for this fungal localization are enhanced adherence of candida species to acrylic, reduced salivary flow under surface of denture, improper fitted denture and poor oral hygiene. Fungal infections have been successfully treated with the systemic antifungal agents since a very long time. The limited spectrum and toxicity of available antifungal agent have concerned for alternative therapies. The use of Morinda citrifolia has emerged as an alternative for treating candida infection compared to commercially available antifungals such as Clotrimazole.

PURPOSE

To evaluate the anti-candida effect of Clotrimazole and Morinda Citrifolia (Noni) on the prevalence of oral candida in denture wearers.

Materials and Methods

A total of sixty completely edentulous subjects wearing dentures since at least last one year were included in the study. They were divided into two groups like group A Clotrimazole and group B Morinda citrifolia. The swabs were collected from the denture and palatal mucosa of both the groups and were subjected to CHROMagar for identification and counting the number of colonies. The test individual were then asked to use Clotrimazole and Morinda citrifolia for 14 days follow up was done on 15^{th} day. Sample collection and microbial procedure in both the groups were carried

in similar manner as explained in order to study candida colonization. The attribute scores were tabulated and submitted to statistical analysis using Wilcoxon match and Mann Whitney U test to evaluate the prevalence of candida species and number of colonies of candida on pre usage and post usage of Clotrimazole and Morinda citrifolia.

Results

The results of the study indicated that the prevalence of candida species were statistically significant in Clotrimazole group and Morinda citrifolia Group on the denture surface. The results also revealed that there was statistically significant difference in the number of colonies on post usage of Clotrimazole and Morinda citrifolia on the denture surface amongst *candida albicans* than *candida glabrata* and *candida tropicalis.*

Conclusion

Within the limitations of this in vivo study, it was concluded that Clotrimazole and Morinda citrifolia (Noni) were effective in reduction of candida among denture wearers. The Clotrimazole was found to be more effective compared to Morinda citrifolia (Noni). This study shows Morinda citrifolia (Noni) may represent a preferred alternative to commercially available Anticandida drugs in treatment of candida in denture wearers.

Key words: *Candida albicans, Candida glabrata, Candida tropicalis,* Clotrimazole, Denture surface, Morinda citrifolia, Palatal surface.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

INTRODUCTION

The life expectancy of human being has increased significantly over the past century. The geriatric population is rapidly growing segment of the whole population. Despite improvement in oral health, tooth avulsion is a major problem in geriatric population which resulted in an increased requirement for prosthodontic rehabilitation. Conventional dentures are the best alternative in restoration of lost teeth that enables individuals with improvement of oral function, enhancement of phonetics, in facilitating social engagement and in leading an aesthetically acceptable life. Dentures have also shown to improve self-esteem in patient. [1]

Despite of several advantages of removable prosthesis, there are some constraints which include accumulation of food debris on the surface of the prosthesis and its removal is difficult.[1] The maintenance of oral cavity along with prosthesis is of chief importance in the prophylactic aspects which require removal and washing of denture after every meal to maintain good oral health. The tissue surface of acrylic resin dentures shows micro-porosities which harbour microorganisms that is challenging to remove. These surface irregularities may lead to surface roughness that facilitates microbial retention that may lead to infections. The porosity and surface texture of acrylic resins allows plaque to penetration into the surface defects and form a mechanical bond to surface irregularities.[1] Dentures tend to collect plaque and stains if they are not cleaned routinely. Dentures should not only be cleaned, but also be relatively decontaminated as the microbial plaque on the tissue surface of the dentures is a significant co-factor in the etiology of denture stomatitis, a common condition in geriatric denture wearers. [1] Alike natural dentition, dentures also require sufficient disinfection to ensure elimination of biofilm development. A routine denture

cleansing regimen should be designed to remove as well as to eliminate the collection of microbial plaque and also to remove mucin, food debris, calculus and exogenous discolourations. Most of the denture wearers report cleaning their dentures regularly. Despite of their cleaning efforts, there is accumulation of the soft debris, bacterial plaque and dental calculus on denture surfaces. [1] The effect of cleaning of the dentures may not be as efficient in elderly as it is in younger people due to reduced vision, dementia and reduced dexterity, hence adversely affecting their self-care ability. The elderly understand that they should clean their prosthesis properly but are not aware of the poor results of their efforts. The role of care-taker becomes more crucial in maintaining daily oral hygiene as the oral hygiene habits have been found to be poor among the elderly. Oral hygiene practices performed by attending nurses have been reported to be inconsistent and highly variable. Reports claim that inspite the poor maintenance of the dentures, the denture wearers get accustomed to wearing unclean dentures. It may be due to improper mechanical cleaning and the relative inadequacy of most commercial products for chemical cleaning of dentures.[1] Various factors such as low education background, low income, poor living conditions, unhealthy lifestyle, inadequate oral hygiene and tobacco use lead to poor oral health among the elderly, which risks their general health.[2] The combination of entrapment of yeast cells in irregularities in denture base and denture relining materials, poor oral hygiene and several systemic factors are the most probable cause for the onset of candidiasis.[3] The most common systemic diseases seen in functionally independent older adults are diabetes, hypertension, arthritis, cancer, chronic obstructive pulmonary disease, mental health conditions, osteoporosis and Parkinson disease.[4]

The oral mucosa becomes more permeable to various harmful agents and more prone to mechanical damage as the age advances. There are mucosal changes seen in

elderly as they use the denture. The ill-fitting dentures with poor oral hygiene, lack of denture cleansing constitute a major predisposing factor for oral fungal infection.[1]

Oral candidiasis, or candida associated stomatitis, is a common problem in elderly patients. A denture wearer is highly prone to develop Candida associated denture stomatitis (CADS), due to the conversion of the normal oral commensal Candida spp. (*Candida albicans*) into a pathogen under favourable conditions. Candidal species which are the commensal organism colonizes in the oral cavities of healthy individuals irrespective of whether they are denture wearers or not.[5] In 20% to 50% of these individuals, candida increases in number as the age advances. Disturbances in the oral balance can lead to candida infection in carriers and are often associated with antibiotic treatment, xerostomia due to disease or multiple medication, impaired local or systemic immune system, neglected oral hygiene, smoking, dietary habits , reduced sight, dementia and reduced manual dexterity, trauma caused by ill-fitting dentures.[6] The tissue conditioning materials and soft liners which have been used in conditioning abused tissue are associated to support the growth of *candida albicans* which may predispose to lesions.[5] The classic triad of oral candidiasis are pseudomembranous, erythematous (atrophic), and hyperplastic variants. In addition, there are a number of other candida-associated lesions where the cause is multifactorial which will be presented in the form of candida associated denture stomatitis, angular cheilitis or angular stomatitis, median rhomboid glossitis, and linear gingival erythema.[7]

Dentures favour accommodation of gram-positive cocci, particularly *streptococci, pneumococci* and *staphylococci.* The most common type of bacteria isolated are *neisseria catarrhalis,* with *staphylococcus albus (epidermidis),* but

streptococci and other types of bacteria are sparsely present.[8] The likelihood of the presence of yeast is also increased in candidiasis with the most frequently isolated species being *candida albicans, candida glabrata, candida tropicalis, candida krusei, candida guilliermondii, candida kefyr, candida parapsilosis.*[5] The *candida albicans* was isolated from 71% of yeast- positive subjects, making it the most predominant yeast in the elderly, followed by *candida glabrata* 29%, *candida tropicalis* 13%, *candida parapsilosis* 9% and *saccharomyces cerevisiae* 11%.[9]

The management of candida associated denture stomatitis is complex procedure because of its multifactorial etiology. The medical strategy adopted includes the use of topical and systemic antifungal drugs, the use of preservatives and disinfectants, irradiation with microwaves, careful removal and control of the plaque present on the denture and on the oral mucosa.[10]

Polyenes (Nystatin and Amphotericin B) and azoles (e.g., Clotrimazole, Fluconazole) are the main drug groups for treating oral superficial candidiasis. For some decades, systemic antifungal agents have been successfully used to prevent mucosal as well as invasive fungal infections. However, due to their side effects like nausea, vomiting and diarrhea and potential emergence of resistant strains, antifungal prophylaxis has not been totally successful. The commonly used antifungals are the polyenes example Nystatin and Amphotericin B and azoles example Fluconazole, Itraconazole, Voriconazole. Interestingly, the biofilm phase of candida is much more resistant to all of these antifungals. The limited spectrum and toxicity of available antifungals and the gradual emergence of resistance to these drugs are of great concern.[7]

Clotrimazole is a well-tolerated fungistatic drug with anticandida and anti-staphylococal activity. Clotrimazole is used for treatment of oral candidiasis and is also effective for prophylaxis in patients undergoing chemotherapy, myeloablative treatment, and transplant recipients and for patients with solid malignant neoplasms. Clotrimazole is an imidazole derivative which works by inhibiting the growth of individual *Candida* or fungal cells by altering the permeability of the fungal cell wall. It binds to phospholipids in the cell membrane and inhibits the biosynthesis of ergosterol and other sterols required for cell membrane production. Clotrimazole causes slow fungal growth or result in fungal cell death. Hence, the search for alternative natural products with less or no side-effects continues and phytochemicals isolated from plants used as traditional medicines are considered as better alternatives. The common side effects of oral formulation include itching, nausea, and vomiting.[11]

Morinda citrifolia commonly known as "Indian mulberry", "cheese fruit", or "Noni" was first discovered in Southeast Asia, is now prevalent almost in all parts of the world.[12] It is called "Yor" in Thailand. It belongs to family Rubiaceae. Morinda citrifolia fruit is oval in shape and will turn from a greenish hue to a yellowish-white colour when it ripens. Morinda citrifolia is reported to have a broad range of therapeutic effect, including antifungal, anti-inflammatory, anti-microbial, anti-carcinogenic, analgesic, and immune enhancement effect.[12] It is also used as an antiviral, antitumor, anthelminthic, and antihypertensive agent. All the part of Morinda citrifolia (roots, bark, leaf, bud and fruit) currently are being used to treat a wide range of health problems.[12] The fruit contains polysaccharide, scopoletin, peroxeronine, vitamins and minerals. The juice of this fruit is popular as a medicinal drink and is used to treat different kinds of illnesses such as muscle pain, arthritis,

diabetes, hypertension, cardiovascular disease, menstrual disorders, gastrointestinal disturbances, and cancers.[13]

It is used in dentistry as an endodontic irrigant, intracanal medicament and has inhibitory effect against caries causing microorganism. It is also used as a mouthwash and disinfectant for artificial prosthesis. [13,14] The Pathogenicity of candida is associated with the ability of the fungi to convert from a cellular yeast to a filamentous form, Morinda citrifolia modifies the microbial balance of host by reducing the overgrowth of pathogens such as candida by interfering with serum induced morphological conversion from cellular to filamentous yeast.[15,16]

The ability of Morinda citrifolia (Noni) to treat candida supersede that of commercially available anticandida agents. They act as prophylactic as well as adjunctive therapy against Candidiasis. Thus, considering the significance of use of natural supplements in the management of oral candidiasis, this study was taken up to compare the anticandida effect of Clotrimazole and Morinda citrifolia on prevalence of oral candida among denture wearers.

NEED FOR THE STUDY

Candida is a normal commensal organism in the oral cavity of healthy people. When the host defense system suffers from any local or systemic alteration *candida albicans* becomes virulent and causes oral candidiasis.

Candida is able to adhere and proliferate to the mucous surfaces as well as to the prosthetic materials. The plaque accumulated on the denture and the poor oral hygiene contributes to oral candidiasis. Dentures have micro pits and micro porosities which makes possible for the yeasts to harbour and thus difficult to eliminate them. Poor oral hygiene and ill-adapted dentures facilitate the adhesion and penetration of the yeast resulting in increased permeability of the epithelium to toxins produced by candida.[10]

Gediatric population due to advancing age have reduce immunity and which brings about change in oral physiology which deteriorates the natural oral defense, leading to candidiasis. They are vulnerable to candidiasis which are provoked by chronic diseases, medication, poor oral hygiene, reduced salivary flow, impairment of the immune system, breakdown of the ability of natural suppression of yeast.[9]

Certain medications have the ability to reduce the salivary flow which induces change and imbalance of the normal microbial communities favouring the over proliferation of candida. Several antifungal agents are commercially available in market, such as Amphotericin B, Clotrimazole, Miconazole, Nystatin, but these agents can alter the micro flora and have several side effects such as nausea, vomiting, gastrointestinal distress, fever, chills and nephrotoxicity. Hence, the search for alternative natural products with less or no side-effects continues.

Morinda citrifolia has various therapeutic effects. Morinda citrifolia modifies the microbial balance of host by reducing the overgrowth of pathogens such as candida by interfering with serum induced morphological conversion from cellular to filamentous yeast.

The research related to antifungal effect of Morinda citrifolia on multispecies oral candida are at sparse. Hence it is required to evaluate the effect of Morinda citrifolia (NONI) on different candida. Therefore this in vivo study was undertaken with an intent to evaluate and compare the antifungal activity of Clotrimazole and Morinda citrifolia against candida species among denture wearers.

HYPOTHESIS

1) NULL HYPOTHESIS:

There is no anticandida effect of Clotrimazole and Morinda citrifolia (NONI) on the prevalence of oral candida in denture wearers.

2) RESEARCH HYPOTHESIS:

There is anticandida effect of Clotrimazole and Morinda citrifolia (NONI) on the prevalence of oral candida in denture wearers

AIMS AND OBJECTIVES

AIM OF THE STUDY:

To evaluate the anti-candida effect of Clotrimazole and Morinda Citrifolia (Noni) on the prevalence of oral candida in denture wearers.

OBECTIVES:

1. To evaluate the incidence of multispecies candida in denture wearers.
2. To evaluate the anti-candida effect of Clotrimazole and Morinda citrifolia (Noni) on prevalence of multispecies candida in denture wearers.
3. To compare the anti-candida effect of Clotrimazole and Morinda citrifolia (Noni) on prevalence of multispecies candida in denture wearers**.**

REVIEW OF LITERATURE

Brian c. muzyka et al. in 1995 gave an overview on oral fungal infection and its appropriate therapy. There are several oral fungal pathogens that often are markers for early signs of immune deterioration. The most common oral fungal infection is caused by the Candida species. It has four clinical presentations- Pseudomembranous, erythematous, hyperplastic and angular cheilitis. In both immunocompetent and immunosuppressed individuals, the prevalent species is *Candida albicans*. *Candida glabrata* and *Candida tropicalis* are also common. Denture stomatitis is a chronic inflammatory condition of denture-bearing mucosa, either localized or generalized. The inflammation is caused by trauma from ill-fitting dentures or from an allergic response to the denture base material along with a superimposed candida infection. There are several predisposing factors of oral candidiasis including drugs immunodeficiency, malignancy. There are several regimes for candida treatment that can be applied topically examples are Clotrimazole and Nystatin. The systemic medication for treatment of candida includes ketoconazole and fluconazole. Clotrimazole is synthetically derived and is available as a cream, lotion, oral rinse, oral troche and vaginal tablet. Clotrimazole alters cell membrane permeability by binding with phospholipids in the fungal cell membrane. Clotrimazole is Fungi static / fungicidal and acts against gram-positive bacteria. Current studies indicate the various etiological factors involved in denture stomatitis and appropriate therapy. [17]

S. R. Lockhart et al in 1999 conducted a study to test whether the increase in candida colonization is age related in a fashion independent of denture use. Candida colonization of the oral cavity increases in the elderly. A predisposing condition is denture use, which also increases in the elderly. The author analyzed the frequency of

carriage, the intensity of carriage, the multiplicity of species, and the genetic relatedness of strains in the oral cavities of 93 test subjects separated into the three age groups: 60 to 69 years, 70 to 79 years, and more than or equal to 80 years. Each group was further subdivided into subjects with and without dentures, and into males and females. The results demonstrate that the frequency of carriage, the intensity of carriage, and multispecies carriage all increase as a function of age and differ according to gender, in both cases independent of denture use, suggesting that the natural suppression of yeast carriage in the oral cavity breaks down in the elderly. In addition, it is demonstrated that *candida glabrata* colonizes the oral cavities of elderly individuals without dentures only after 80 years of age, suggesting that there are age-related compromising conditions other than denture use in this most elderly age group.[9]

Anna R. Dixon et al in 1999 presented a review article on noni being the traditional Polynesian medicine. They presented the list of medicinal use of Morinda citrifolia in south East Asia and Polynesia. Various parts of Morinda citrifolia are used for medicinal purpose. The fruit, flower, leaves, bark and root. Each part has its own medicinal application and are used to treat various disorders. Noni can also be applied topically for sprains and swollen limbs. It also plays an important role in healing deep open wounds. Leaves of noni were applied for wounds, bruises, sores, and boils. The noni juice is mixed with water and drunk one to three times a day empty stomach. Noni juice has been used to treat eye, skin and mouth and throat infection also diabetes, high blood pressure, headache, digestion and arthritis. [18]

Wang Mi an-ying et al. in 2002 presented a review article that gives an overview on Morinda citrifolia (NONI) and its recent advances. It has been reported to have broad

range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumor, anti-helminthic, analgesic, hypotensive, anti-inflammatory and immune enhancing effect. NONI plant is used as an alternative to medicine for different kind of illness such as arthritis, diabetes, high blood pressure, muscle aches and pains, menstrual difficulties ,headache, heart diseases , AIDS, cancer , gastric ulcer , sprains , etc.[19]

Siriorn Paritpokee et al in 2005 evaluated CHROMagar candida using a battery of yeast isolates, which represent the most commonly encountered human yeast pathogens, as a possible means of more rapid identification. 139 yeast isolates consisting of 15 *candida albicans*, 17 *candida parapsilosis*, 16 *candida glabrata*, 17 *candida tropicalis*, 13 *candida krusei*, 9 *candida lusitaniae*, 2 *candida rugosa*, 3 *candida kefyr*, 16 *candida neoformans*, 10 *rhodotorula species*, 13 *saccharomyces species*, 8 *trichosporon species* were studied. These were inoculated onto CHROMagar candida medium and incubated at 37°C, according to manufacturer's suggestions. The colours and colonial features were observed at 24 and 48 hours and compared to the conventional identification. All *candida albicans* and *candida krusei* isolates had species-specific colony characteristics and colour at both 24 and 48 hours. At 24 hours, 6 out of 8 *trichosporon species* isolates showed unique flat-light blue colonies. The rest of the yeast isolates were underdeveloped at 24 hours i.e colours were non-distinct. At 48 hours, 10 out of 17 *candida parapsilosis* were light pink, whereas the remaining 7 were creamy white. 13 out of 16 *candida glabrata* were also pink and the remaining 3 were creamy white at 48 hours. We could not reliably differentiate *candida parapsilosis* from *candida glabrata* at 48 hours, but the colour was often different from other yeasts. The colour and colonial characteristics of the following yeasts were present at 48 hours: 16 out of 17 *candida tropicalis* were metallic blue, all 3 *candida kefyr* were light pink with pink center, 14 out of 16

candida neoformans were white, 9 out of 10 of *candida rugosa* were orange-pink, 7 out of 8 *trichosporon* were light blue, and all 13 Saccharomyces species were purple. The article concluded that CHROMagar candida is a useful method for identifying certain yeasts, such as *candida albicans* and *candida krusei*. Colours were developed for some yeasts, such as *candida albicans* and *candida krusei*, by 24 hours incubation, whereas many strains required 48 hours incubation. Although we could not distinguish *candida parapsilosis* from *candida glabrata* by colour, most isolates could be distinguished from other *non-candida glabrata*, *non- candida parapsilosis* isolates at 48 hours.[20]

Daniluk T et al in 2006 determined the fungi occurrence rate in the oral cavity of denture wearer patients in comparison to those without dentures. Samples for mycological examinations from the tongue dorsa, palatal mucosa, and mucosal surfaces of dentures were collected from patients with dentures while tongue and palate swabs were taken from those without dentures. All oral specimens were placed on Sabouraud glucose agar. The results were evaluated. Dental and mycological examinations were performed in 95 patients, out of which 57 (60.0%) used complete or partial dentures and 38 (40.0%) had their own dentition (without dentures). The occurrence rate of oral *Candida albicans* in patients with dentures was higher than in patients without dentures.[21]

Saswati Banerjee et al. in 2006 carried out a study to evaluate whether Morinda citrifolia (Noni) interferes with the Serum-induced formation of filamentous Structures in *Candida albicans.* The *Candida albicans* was grown overnight with noni extract and the growth was monitored measuring the optical density at 600nm. The cells were then inoculated in solid media and liquid media. The study demonstrated

that noni contains a water-soluble component which has a potential therapeutic value in preventing the formation of filamentous structures in candida albicans and aspergillosis.[14]

Crispian Scully et al in 2007 presented an overview of the management issues associated with systemic diseases that are more common in older adults than in younger people, even among those who are functionally independent. Systemic diseases are more common in older adults than in younger people, even among those who are functionally independent. Dentists should understand how these diseases can affect the dental care of their aging patients. To provide good oral health care, dental professionals must understand the special needs of older people and their ability to undergo and respond to care; they should work closely with the rest of the health care team and they should be prepared to manage emergencies.[4]

Jensen et al. in 2007 presented a review on Morinda citrifolia its composition, methods and oral care. Morinda citrifolia has an excellent antimicrobial property and is biocompatible. It is beneficial in treating one or more of the dental disorders like periodontal disorders, such as gingivitis and periodontitis, tooth decay, halitosis, and mouth irritation. It sufficiently reduces the plaque formation. The fruit is juicy, bitter, dull yellow and contains seeds. Morinda citrifolia produces xeronine. Xeronine is an active dormant enzyme found in small intestine. Morinda citrifolia can be incorporated in dentifrice powder, gel, paste mouthwash, tablets and can be made commercially available.[22]

Sathish Kumar Jayaraman et al. in 2008 the study was done to determine antifungal potential of Morinda citrifolia fruit extracts. The yield of Morinda citrifolia extract was made using three solvents i.e. methanol, hexane, and ethyl acetate. Potato

dextrose agar was prepared and 1 mL of fruit extract was added to 100 mL of medium. A loop full of culture was placed on the center of the plate. Controls were maintained. The growth of the fungal cultures was measured and compared with their respective control plates. Results demonstrated that the methanol extract of Morinda citrifolia fruits remarkably inhibited the mycelia growth of all the fungi.[23]

Jainkittivong et al in 2009 evaluated the antifungal activity of Morinda citrifolia fruit extract against *candida albicans*. The antifungal activity of Morinda citrifolia extract against *Candida albicans* was tested at various concentration (10, 20, 30, 40, 50, and 60mg/ml) and for different contact time (15, 30, 45, 60,75and 90 minutes). The colony forming units were then evaluated on each agar plate. Results demonstrated that Morinda citrifolia fruit extract at a concentration of 50 mg/ml partially inhibited candida growth at 15 min contact time and completely inhibited candida growth at contact time of 30 min. The concentration of 60mg/ml showed complete inhibitory effect at all contact times.[13]

M Rathee et al in 2009 presented a review article that gives an overview of various methods and the materials used for cleaning dentures for geriatric denture wearers. It also states that it is the responsibility of the patient to maintain oral hygiene through a daily home care routine and it is the obligation of the dentist to motivate and instruct the patient and provide the means and methods for plaque control. Denture therapy for geriatric patient is expected to be in high demand owing to expanding proportion of elderly population. Oral hygiene has been found to be poor among the elderly. Patients are either unable or unmotivated to clean their dentures adequately. A simple effective denture cleaning methods that removes and/or kills micro-organisms on acrylic resin denture are desirable.[1]

Seema Pattanaik et al in 2010 published a review article on denture stomatitis regarding the various etiological factors like mechanical irritation by the dentures, traumatic occlusion, an accumulation of microbial plaque, fungal infection, a toxic or allergic reaction to components of the denture material contributing to denture stomatitis and its treatment. Denture stomatitis is a prevalent and long standing problem in complete denture wearers. Post denture placement produces significant changes in the oral environment that may have an adverse effect on the integrity of the oral tissues. Treatment of denture induced stomatitis differs depending on the causes of the disease. In most of the patient, the elimination of mechanical and traumatic factors, the consistent use of oral hygiene measures, and the administration of local anti-mycotic therapy usually enables the inflammatory lesions to heal rapidly.[24]

Usha et al in 2010 evaluated the antimicrobial activity of Morinda citrifolia against various organism. *Morinda Citrifolia* is one of the most important traditional Polynesian medicinal plants. This small evergreen tree is native from South Eastern Asia to Australia and now it has a Pan tropical distribution. It has antifungal, antibacterial, anti-inflammatory and antiviral activities. *Morinda citrifolia L.* was studied for its antimicrobial activity. The leaves of this plant were dried, powdered and different extracts were prepared using different solvents like benzene, chloroform, ethyl acetate, ethanol and water. Four organisms, namely *E.coli, Staphylococcus aureus, Candida albicans* and *Aspergillus niger,* were used for investigation. The activity of each solvent extract was checked on each organism by disc diffusion method and then the zone size of each was measured. The results of our antimicrobial assay revealed that plant.[25]

Carmen Salerno et al in 2011 presented a systematic review article on candida associated denture stomatitis. *Candida albicans* is a dimorphic yeast strongly gram positive able to live as normal commensal organism in the oral cavity of healthy people. It is the yeast more frequently isolated in the oral cavity. Under local and systemic factors related to the host conditions, it becomes virulent and responsible of oral diseases known as oral candidiasis. It has been shown that the presence of denture is a predisposing factor to the onset of pathologies related to *candida albicans*. Clinical studies have shown that *candida albicans* is not only able to adhere to the mucous surfaces, but also to stick to the acrylic resins of the dental prostheses. Both the plaque accumulated on the denture and the poor oral hygiene contributes to the virulence of candida, offering the clinical picture of candida associated denture stomatitis. The therapeutic strategies currently adopted in the clinical practice to overcome these fungal infections provide for the use of topical and/or systemic antifungal and topical antiseptics and disinfectants, the irradiation with microwaves and the accurate mechanical removal of the bacterial plaque from the denture surfaces and from the underlying mucosa. This article also suggests that a correct oral hygiene is important for the control of the bacterial biofilm present on the denture and on the oral mucosa and it is the fundamental base for the prophylaxis and the therapy of the candida associated denture stomatitis.[26]

Kamiar Zomorodian et al in 2011 investigated risk factors associated with progression to candida related denture stomatitis in patients using complete dentures, and genetically identified candida isolates associated with disease and colonization. Candida species are considered the primary causative agents of denture stomatitis, but their role in colonization and disease in denture wearers remains undefined. 114 retirement home residents were recruited for this study, from which oral mucosa

samples were collected and cultured following oral cavity examination. Morphologic analysis was used to identify potential yeast positive cultures, which were then characterized further by restriction fragment length polymorphism (RFLP) analysis. *Candida albicans* was the most frequently recovered species followed by *candida glabrata* and *candida tropicalis*. In addition, 16 isolates of *candida dubliniensis* were recovered, which was the first identification of this species in clinical samples from Iran. Study demonstrated a significant association between the duration of denture wear and oral candidiasis. Furthermore, it was noted a high prevalence of *candida dubliniensis* in complete denture wearers.[27]

Sonia Silva et al in 2012 in their review article provides information on the current state of knowledge on the biology, identification, epidemiology, pathogenicity and antifungal resistance of *candida glabrata, candida parapsilosis* and *candida tropicalis*. The incidence of infections caused by Candida species has increased considerably over the past three decades, mainly due to the rise of the AIDS epidemic, an increasingly aged population, higher numbers of immunocompromised patients and the widespread use of indwelling medical devices. *Candida albicans* is the main cause of candidiasis; however, *non-candida albicans candida (NCAC)* species such as *candida glabrata, candida tropicalis* and *candida parapsilosis* are now frequently identified as human pathogens. The apparent increased emergence of these species as human pathogens can be attributed to improved identification methods and also associated with the degree of diseases of the patients, the interventions that they were subjected and the drugs used. Candida pathogenicity is facilitated by a number of virulence factors, most importantly adherence to host surfaces including medical devices, biofilm formation and secretion of hydrolytic enzymes. Furthermore, despite extensive research to identify pathogenic factors in fungi, particularly in *candida*

albicans, relatively little is known about non-*candida albicans* candida (NCAC) species.[28]

Najla S.Dar-Odeh et al in 2012 evaluated the role of antifungal agents in denture stomatitis. Denture-associated stomatitis (DAS) (*Candida*-associated denture stomatitis, chronic atrophic candidiasis, denture-sore mouth) is a chronic infection of the oral mucosa caused solely by *Candida* species or in association with bacteria. The role of *Candida*, and specifically *C. albicans*, in development of denture stomatitis is associated with pathogenic overgrowth of *Candida* on denture surfaces and the oral mucosa, and is widely accepted as a leading etiological factor. There are several antifungal agents used in treatment of denture stomatitis.

Antifungal agents are either polyenes (Nystatin and Amphotericin B) or azoles which are classified into: Imidazoles (Clotrimazole, Econazole, Fenticonazole, Isoconazole, Ketoconazole, Miconazole, Tioconazole); and Triazoles (Fluconazole, Itraconazole). Some of the drugs can be applied topically and some can be used in systemic forms. It was shown that most *Candida* species are susceptible to topical antifungal drugs like Amphotericin B, Nystatin Miconazole and Clotrimazole. Systemic medication are given in patients with poor compliance. Antifungal drugs should be used alongside denture care. Antifungal drugs certainly have a place in treating denture stomatitis.[29]

Vinaya Bhat et al. 2013 determined the prevalence of candida associated denture stomatitis and speciation of candida among complete denture wearers of south west costal region of Karnataka. 55 completely edentulous patients wearing complete denture prosthesis at least for the past one year was selected. Swabs were collected from the tissue surface of maxillary denture samples were cultured on Sabouraud dextrose plate (SDA) to get pure culture of candida culture. The phenotypes were

defined by on culture on CHROMagar candida plates. The different species were obtained by the colour displayed by them as *Candida albicans* (light green), and *Candida glabrata* (pink) and *Candida tropicalis* (blue to metallic blue). The later species were identified by gram staining and later confirmed by germ tube test, corn Meal agar for chlymydospore formation and biochemical test. This study concluded that candida associated stomatitis is prevalent in denture wearers. Males are affected more than females. *Candida albicans* is the most prevalent of candida followed by C. tropicalis and C. glabrata.[30]

Jonas Glang et al. in 2013 determined the effect of Morinda citrifolia L. fruit juice on gingivitis and periodontitis. 11 were selected and divided into 5 in control group and 6 in test group (noni juice). Bacterial samples were taken at the beginning. Papillary bleeding index and the plaque index was also evaluated. Patients in noni group was asked to additionally rinse with 30 ml noni juice plus 30 ml water in mouth for 2min with subsequent swallowing twice a day for total duration of four weeks. The antimicrobial profile and sensitivity of bacteria towards noni juice was investigated. The papillary bleeding index in noni group had a highly significant improvement after four weeks. An in vitro experiment was done with aerobic and anaerobic to evaluate antibacterial activity of noni juice. A complete zone of inhibition was observed at noni juice dilution of 1:2 and 1:4. The investigation showed the presence of good oral hygiene and administration of noni juice was a promising treatment of gingivitis and periodontitis.[31]

Barani et al. in 2014 determined antifungal activity of Morinda citrifolia (NONI) extract against *candida albicans*. Antifungal activity was determined using disc diffusion method. Samples were loaded at various concentration (1000µg, 500µg,

250µg and 100µg). The samples were compared with positive control amphotericin B. The microbial growth was determined by measuring the diameter of zone of inhibition. Results showed that Morinda citrifolia extract at 1000µg/ml concentration effectively inhibited the growth of *Candida albicans* compared with positive control amphotericin B. Morinda citrifolia fruit extract had an anti-fungal effect on C. albicans and the inhibitory effect varied with concentration.[32]

Vennila Srinivasahan et al. in 2014 evaluated the antimicrobial activities of hydroethanolic extract of Morinda citrifolia fruit. Hydroethanolic extract of Morinda citrifolia was prepared using 50% ethanol. Antimicrobial activity was screened using disc diffusion method. The antibacterial activity of hydroethanolic extract of M.citrifolia at 100µg/ml concentration was found to be more effective against Klebsiella pneumonia than other tested organism. At concentration of 20 µg/ml antifungal activity of Morinda citrifolia was effective against Aspergillus fumigatus. Thus, Morinda citrifolia extract has a dose dependent antimicrobial activity against tested bacteria and fungi. Morinda citrifolia constitutes bio active principles responsible for antibacterial and antifungal activity including medicinal values and physiological activities.[33]

Carla Garcia-Cuesta et al. in 2014 gave a comprehensive review on candidiasis. Candidiasis or oral candidosis is one of the most common human opportunistic fungal infections of the oral cavity. This pathology has a wide variety of treatment which has been studied until these days. The present study offers a literature review on the treatment of oral candidiasis, with the purpose of establish which treatment is the most suitable in each case. Searching the 24 latest articles about treatment of candidiasis it concluded that the incidence depends on the type of the candidiasis and

the virulence of the infection. Although Nystatin and Amphotericin B were the most drugs used locally, Fluconazole oral suspension is proving to be a very effective drug in the treatment of oral candidiasis. Fluconazole was found to be the drug of choice as a systemic treatment of oral candidiasis. Due to its good antifungal properties, its high acceptance of the patient and its efficacy compared with other antifungal drugs. But this drug is not always effective, so we need to evaluate and distinguish others like Itraconazole or Ketoconazole, in those cases when *Candida* strains resist to Fluconazole.[34]

Andréa Araújo De Vasconcellos et al. in 2014 presented a review on *Candida* associated Denture Stomatitis. *Candida*-associated denture stomatitis is a common fungal infection that affects removable denture wearers. Although *Candida spp.* are considered commensal fungal in the oral cavity, changes in local and/or systemic predisposing factors related to the host conditions may lead to pathogenic form and cause disease. Local factors such as irradiation, trauma, xerostomia, complete denture wearers, poor dental hygiene, smoking, carbohydrate-rich diet contribute as a risk factors in development of diseases. Systemic factors such as diabetes, chemotherapy, hemophilia and immunosuppressed patients contribute to candida associated denture stomatitis. The therapeutic strategies commonly adopted in the clinical practice are the use of topical or systemic antifungal, in addition to removing mechanically the plaque from denture surfaces and from underlying mucosa and giving instructions about the correct oral hygiene to the patient.[35]

Bharthi Prakash et.al in 2015 evaluated the prevalence of candida spp. among healthy denture and non-denture wearers with respect to hygiene and age. 100 subjects were included in the study, among which 50 were denture wearer and 50

were non denture wearer aged between 35- 80 years. Sample collection for denture wearers were done from the tissue bearing area of the upper denture and for the non-denture wearers was done from palatal mucosa. This loopful of suspension was placed on sabouraud dextrose (SD) agar containing gentamycin and chloramphenicol. Identification was done by the growth of candida on SDA. Results demonstrated denture wearer harbour a mixed species of candida which is predominantly *candida albicans* (58%), followed by *candida tropicalis* (28%), *candida glabrata* (2%) wherein all the age group showed more than 2 different candida species. In contrast non denture wearers, *candida albicans* was observed to be dominant (96.2%) species. In comparison to non-denture wearers, multispecies candida colonized the denture thus presenting higher risk of candidiasis especially with increasing age .[36]

A.Shafath Ahmed et al. in 2015 determined the antibacterial efficacy and the effect of Morinda citrifolia L. mixed with irreversible hydrocolloid for dental impression .Twenty volunteer were randomly divided into 2 groups. Group A 30 ml extract of Morinda citrifolia L was diluted in 30ml of water and was mixed with irreversible hydrocolloid. Group B 30 ml of deionized water was mixed with irreversible hydrocolloid material to make the impression and the surface roughness and dimensional stability was evaluated. The results demonstrated that the extract of Morinda citrifolia with irreversible hydrocolloid decreased the percentage of organism when compared to water but did not affect the surface quality or dimensional stability.[37]

Dheeraj Sharma et al in 2015 gave a comprehensive review of etiopathogenesis and management and current trends in management of denture stomatitis. Denture stomatitis is the most prevalent and long-standing problem in denture wearers. The

etiopathogenesis of denture stomatitis is multifactorial and complex to understand. The placement of denture produces significant changes in the oral environment and adversely affects the integrity of oral tissues. The combination of entrapment of yeast cells in irregularities in denture-base and denture relining materials, poor oral hygiene and several systemic factors is the most probable cause for the onset of this infectious disease. Hence colonization and growth on prostheses by candida species are of clinical importance.[3]

Jolanta E Loster et al. in 2016 evaluated the intensity, genera, and frequency of yeasts in the oral cavity of complete denture wearers in terms of subject gender and age. 920 patients (307 males and 613 females) with complete upper dentures were selected for the study and divided into four age groups of 50 years, 51–60, 61–70, and 70 years. Yeast samples were taken as a smear from the palate. There was a statistically significant relationship between the intensity of yeast growth and the gender. In every age group, the number of infection-free individuals was greater among males than females. Intermediate, intense, and abundant growth of yeast occurred most frequently in the youngest group of females. The genera of Candida species and the frequency of yeast infection in denture wearers appear to be influenced by both age and gender.[38]

Sri Rezeki et al. in 2017 performed a vivo study in Wistar rats to determine the effect of Morinda citrifolia leaves extract on wound healing percentage in oral mucosa. Traumatic ulcer is a lesion in oral mucosa caused by trauma. Traumatic ulcer resulting pain can disturb activities and metabolism. Noni leaves (Morinda citrifolia L) contain flavonoid, saponin, and tannin as anti-inflammatory, anti-bacterial, and antioxidant to accelerate healing process. This research aimed to determine the effect of Morinda

citrifolia L leaves extract on healing percentage of traumatic ulcer in oral mucosa. Ulcer was made by put hot burnisher on lower labial mucosa of twelve Rattus norvegicus for a second. They were divided into three groups. First group was given Morinda citrifolia L leaves extract, control group were given triamcinolone acetonide 0,1% and aquades. The research was performed for 10 days by measuring ulcer diameter and application of this extract. Morinda citrifolia L leaves extract has no significant effect on wound healing percentage of traumatic ulcer compare to positive control and negative control.[39]

Dhivya Dilipkumar et al in 2017 evaluated the repercussion of Noni mouthwash on surface characterization of nickel‑titanium arch wire. Pre-formed 0.014-inch NiTi arch wire were used. The study comprised of two samples, one control and one test sample which were 25mm in length. Control sample was stored at room temperature without any manipulation while test sample was immersed in Noni mouthwash solution for 1.5 hours, after which the test specimen was removed from the mouthwash solution and rinsed with distilled water. Both control and test samples were sent for scanning electron microscopy analysis, to qualitatively characterize the topography of the wire surface. Electron dispersion spectrum analysis was done to evaluate the various components of both the wires. Noni mouthwash did not have significant influence on the surface roughness or altered the composition of the Ni-Ti wire. Hence, Noni mouthwash may be prescribed as a natural, non-destructive prophylactic agent for orthodontic patients.[16]

Shruti Nayak et al in 2017 conducted a comparative study of candida by conventional and CHROMagar method in non-denture and denture wearers by oral rinse technique. The study was performed to isolate, quantify, and speciate candidal

species in non-denture wearers (controls) and denture wearers (study group) by the oral rinse technique. Isolation was done using Sabouraud dextrose agar (SDA). Speciation was done using conventional methods like the germ tube test, carbohydrate fermentation test, urease test, as well as the CHROMagar method. Salivary samples for candida evaluation were collected from the subjects in sterile sample containers, using the oral rinse technique. *Candida glabrata* was the most commonly found species among denture wearers and non-denture wearers both by conventional and CHROMagar methods. In males, *candida albicans* was the predominant species, whereas *candida glabrata* was the predominant species in females. Candidal colonization was higher in denture wearers compared to non-denture wearers, especially among females. It was concluded, both conventional and CHROMagar methods can be used for isolation and speciation of candida but the CHROMagar method is more rapid compared to the conventional methods. Candidal colonization was higher in denture wearers compared to non-denture wearers, especially in females.[5]

Hussain Mookhtiar et al in 2018 presented a review article that overviewed Morinda citrifolia in the field of endodontics. A literature review was conducted based on the data based such as PubMed, Google Scholar, Scopus, using keywords “Herbal Medicine” and “M.citrifolia”. A better and advanced search was carried out the keywords using “Endodontics”, “Root Canal Therapy”,“ Irrigation”, “Herbal Endodontics”, “ Endodontic Disinfectant”. The MCJ is used as an endodontic irrigant along with EDTA and as an adjuvant to sodium hypochlorite and chlorhexidine due to its better smear layer removal properties and antimicrobial activity. It does not have any major side effect than as compared with sodium hypochlorite and Chlorhexidine. It also helps in caries prevention and control. So Morinda citrifolia is used as an

alternative to sodium hypochlorite in irrigation of root canal, intracanal medicament, smear layer removal and antimicrobial properties.[15]

Ekta Choudhary et al. in 2018 evaluated the efficacy of commercial preparation of Morinda citrifolia and Triphala juice against *E.faecalis* and *candida albicans.* The study was conducted on 84 permanent extracted human teeth. After decoronation and biomechanical preparation, teeth were inoculated (with *E. faecalis* and *C. albicans*) and root sections were divided randomly into four experimental (MCJ, *Triphala* juice, 1% NaOCl, and 2% chlorhexidine [CHX]) among which two were the control groups (preservative control and distilled water). Colony‑forming units (CFUs) obtained for each group were counted on Sabouraud's dextrose and brain heart infusion culture plates. The overall antimicrobial effects of different irrigants were maximum for CHX, whereas MCJ and *Triphala* juice also showed significant reductions.[40]

Yufri Aldi et al in 2019 evaluated the effect of NONI fruit extract (Morinda Citrifolia L.) in gingivitis patient. 15 students were reviewed with the respective mouth washes and were divided into 3 groups. Group 1 consist of extract of noni fruit 10g, Group2 chlorhexidine 200 mg and Group 3 consist of mixture of noni10g and chlorhexidine 200mg. The mouthwash was used after brushing teeth every morning and night for seven consecutive day. For every gargle 15ml for 30 sec. The gingival score was then evaluated. The results analysis showed that there was a significant decrease in gingival index in gargling group with gargle extract of NONI fruit. This is due to the anti-inflammatory and antibacterial effect. Even the use of Chlorhexidine mouthwash showed decreased inflammation. All the mouthwash can be used as an antiseptic the extract noni should be used more than another mouthwash because it gives the same

effect as chlorhexidine and also has minimal side-effects, economical and efficacious.[41]

Monika Singh et al. in 2019 evaluated the antimicrobial effectiveness of propalis, Morinda citrifolia juice, Sodium hypochlorite and chlorhexidine on enterococcus faecalis and *candida albicans* as endodontic irrigant. Standard ATCC strains of enterococcus faecalis and candida albicans were inoculated into peptone water and was incubated for 37 ˚ C for 3 to 4 hours to attain the turbidity to Mc Farland CFU. Different solutions were prepared and different concentration were obtained of propalis (2.5% and 5%), Morinda citrifolia juice (1.5%, 0.75% and 3%), sodium hypochlorite (3%, 2.5% and 0.5%) and chlorhexidine (1%, 0.5%and 0.22%). Disc preparation was done and the zone of inhibition was measured according to Hi-media scale. There was no zone of inhibition for 0.75%, and 1.5% concentration of Morinda citrifolia and a positive relation between increasing concentration and zone of inhibition. 100% of Morinda citrifolia showed highest zone if inhibition. Morinda citrifolia showed a greater zone of inhibition for candida albicans when compared with propalis. Propalis showed a greater zone of inhibition than former for *E.faecalis.*[42]

METHODOLOGY

SOURCE OF DATA:

This in vivo study was conducted on complete denture patients reporting to the Department of Prosthodontics and Crown and Bridge, KAHER KLE V.K. Institute of Dental Sciences, Belagavi. The old denture wearers between the age group of 50 years and above were considered for the study. Microbiological procedures were carried out in the Department of Microbiology, KAHER JNMC, Belagavi. A written and informed consent of all the study subjects in their own language were obtained. This study investigated the anticandida effect of Clotrimazole and Morinda citrifolia on prevalence of oral candida among denture wearers.

METHOD OF COLLECTION OF DATA.

SAMPLE SIZE:

Sixty healthy completely edentulous subjects, wearing dentures for at least one year and having no systemic medical conditions were randomly selected for the study. The study subjects were divided into 2 groups based on usage of Clotrimazole and Morinda citrifolia (Noni).

- Group A (n=30) denture wearers in Clotrimazole group.
- Group B (n=30) denture wearers in Morinda citrifolia (Noni) group.

INCLUSION CRITERIA:

1. Complete denture wearers of above 50 years without any medical history.

2. History of denture wearing since at least last 1 year.

3. Patients without any habits like smoking, tobacco chewing and alcohol.

4. All patients should be ambulatory and healthy.

EXCLUSION CRITERIA:

1. Patient with underlying systemic conditions.

2. Use of any systemic medications.

3. Use of antifungal agents and / or use of mouth rinses.

4. Inability to perform / understand the experimental procedures.

Table 1: Materials used in the study.

Material	Description	Manufacturer
Sterile cotton swabs	-	-
CHROMagar	LOT 0000395188	Hi Media, Belagavi
Clotrimazole	11190635	GLENMARK, Mumbai
Morinda citrifolia (Noni)	11316007000702	PRiiS, Kerala

Table 2: Armamentarium used in the study. (Figure 1)

Armamentarium	Description	Manufacturer
Test tube	-	Borosil
Petri plates	-	-
Biological oxygen demand incubator	Model C1-65 Sr.no- ZBCI-08444	Remi elektrotechnik ltd, India
Straight wire	Nichrome	-

METHODOLOGY:

1) Determination of Minimum Inhibitory Concentrations for Morinda citrifolia (Noni):

A standard procedure for performing the Minimum Inhibitory Concentration test was followed. Candida was revived by plating on Sabouraud dextrose agar and was incubated at 37^{o}C. Isolated colonies were transferred to sterile Sabouraud dextrose broth and were incubated. The growth concentration was adjusted to 10^5 organisms / ml by using 0.5 McFarland's turbidity standard. 200 µl of the Sabouraud dextrose broth was added in six Minimum Inhibitory Concentration tubes. In first Minimum Inhibitory Concentration tube containing 200 µl broth, 200 µl of stock (Noni) was added. After mixing well, 200 µl was transferred in second Minimum Inhibitory Concentration tube. This was continued till 6th tube. From the last well 200 µl was discarded. By following this two-fold serial dilution, the concentration of the Noni was achieved i.e. 200, 100,50,25,12.5,6.25mg/ml. Then to each such test tube 200 µl of earlier prepared strains of candida was added, so that the final volume per tube was 400 µl. The tubes were then incubated for 48 hours at 35^{o}C. The turbidity was then compared. Turbidity in the Minimum Inhibitory Concentration tube indicated growth of the candida implying that it was resistant to Noni. The influence of the pure Noni extract on the growth of candida was investigated by re-plating aliquot on Sabourad dextrose agar plate incubated for 24hr at 37˚C.[43] Based on the results MIC was established at a concentration of 100% i.e. 200 mg/ml.

2) Determination of Cytotoxicity of Morinda citrifolia (Noni).

The MTT solution was prepared using 5 mg in 1 ml of Phosphate Buffer Saline (PBS pH7.4) and Cytotoxicity Assay was performed on mouse fibroblast stain L929. In vitro growth inhibition effect of NONI was assessed by ELISA reader by determination of conversion of MTT into "Formazan blue" by living cells. The 50µl of 4000 cells/ml cell suspension was seeded into each well in a 96 well micro titer plate and final volume was made up to 150 µl by adding Dulbecco's Modified Eagle Medium (DMEM) media. Dilutions of the NONI was prepared at a concentration of 60%, 80% and 100% in DMEM media.100µl of the NONI of different concentrations was added to the wells and incubated for 24 hours, in presence of 5 % CO_2, at 37^0C into CO_2 incubator. After 24 hours, 20µl of 5 mg/ ml MTT reagent was added to the wells. The plate was incubated for 4hours in dark place at room temperature. (The plate was covered with Aluminum foil, since MTT reagent is photosensitive). The supernatant was carefully removed without disturbing the precipitated Formazan crystals and 100 µl of Dimethylsulfoxide (DMSO) was added to dissolve the crystals formed. The optical density (OD) was measured at wavelength of 492 nm. The study was performed in triplicates. The result represents the mean of three readings. Results demonstrated that favorable cell viability was seen with the different concentration of Noni tested. The 60%, 80% and 100% Noni concentration resulted 97.1%, 96.2% and 92.12% cell viability respectively.

FORMULA:

$$\text{Surviving cells (\%)} = \frac{\text{Mean OD of test compound}}{\text{Mean OD at control (untreated cells)}} \times 100$$

3) Microbial analysis:

The study was conducted on completely edentulous subjects wearing dentures since at least last one year who reported to the Department of Prosthodontics and Crown and Bridge, KLE's V.K. Institute of Dental Sciences, Belagavi. These denture wearers were divided into two groups.

- Group A consist of (n=30) denture wearers used Clotrimazole
- Group B consist of (n=30) denture wearer used Morinda Citrifolia (Noni)

Samples were collected by passing sterile cotton swabs across the palatal mucosa and tissue fitting palatal surface of maxillary denture of each test individual (Figure2,3). They were then placed in the plugged test- tubes and sent to the laboratory for plating within 4 hours. (Figure 4)

Identification of candida using CHROMagar:

The swab was gently rolled over the surface of CHROMagar plate along its diameter. (Figure 5). A sterile inoculation loop was used to spread the sample over the surface to get a lawn culture. Plates were then incubated in biological oxygen demand (BOD) at 25 ± 2°C for 24 hours[44] (Figure 6). For the identification of candida colonies, the inoculated agar plates had been divided into two parts as denture surface and palatal mucosa. The number of colonies were scored as colonies forming per unit (CFU) and the colour identification of different candida organisms like *candida albicans, candida tropicalis,* and *candida glabrata* were done on CHROMagar plates. CHROMagar is a special media that yields microbial colonies of varying pigmentation, which is due to the chromogenic substrates that react with the enzymes

secreted by the candida species. Speciation of candida was done by the colour that was exhibited by each species on CHROMagar plate.[5] The light green colonies were suspected of *candida albicans,* metallic blue colonies with or without halo suggested of *candida tropicalis* and white to light pink colonies suggested of *candida glabrata*.[44,45] Presumptive identification were made by colour and morphology of the colonies as per the manufacturer's instructions.

4) Clotrimazole and Morinda citrifolia usage:

The study subjects in group A were instructed to apply Clotrimazole and group B Morinda citrifolia on the intaglio surface of cleaned maxillary denture and on to the palatal surface, and to use the denture in close contact with palatal mucosa. Test individuals were asked to use the Clotrimazole and Morinda citrifolia twice daily for 14 days. Follow up of the patient was done on 15th day. To study the effect of Clotrimazole and Morinda citrifolia on colonization of different candida, sample collection and microbiological procedures was carried out in a similar manner as explained earlier. The resultant colony forming units of different candida in different groups were tabulated. The resultant pre-test and post test data in both the groups were subjected to statistical analysis to draw the conclusion from the resultant data. (Figure7-12)

FLOWCHART OF THE METHODOLOGY

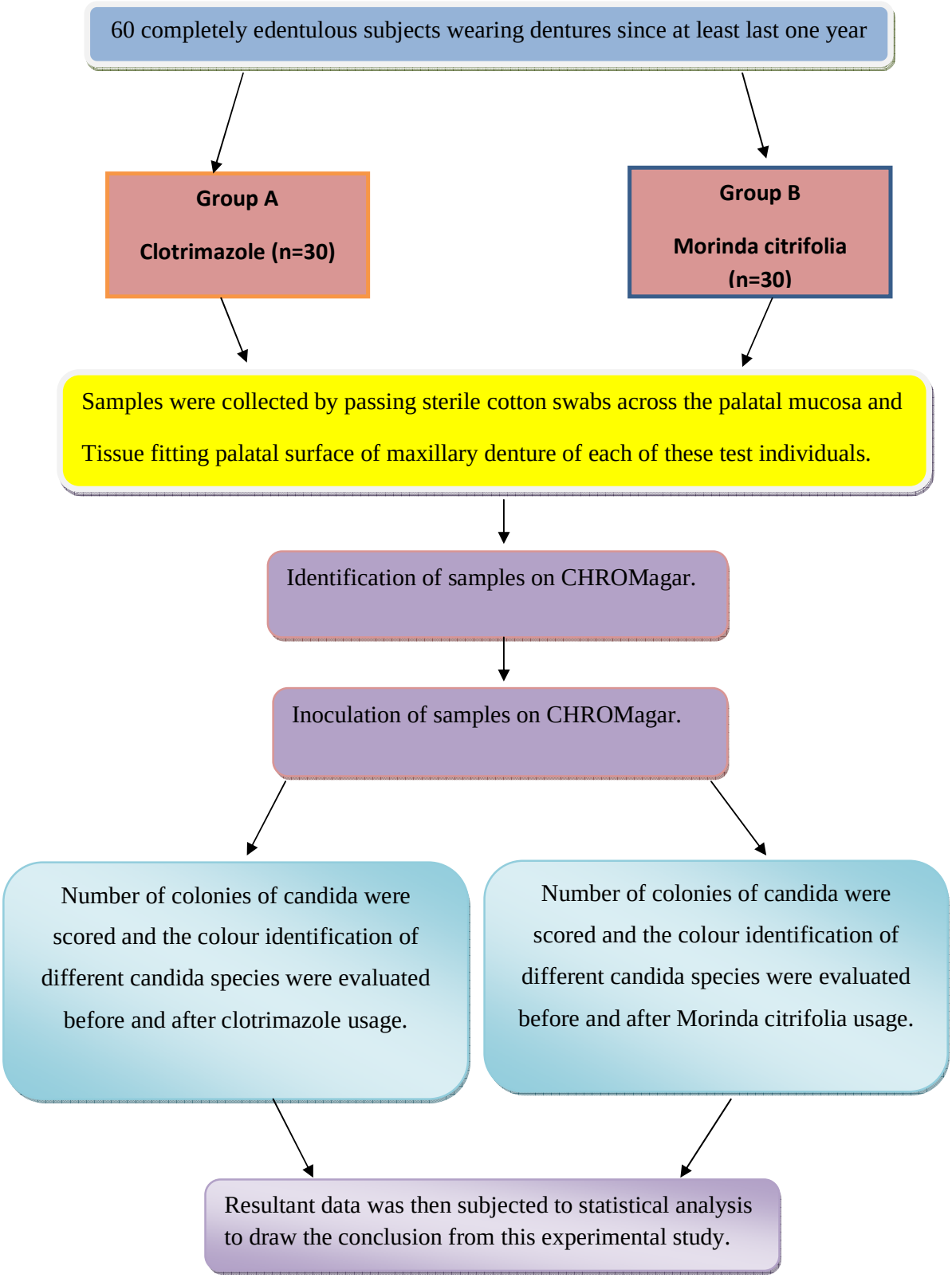


FIGURE NO 1: MATERIALS USED IN THE STUDY

FIGURE NO 2: SAMPLE COLLECTION FROM DENTURE SURFACE

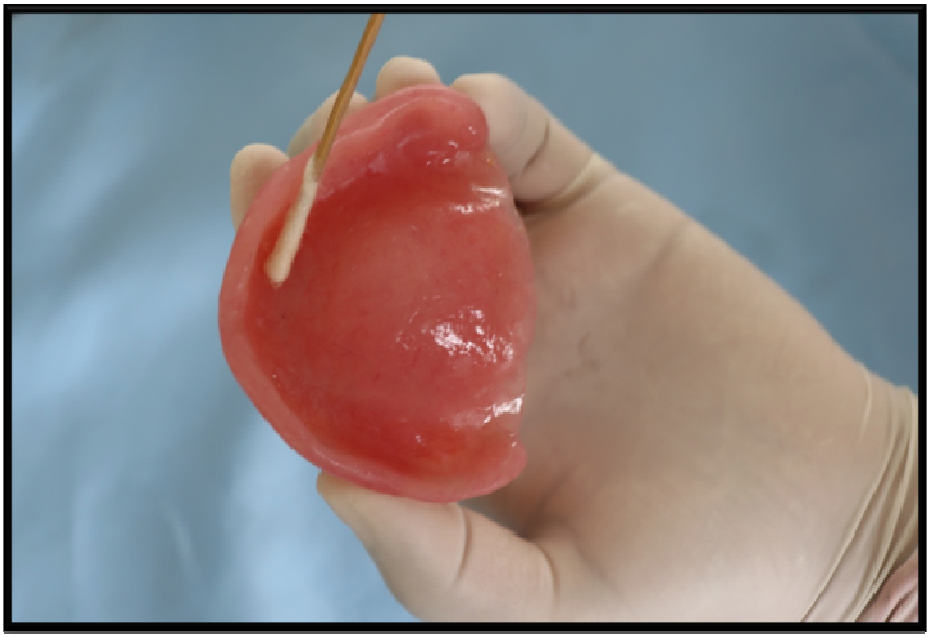


FIGURE NO 3: SAMPLE COLLECTION FROM PALATAL SURFACE

FIGURE NO 4: SWABS TRANSPORTED TO THE LABORATORY

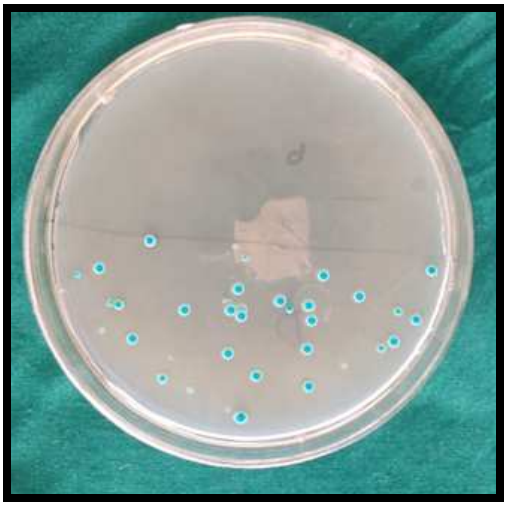
SWABS FROM DENTURE AND PALATAL SURFACE

FIGURE NO 5: CULTURE OF SWABS ON CHROMAGAR PLATE

FIGURE NO 6: INCUBATOR USED FOR INCUBATION OF AGAR PLATES

FIGURE NO 7: IDENTIFICATION OF CANDIDA ALBICANS (LIGHT GREEN COLOUR COLONIES) ON THE DENTURE SURFACE AND PALATAL MUCOSA USING CHROMAGAR

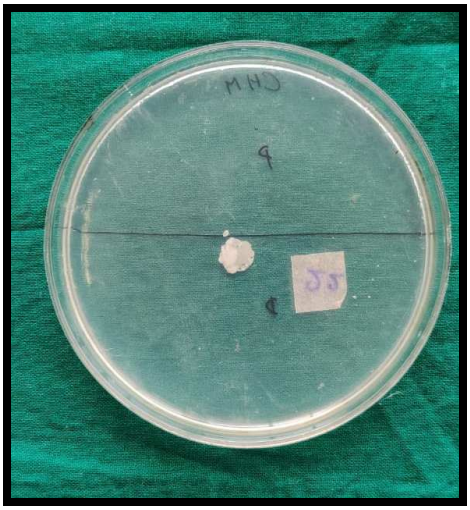
PRE-USAGE OF NONI



POST-USAGE OF NONI

FIGURE NO 8: IDENTIFICATION OF CANDIDA ALBICANS (LIGHT GREEN COLOUR COLONIES) ON THE DENTURE SURFACE AND PALATAL MUCOSA USING CHROMAGAR

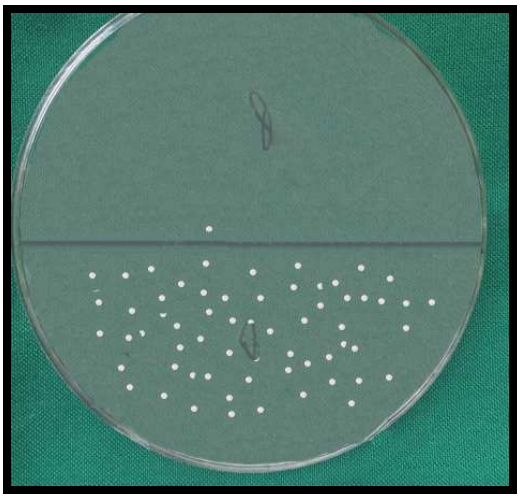
PRE-USAGE OF CLOTRIMAZOLE



POST-USAGE OF CLOTRIMAZOLE

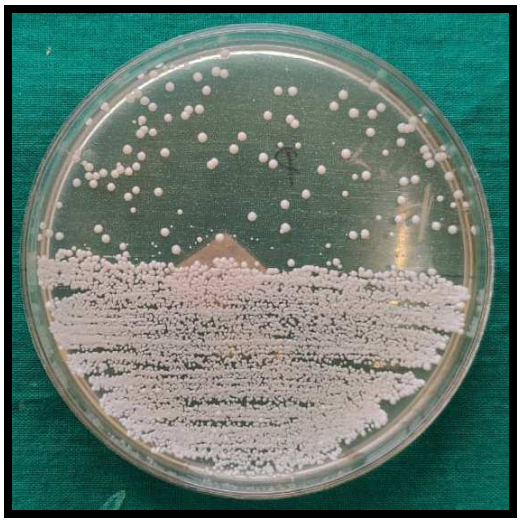
FIGURE NO 9: IDENTIFICATION OF CANDIDA GLABRATA (WHITE TO LIGHT PINK COLOUR COLONIES) ON THE DENTURE SURFACE AND PALATAL MUCOSA USING CHROMAGAR

PRE-USAGE OF NONI

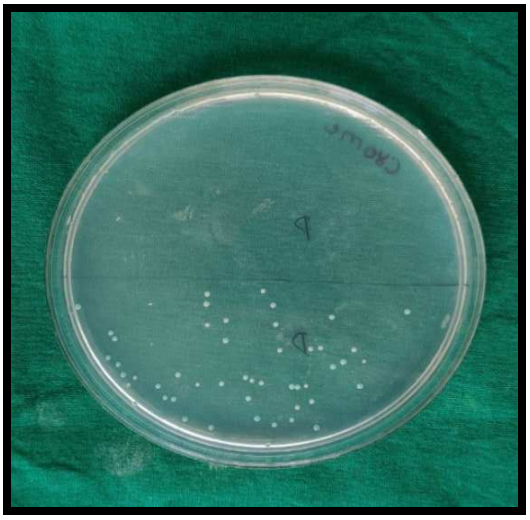


POST-USAGE OF NONI

FIGURE NO 10: IDENTIFICATION OF CANDIDA GLABRATA (WHITE TO LIGHT PINK COLOUR COLONIES) ON THE DENTURE SURFACE AND PALATAL MUCOSA USING CHROMAGAR

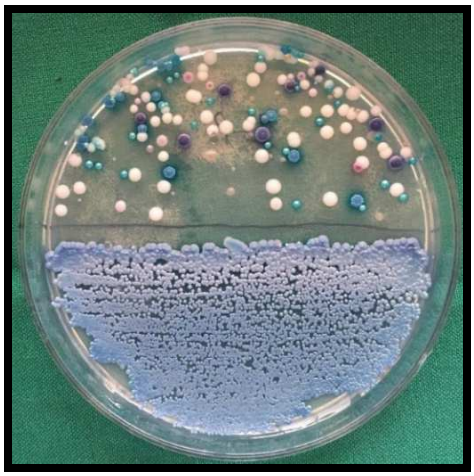


PRE-USAGE OF CLOTRIMAZOLE

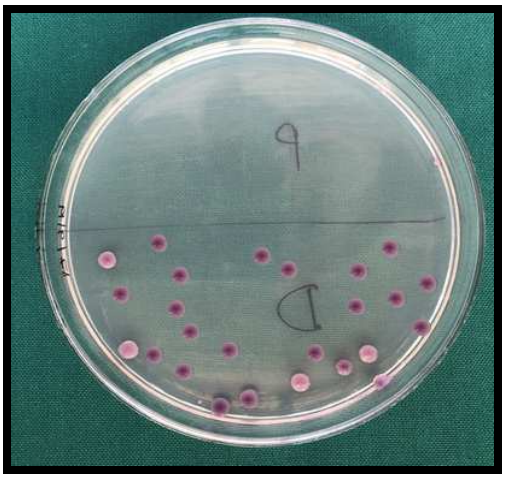


POST -USAGE OF CLOTRIMAZOLE

FIGURE NO 11: IDENTIFICATION OF CANDIDA TROPICALIS (METALLIC BLUE COLOUR COLONIES) ON THE DENTURE SURFACE AND PALATAL MUCOSA USING CHROMAGAR



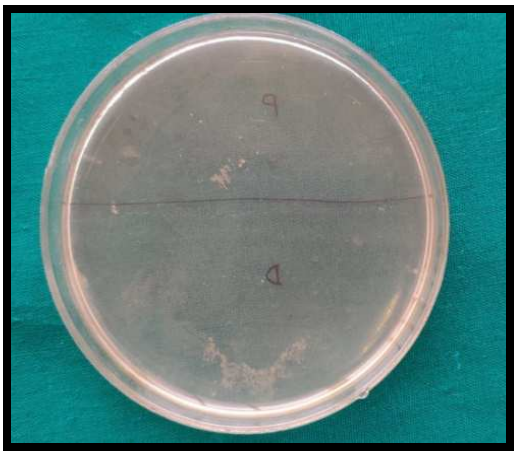
PRE -USAGE OF NONI



POST -USAGE OF NONI

FIGURE NO 12: IDENTIFICATION OF CANDIDA TROPICALIS (METALLIC BLUE COLOUR COLONIES) ON THE DENTURE SURFACE AND PALATAL MUCOSA USING CHROMAGAR

PRE-USAGE OF CLOTRIMAZOLE



POST-USAGE OF CLOTRIMAZOLE

RESULTS

The present study evaluated the effects of Morinda citrifolia and Clotrimazole on the prevalence of oral candida organisms in denture wearers aged 50 years and above on two different surfaces: denture surface and palatal mucosa. The complete denture wearers were evaluated for the prevalence of candida species and the number of colonies of candida were counted on pre usage and post usage of Morinda citrifolia and Clotrimazole by identification using CHROM agar.

Table 3and Graph1: Pair wise comparison of number of candida colonies on pre usage and post usage of Clotrimazole on denture and palatal surface using Mann Whitney U test

Table 4 and Graph 2: Comparison of number of colonies on pre usage and post usage of Clotrimazole on denture and palatal surface using Wilcoxon matched pairs test.

Table 5 and Graph3: Pair wise comparison of number of candida colonies on pre usage and post usage of Morinda citrifolia on denture and palatal surface using Mann Whitney U test

Table 6 and Graph4: Comparison of number of colonies on pre usage and post usage of Morinda citrifolia on denture and palatal surface using Wilcoxon matched pairs test.

Table7 and Graph 5: Pair wise comparison with respect to pre usage and post usage of Clotrimazole and Morinda citrifolia on denture surface among different candida organisms by Mann-Whitney U test

Table 8 and Graph 6: Comparison of pre usage and post usage of Morinda citrifolia and Clotrimazole on the denture surface by Wilcoxon matched pairs test.

Table 9 and Graph 7: Pair wise comparison with respect to pre and post usage of Clotrimazole and Morinda citrifolia on palatal surface among different candida organisms by Mann-Whitney U test

Table 10 and Graph 8: Comparison of pre usage and post usage of Morinda citrifolia and Clotrimazole on the palatal surface by Wilcoxon matched pairs test.

The resultant values of pre usage and post usage of Clotrimazole and Morinda citrifolia on the denture surface and palatal mucosa were subjected to statistical analysis to draw the conclusion from the experimental data. Descriptive statistical measures such as mean and standard deviation were computed for all the study groups. Pair-wise comparison of the test group were done using Mann-Whitney U test and comparison in between the pre usage and post usage of Morinda citrifolia and Clotrimazole were done using Wilcoxon matched pairs test. The p value of <0.05 was considered statistically significant.

The mean, standard deviation was calculated for number of candida colonies on pre usage and post usage of Clotrimazole on denture and palatal surface (Table 3 and Graph 1). On pair wise comparison of Mann-Whitney U test among candida tropicalis and candida glabrata there was no statistically significant difference seen in the colony count in pre usage and post usage of Clotrimazole. Candida albicans did shows mean significance change on denture (2.80 ± 1.95) and palatal (0.55 ± 0.77) in pre usage, whereas no significant mean difference among the post usage. ($p<0.0001$)

On comparison between pre usage and post usage of Clotrimazole on candida colonies using Wilcoxon matched pair test (Table 4 and Graph 2). Denture surface showed a statistically significant reduction in mean colony count among the candida albicans (2.74 ± 1.91) ($p<0.0001$) and candida glabrata (0.39 ± 1.02) ($p<0.0180$). On evaluation on palatal surface a significant difference was seen in pre usage to post

usage among candida albicans (0.50±0.78) ($p<0.0024$) and candida glabrata (0.41± 1.03) ($p<0.0117$) colonies whereas there was no statistically significant difference noticed among the candida tropicalis (-0.06 ± 0.56) ($p=0.5930$).

The mean, standard deviation was calculated by Mann- Whitney U test for number of candida colonies on pre usage and post usage of Morinda citrifolia on denture and palatal surface (Table 5 and Graph 3).On evaluation there was statistically significant difference seen among the mean candida albicans ($p<0.0001$) on pre usage (2.36± 1.91) and post usage (0.35±0.64) on denture and palatal surface. There was no statistically significant change noticed in the candida tropicalis and glabrata.

On pair wise comparison between pre usage and post usage of Morinda citrifolia on candida colonies using Wilcoxon matched pair test (Table 6 and Graph 4). Denture surface showed a statistically significant reduction in colony count among the candida albicans (1.21±1.71) ($p<0.0004$) and candida tropicalis (0.27±0.71) ($p<0.0277$). Examination on Palatal surface showed a statistically significant difference among the candida albicans (0.29±0.57) ($p<0.0077$) and candida glabrata (0.31± 0.95) ($p=0.0277$). There was no difference noticed among candida tropicalis (0.05± 0.38) ($p=0.6547$).

The mean, standard deviation were calculated by Mann-Whitney U test for the number of candida colonies on pre usage and post usage of Morinda citrifolia and Clotrimazole on the denture surface (Table 7 and Graph 5) .The mean values of the change in the number of mean candida albicans colonies between the pre usage(0.06±0.34) and post usage(1.15±0.82) of Morinda citrifolia and Clotrimazole on denture surface showed a statistically significant reduction ($p<0.0001$*) in colony count when compared to candida tropicalis and glabrata.

On comparison between the pre usage and post usage of Morinda citrifolia and Clotrimazole on the denture surface using Wilcoxon matched pair test (Table8 and graph 6), statistically significant differences were found in the number of colonies of candida albicans in Clotrimazole Group (2.74±1.91) ($p<0.0001$*) and Morinda citrifolia Group (1.21±1.71) ($p<0.0004$*). Also mean statistically significant difference was found in number of colonies candida glabrata species in Clotrimazole group (0.39±1.02) ($p=0.0180$*).

The mean, standard deviation and coefficient of variation were calculated for the number of colonies of candida on pre usage and post usage of Morinda citrifolia and Clotrimazole on the palatal mucosa.

On pair-wise comparison of pre usage and post usage of Morinda citrifolia and Clotrimazole on the palatal mucosa by Mann-Whitney U test (Table 9 and Graph 7), there was no statistically significant differences found in the number of colonies among the two groups.

On comparison between change in the number of candida colonies between the pre usage and post usage of Morinda citrifolia and Clotrimazole on the palatal mucosa in both the groups using Wilcoxon matched paired test (Table 10 and Graph 8), there was highly statistically significant difference for candida albicans (0.50±0.78) $p<0.0024$*(Clotrimazole group),(0.29±0.57) $p<0.0077$* (Morinda citrifolia Group) and glabrata (0.41±1.03) ($p=0.0117$*)(Clotrimazole group), (0.0±0.24) $p<0.0277$* (Morinda citrifolia group) when compared to candida tropicalis (-0.006±0.56) $p=0.5930$ (Clotrimazole group), (0.36±1.02)$p=0.6547$ (Morinda citrifolia group)

The results of the study indicated that the prevalence of candida species were statistically significant in Clotrimazole group and Morinda citrifolia Group on the

denture surface .The results also revealed that there was statistically significant difference in the number of colonies post usage of Clotrimazole and Morinda citrifolia on the denture surface amongst candida albicans compared to candida glabrata and candida tropicalis.

Table 3: Pair wise comparison of number of candida colonies on pre usage and post usage of Clotrimazole on denture and palatal surface using Mann Whitney U test

Organism	Times	Denture (Group A)			Palatal (Group A)			U-value	p-value	p-value
		Mean	SD	Mean rank	Mean	SD	Mean rank			
Candida albicans	Pre usage	2.80	1.95	40.20	0.55	0.77	20.80	159.00	4.2949	0.0001*
	Post usage	0.06	0.34	30.52	0.05	0.26	30.48	449.50	0.0000	1.0000
Candida tropicalis	Pre usage	0.12	0.45	31.00	0.06	0.31	30.00	435.00	0.2144	0.8303
	Post usage	0.14	0.46	30.97	0.12	0.45	30.03	436.00	0.1996	0.8418
Candida glabrata	Pre usage	0.40	1.03	29.95	0.44	1.04	31.05	433.50	-0.2366	0.8130
	Post usage	0.01	0.05	30.48	0.03	0.16	30.52	449.50	0.0000	1.0000

***p<0.05; SD: Standard Deviation**

This table signifies that there is statistically significant change in colony count of *candida albicans* (p=0.0001*) on pre usage of Clotrimazole. No significant difference seen on pre and post usage of Clotrimazole on *candida tropicalis* and *candida glabrata*.

Table 4: Comparison of number of colonies on pre usage and post usage of Clotrimazole on denture and palatal surface using Wilcoxon matched pairs test

Sample	Organisms	Times	Mean Diff.	SD Diff.	% Of change	Z-value	P-value
Denture (Group A)	*Candida albicans*	Pre usage to Post usage	2.74	1.91	97.80	4.2857	0.0001*
	Candida tropicalis	Pre usage to Post usage	-0.02	0.67	-19.72	0.1348	0.8927
	Candida glabrata	Pre usage to Post usage	0.39	1.02	97.49	2.3664	0.0180*
Palatal (Group A)	*Candida albicans*	Pre usage to Post usage	0.50	0.78	91.17	3.0400	0.0024*
	Candida tropicalis	Pre usage to Post usage	-0.06	0.56	-104.66	0.5345	0.5930
	Candida glabrata	Pre usage to Post usage	0.41	1.03	93.21	2.5205	0.0117*

***p<0.05; SD: Standard Deviation**

The table shows statistically significant difference seen in number of *candida albicans* and glabrata colonies in denture and palatal surface pre and post usage of Clotrimazole,($p<0.0001$) whereas no statistically significant difference was seen on denture and palatal surface among *candida tropicalis*

Table5: Pair wise comparison of number of candida colonies on pre usage and post usage of Morinda citrifolia on denture and palatal surface suing Mann Whitney U test

Organism	Times	Denture (Group B)			Swab (Group B)			U-value	p-value	p-value
		Mean	SD	Mean rank	Mean	SD	Mean rank			
Candida albicans	Pre usage	2.36	1.91	40.77	0.35	0.64	20.23	142.00	4.5462	0.0001*
	Post usage	1.15	0.82	41.03	0.06	0.23	19.97	134.00	4.6645	0.0001*
Candida tropicalis	Pre usage	0.50	1.31	32.97	0.07	0.37	28.03	376.00	1.0867	0.2772
	Post usage	0.22	0.68	32.03	0.02	0.09	28.97	404.00	0.6727	0.5011
Candida glabrata	Pre usage	0.32	1.01	29.60	0.38	1.09	31.40	423.00	-0.3918	0.6952
	Post usage	0.25	0.62	31.68	0.07	0.22	29.32	414.50	0.5175	0.6048

***p<0.05; SD: Standard Deviation**

It means that there was statistically significant difference in *candida albicans* colonies (p<0.0001) on pre and post usage of Morinda citrifolia. There was no statistically significant difference seen among the *candida tropicalis* and *candida glabrata*.

Table 6: Comparison of number of colonies on pre usage and post usage of Morinda citrifolia on denture and palatal surface using Wilcoxon matched pairs test

Sample	Organisms	Times	Mean Diff.	SD Diff.	% of change	Z-value	P-value
Denture (Group B)	*Candida albicans*	Pre usage to post usage	1.21	1.71	51.16	3.5466	0.0004*
	Candida tropicalis	Pre usage to post usage	0.27	0.71	55.45	2.2014	0.0277*
	Candida glabrata	Pre usage to post usage	0.07	0.60	21.73	0.1348	0.8927
Swab (Group B)	*Candida albicans*	Pre usage to post usage	0.29	0.57	83.03	2.6656	0.0077*
	Candida tropicalis	Pre usage to post usage	0.05	0.38	76.39	0.4472	0.6547
	Candida glabrata	Pre usage to post usage	0.31	0.95	82.43	2.2014	0.0277*

***p<0.05; SD: Standard Deviation**

The table shows that there was a statistically significant difference seen among the *candida albicans* (p<0.0004) and tropicalis (p<0.0277) in pre and post usage of Morinda citrifolia on denture surface. Whereas no significant change seen among *candida glabrata* on denture surface. When palatal surface was examined, there was statistically significant change seen among *candida albicans* (p<0.0077) and *candida glabrata* (p<0.0277), no significant change seen among *candida tropicalis.*

Table7: Pair wise comparison on pre usage and post usage of Clotrimazole and Morinda citrifolia on denture surface among different candida organisms by Mann-Whitney U test

Organism	Times	Group A (Denture)			Group B (Denture)			U-value	p-value	p-value
		Mean	SD	Mean rank	Mean	SD	Mean rank			
Candida albicans	Pre usage	2.80	1.95	32.20	2.36	1.91	28.80	399.00	0.7466	0.4553
	Post usage	0.06	0.34	20.03	1.15	0.82	40.97	136.00	-4.6349	0.0001*
Candida tropicalis	Pre usage	0.12	0.45	28.53	0.50	1.31	32.47	391.00	-0.8649	0.3871
	Post usage	0.14	0.46	29.98	0.22	0.68	31.02	434.50	-0.2218	0.8245
Candida glabrata	Pre usage	0.40	1.03	31.82	0.32	1.01	29.18	410.50	0.5766	0.5642
	Post usage	0.01	0.05	28.42	0.25	0.62	32.58	387.50	-0.9166	0.3593

***p<0.05; SD: Standard Deviation**

The table shows that there was statistically significant change seen among the post usage of Clotrimazole and Morinda citrifolia on denture surface among *Candida albicans* colonies (p<0.0001*), whereas no statistically significant difference seen among the *candida tropicalis* and *candida glabrata* colonies.

Table8: Comparison of pre usage and post usage of Clotrimazole and Morinda citrifolia on the denture surface by Wilcoxon matched pairs test.

Groups	Organisms	Times	Mean Diff.	SD Diff.	% of change	Z-value	P-value
Group A (denture)	*Candida albicans*	Pre usage to post usage	2.74	1.91	97.80	4.2857	0.0001*
	Candida tropicalis	Pre usage to post usage	-0.02	0.67	-19.72	0.1348	0.8927
	Candida glabrata	Pre usage to post usage	0.39	1.02	97.49	2.3664	0.0180*
Group B (denture)	*Candida albicans*	Pre usage to post usage	1.21	1.71	51.16	3.5466	0.0004*
	Candida tropicalis	Pre usage to post usage	0.27	0.71	55.45	2.2014	0.0277*
	Candida glabrata	Pre usage to post usage	0.07	0.60	21.73	0.1348	0.8927

***p<0.05; SD: Standard Deviation**

It shows that there was statistically significant difference seen among the *candida albicans* (p<0.0001*) and glabrata (p<0.0180) colonies on denture surface among Clotrimazole group**.** When compared Morinda citrifolia there was statistically significant difference seen on denture surface among *candida albicans* (p<0.0004) and *candida tropicalis* (p<0.0277) group whereas no significant change in number of colonies among glabrata**.**

Table9: Pair wise comparison of Pre usage and post usage of Clotrimazole and Morinda citrifolia on palatal surface among different candida organisms by Mann-Whitney U test

Organism	Times	Group A (Palatal Swab)			Group B (Palatal Swab)			U-value	p-value	p-value
		Mean	SD	Mean rank	Mean	SD	Mean rank			
Candida albicans	Pre usage	0.55	0.77	32.77	0.35	0.64	28.23	382.00	0.9979	0.3183
	Post usage	0.05	0.26	30.03	0.06	0.23	30.97	436.00	-0.1996	0.8418
Candida tropicalis	Pre usage	0.06	0.31	30.48	0.07	0.37	30.52	449.50	0.0000	1.0000
	Post usage	0.12	0.45	31.03	0.02	0.09	29.97	434.00	0.2292	0.8187
Candida glabrata	Pre usage	0.44	1.04	31.52	0.38	1.09	29.48	419.50	0.4435	0.6574
	Post usage	0.03	0.16	29.52	0.07	0.22	31.48	420.50	0.4287	0.6681

P<0.05; SD: Standard Deviation

The table shows that there was no significant change seen on pre and post usage of Clotrimazole and Morinda citrifolia on the palatal surface among all the three candida studied.

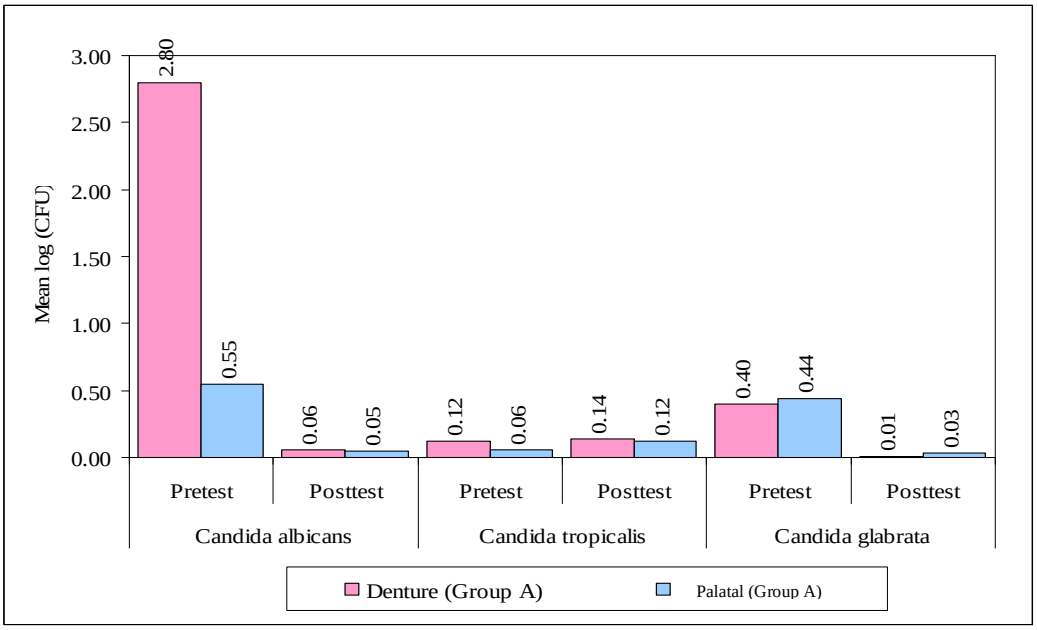
Table 10: Comparison of pre usage and post usage of Morinda citrifolia and Clotrimazole on the palatal surface in two groups (A&B) by Wilcoxon matched pairs test.

Groups	Organisms	Times	Mean Diff.	SD Diff.	% of change	Z-value	P-value
Group A (Palatal Swab)	*Candida albicans*	Pre usage to post usage	0.50	0.78	91.17	3.0400	0.0024*
	Candida tropicalis	Pre usage to Post usage	-0.06	0.56	-104.66	0.5345	0.5930
	Candida glabrata	Pre usage to Post usage	0.41	1.03	93.21	2.5205	0.0117*
Group B (Palatal Swab)	*Candida albicans*	Pre usage to Post usage	0.29	0.57	83.03	2.6656	0.0077*
	Candida tropicalis	Pre usage to Post usage	0.36	1.02	95.81	0.4472	0.6547
	Candida glabrata	Pre usage to Post usage	0.00	0.24	1.05	2.2014	0.0277*

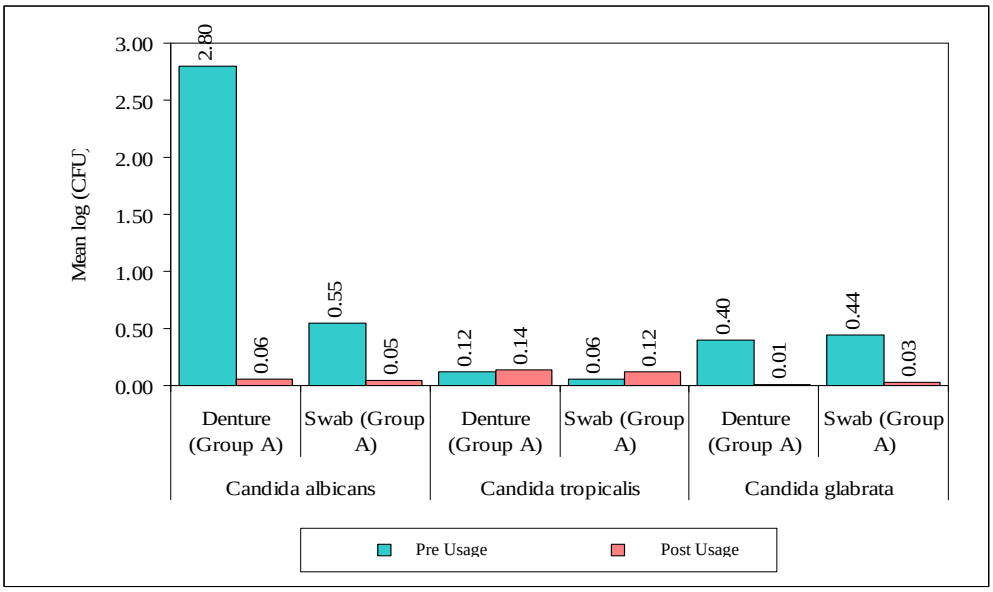
P<0.05; SD: Standard Deviation

The table shows that there is statistically significant change seen among the *candida albicans* and *candida glabrata* colonies on palatal surface after pre and post usage of Clotrimazole and Morinda citrifolia ($p < 0.0077$ and $p<0.0277$). There is no statistically significant difference seen among the *candida tropicalis* colonies in pre and post usage of both the groups.

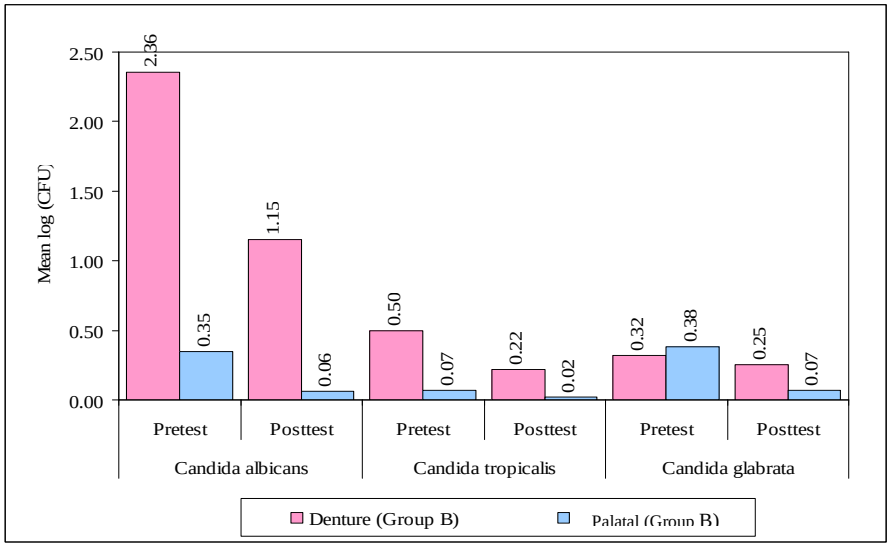
Graph 1: Pair wise comparison of number of candida colonies on pre usage and post usage of Clotrimazole on denture and palatal surface using Mann Whitney U test



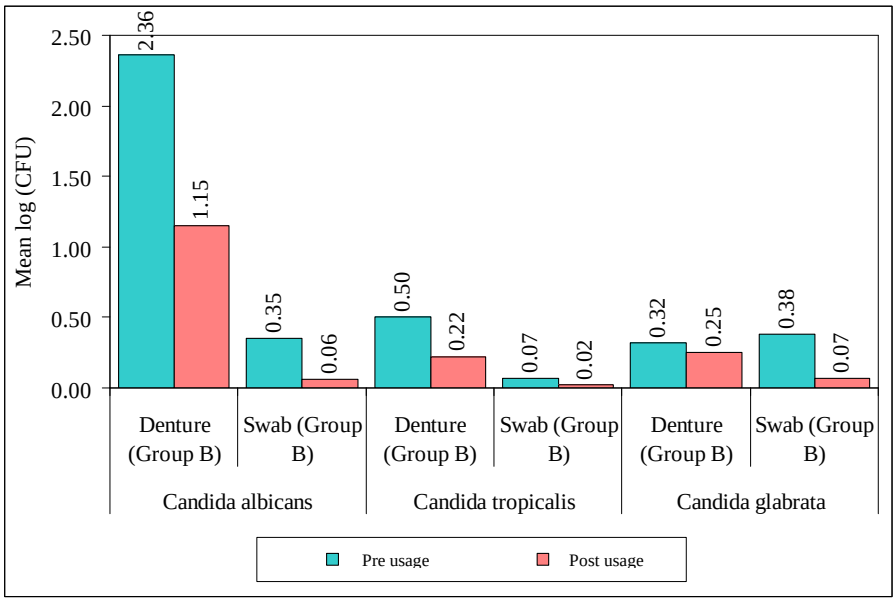
Graph 2: Comparison of number of colonies on pre usage and post usage of Clotrimazole on denture and palatal surface using Wilcoxon matched pairs test



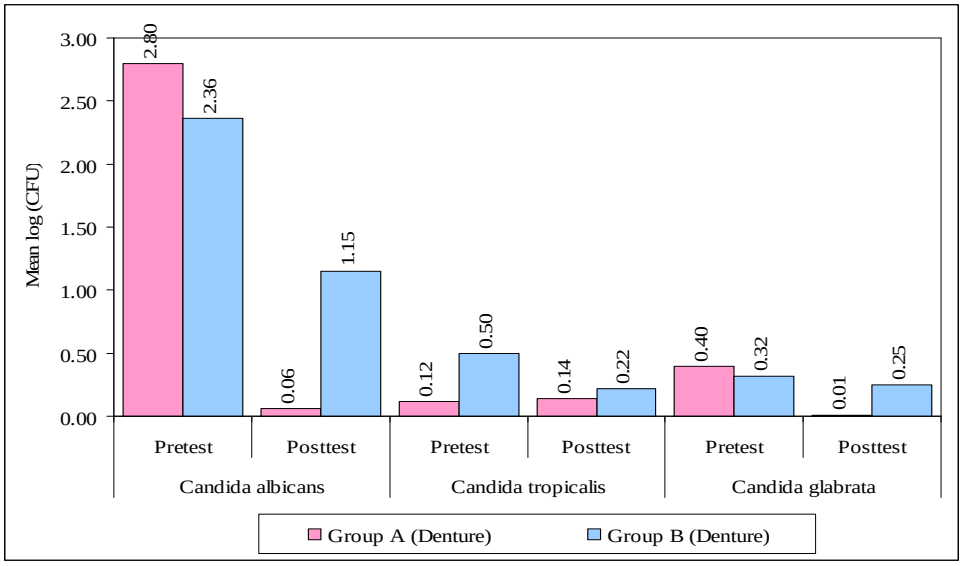
Graph 3: Pair wise comparison of number of candida colonies on pre usage and post usage of Morinda citrifolia on denture and palatal surface suing Mann Whitney U test



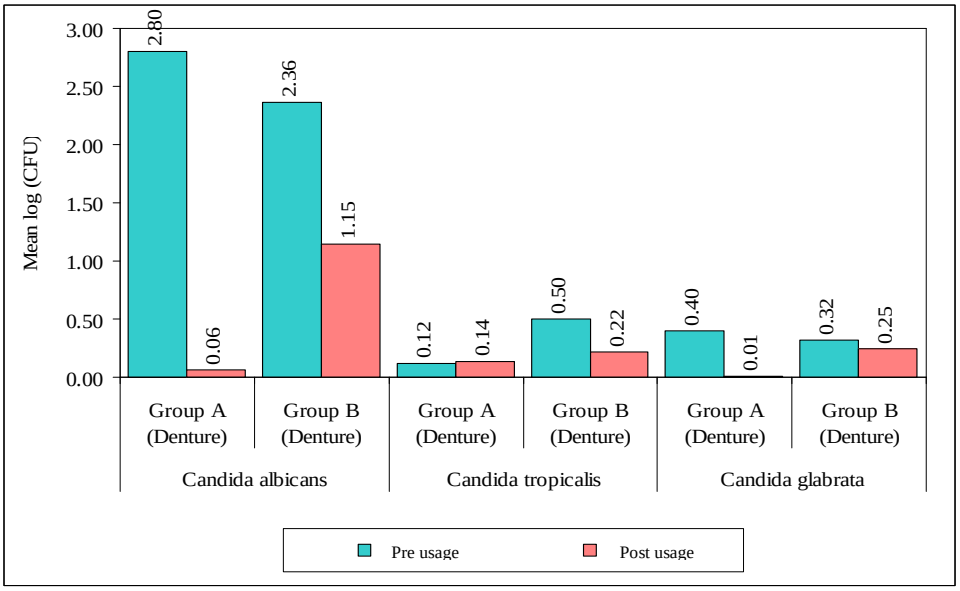
Graph 4: Comparison of number of colonies on pre usage and post usage of Morinda citrifolia on denture and palatal surface using Wilcoxon matched pairs test



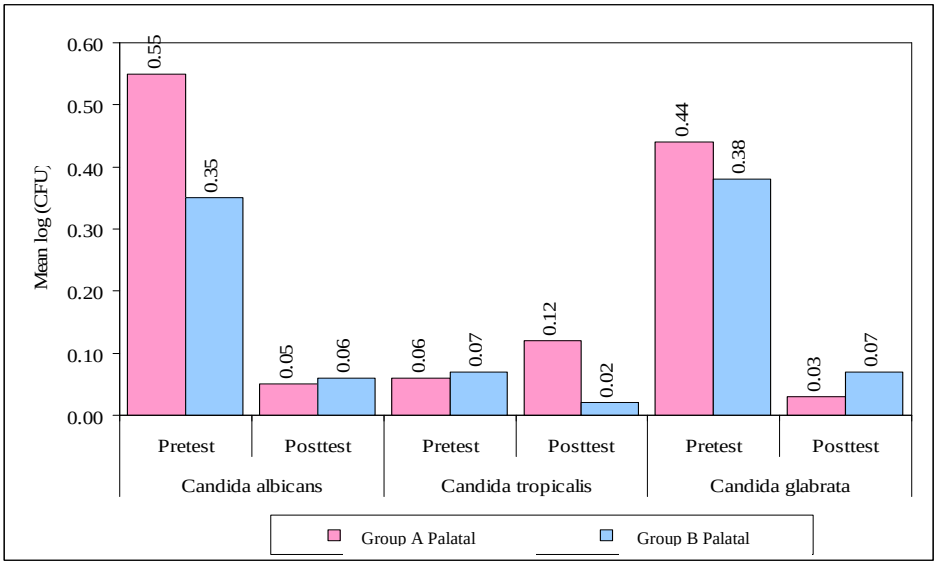
Graph 5: Pair wise comparison on pre usage and post usage of Clotrimazole and Morinda citrifolia on denture surface among different candida organisms by Mann-Whitney U test



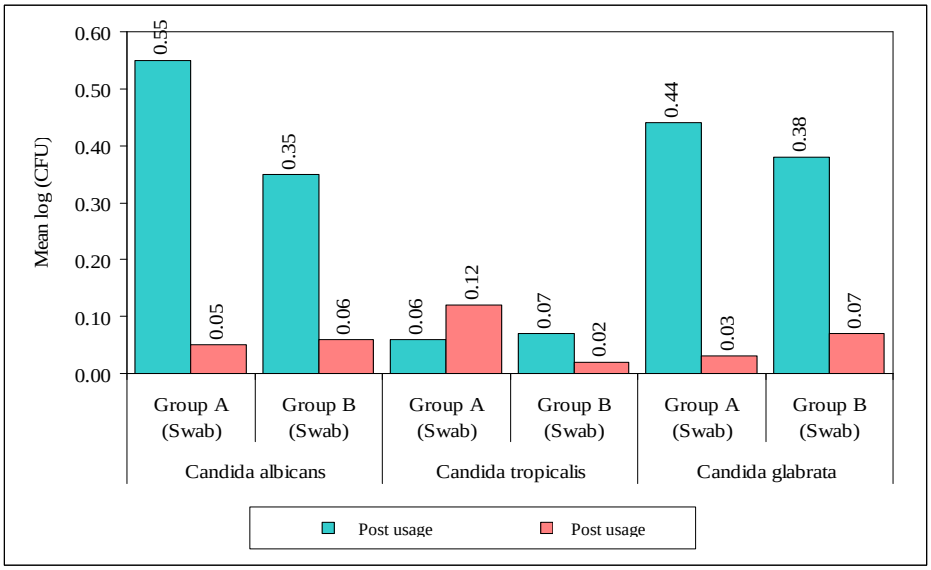
Graph 6: Comparison of pre usage and post usage of Morinda citrifolia and Clotrimazole on the denture surface by Wilcoxon matched pairs test



Graph7: Pair wise comparison of pre usage and post usage of Clotrimazole and Morinda citrifolia on palatal surface among different candida organisms by Mann-Whitney U test



Graph 8: Comparison of pre usage and post usage of Morinda citrifolia and Clotrimazole on the palatal surface in two groups (A&B) by Wilcoxon matched pairs test.



DISCUSSION

The global rise in life expectancy of human population is increased due to advancement in medical technology. Dental status is considered to be an important contributing factor to health and adequate nutrition in elderly. It has been recognized that poor oral hygiene and dental avulsion in the elderly can affect nutrition intake, psychosocial impact, including psychological discomfort. This in turn can affect general health, oral health and can lead to oral candidiasis. [46]

Candida species are the normal commensal of oral microbial habitat.[47] Candida species are opportunistic fungi that cause mucosal and deep-tissue lesions in humans. The presence of fungus on mucosal surfaces is thought to be a key risk factor for candida infection.[48] Candida-associated denture stomatitis has a complex pathogenesis. It's most common among denture wearers, with predominant localization on the palatal mucosa and denture tissue surface.[10] The predisposing local factors for this favourable localization are adherence of candida species to acrylic surface, xerostomia under the surfaces of the denture fittings, ill-fitting dentures, and poor oral health.[5]Microbial plaque formation on the intaglio surface of dentures and poor oral hygiene, according to M Rathee et al, is an important co factor in the aetiology of candidiasis.[1]

Different techniques and media have been used in the speciation of candida. According to study conducted by Sangeeta Khadka et al in terms of laboratory-based identification, conventionally *Candida* species can be identified by their growth characteristics in SDA culture media and expression using germ tube, but of these test lack the species level identification.[49] According to a study conducted by Sanjeev

Kumar et al proposed that when we compared technically challenging, time consuming, and expensive conventional procedures, Speciation of Candida species using CHROMagar on the basis of colour distinction has several advantages of rapid identification, technical simplicity, cost effectiveness, and reliability. Chromogenic medium was helpful in identifying multispecies candida infections.[44]

CHROMagar for the identification of medically important yeast in routine clinical microbiology laboratory and it was observed that CHROMagar is a simple, quick, and economical approach for identifying such species with high sensitivity and specificity.[50] According to study conducted by Vijaya d et al proposed that CHROMagar contains certain chromogenic substance which when reacted with enzymes of microorganism gives various colour differentiation. Based on these advantages, it was decided to evaluate the candida colonization on the denture surface and palatal mucosa by using CHROMagar.[51]

Fungal infections have been successfully treated by systemic antifungal agents since a very long time. Clotrimazole is a broad-spectrum antimycotic that is commonly used to treat *Candida albicans* and other fungal infections. It is used in topical treatment of oropharengeal candidiasis. It is classified as a fungi static rather than a fungicidal**.** Clotrimazole is an over-the-counter drug that is relatively safe and free of major adverse effects. Clotrimazole inhibit the microsomal cytochrome p 450, which is the crucial step in fungi synthesis. Topical application of Clotrimazole is of choice to treat localized candidiasis.[11]

Few antifungal drugs have an undesirable side effects like nausea, vomiting and diarrhoea and cause profound effect on general health. It has been found that candida is resistant to some of the commonly used antifungals like Nystatin,

Amphotericin B, Fluconazole, Itraconazole, and Voriconazole. The limited spectrum and toxicity of available antifungals and the gradual emergence of resistance to these drugs are a concern; thus, alternative therapies are required on urgent basis.[11]

The use of herbal antifungal agent has an emerging trend in treating fungicides. Due to its low side effects their use has been beneficial to many. The search of this fungicide and antimicrobial from the natural source has intensifies in response to limit the currently available antifungal drug system. Morinda citrifolia commonly called as "NONI" is a popular medicine in the field of Ayurveda. The use of Morinda citrifolia is reported to have broad range of therapeutic effects, including antimicrobial, anti-inflammatory, anticancer, analgesic and immune enhancement. This traditional medicine is also has been used to treat arthritis, diabetes, cancers, gastric ulcers, and drug addiction. Morinda citrifolia may be used to treat candida infection, as mouthwash or topical medicament and as an disinfectant for denture.The results of the study performed by Aree jainkittinvong et al demonstrate the extract of M citrifolia has antifungal effect on various candida species and this inhibitory effect varies in contact time.[13]

According to study conducted by Bharathi Prakash et al dentures are basically made of polymers which have micro pores that cause candida species to adhere and colonize. These microbes form oral environment and colonize on denture surface to form a biofilm which depend upon oral hygiene practices of host. If the immune system of the host is weaker then candida species predominate and cause oral infections like denture stomatitis. Candida species forms their biofilm on denture surface and are therefore isolated from denture plaque than dental plaque. Denture insertion thus accelerates the biofilm formation and colonization.[36] According to

study conducted by Vanden Abbeele et al and Bharathi Prakash et al the most predominant species isolated among the candida are the *candida albicans* followed by *candida tropicalis, candida dubliensis.* Dentures create a microenvironment that promotes candida growth, which thrives in a low-oxygen, low-pH, and anaerobic environment. There are over 350 candidal species, with about ten of them capable of colonizing the oral cavity.[5]

The present in vivo study was undertaken to evaluate and compare the effectiveness of Morinda citrifolia and Clotrimazole on the prevalence of oral candida in the elderly denture wearers.

A total of sixty completely edentulous subjects wearing dentures since at least last one year were included in this study. They were then divided into two different groups that i.e group A used Clotrimazole and group B used Morinda citrifolia on denture as well as palatal surface.

The swabs were collected from the denture surface and palatal mucosa of both the group before and after usage of the respective agents and were subjected to CHROMagar. CHROMagar helped in colour identification of different candida species and scoring the number of candida colonies.

The results of the present study revealed that there was mean reduction in the number of colonies on the denture surface after Morinda citrifolia and Clotrimazole usage. Clotrimazole showed highest reduction in colony counts of *candida albicans* and glabrata species than Morinda citrifolia after the usage. Statistically significant difference was seen among the colony forming units in Clotrimazole usage on denture surface than Morinda citrifolia. No statistically significant difference seen on colony

forming unit on palatal surface in Clotrimazole and Morinda citrifolia. In this study there was statistically significant reduction *candida albicans* species in both the groups. But the marked reduction in colony count were more in Clotrimazole than Morinda citrifolia.

Our results indicate that the denture surface showed more reduction in the number of candida colonies after Clotrimazole and Morinda citrifolia usage as compared to palatal mucosa which may be because the Clotrimazole and Morinda citrifolia have direct application was done on the denture surface.

The present study showed that *candida albicans* was the most prevalent species in the oral cavity of elderly complete denture wearers. It also suggested that the decrease in the number of colonies after Morinda citrifolia and Clotrimazole usage was observed more with *candida abicans* as compared to other candida species. Several multispecies were isolated like *candida tropicalis, candida glabrata, candida krusei, candida parapsilosis,* etc. This was in agreement with the previously conducted study by Lockhart et al and Vinaya Bhat et al which also proved *candida albicans* as the most commonly isolated candida species in the elderly individuals.[9] *Candida albicans* have property to degrade the proteins in yeast and hyphal form, whereas c glabrata does not show this capacity. This shows that *candida albicans* can attack more of deeper tissue making it more widespread among the oral infection.[9,]

Clotrimazole is the gold standard antifungal agent. But Morinda citrifolia can be used as a therapeutic alternative to the standard drugs. The application of these agent on the palatal mucosa might sometimes activate the immune response which might be responsible for antifungal and microbial effect.[11]

The study demonstrated that the Morinda citrifolia contains certain water-soluble products that interferes with the conversion of *candida albicans* morphologically and thus have potential beneficial values against candidiasis. This is in accordance with previous study by Banerjee et al and Barani k et al**.**[12,14]

Based on the above finding this study showed that the tested Morinda citrifolia product may represent an alternative method to reduce Candida colonization, thus preventing candida infections.

LIMITATIONS

- Three different candida species that is *candida albicans, candida tropicalis* and *candida glabrata* are considered, including other candida species might have differed the results.
- Patients who are healthy without any systemic disease have been considered. Involving patients on drug therapy and having systemic medical condition might add new information to this study.
- Topical application of Morinda Citrifolia in the form of juice was done in this study. Using any other form of Morinda citrifolia may or of some other brand and different way of application may attribute variable results.
- To generalise the effect of Morinda citrifolia, a larger sample size with diverse population might attribute better understanding about the usage of these natural therapeutic agents.

CLINICAL IMPLICATION

In this study, the results showed that Morinda citrifolia and Clotrimazole had a definite influence on the reduction of candida colonies on the denture surface and palatal mucosa among the denture wearers. Commercially available antifungal agent such as Clotrimazole showed a significant decrease in colony count when compared to the herbal antifungal agent. A greater decrease was seen among the *candida albicans* colony when compared to tropicalis and glabrata in both the groups. Therefore, Morinda citrifolia juice can be considered as a beneficial promising and alternative treatment for candida infection in the elderly denture wearer patient compared to the commercially available antifungal agents.

SCOPE OF THE STUDY

- The present study evaluated the effect of Clotrimazole and Morinda citrifolia on the prevalence of candida species in denture wearers.
- The number of candida colonies present on the denture surface and palatal mucosa was identified using CHROMagar, before and after Clotrimazole and Morinda citrifolia usage.
- This research project, although was carried out over a period of three years, has been limited in its scope due to restricting factors such as long -time clinical trials and follow up studies.
- Further research is suggested to assess the effect of Morinda citrifolia and Clotrimazole on the prevalence of candida species in denture wearers among the subjects with wider age groups.
- Further studies are suggested to include not only healthy patients but also the patients who are medically compromised and patients with history of systemic medical conditions.

CONCLUSION

Within the limitations of this in vivo study the following conclusions were drawn. Clotrimazole was more efficient in reduction of colony count among the denture wearers when compared to Morinda citrifolia.

Morinda citrifolia (Noni) was effective in reducing the colonization of candida in elderly denture wearers. Our study shows that use of Morinda citrifolia (Noni) may represent an alternative to commercially available drugs in the management of candida infection in elderly denture wearers.

SUMMARY

The present in vivo study was carried out to evaluate the effect of Morinda citrifolia and Clotrimazole on the prevalence of different candida organisms in denture wearers on the denture surface and palatal mucosa.

A total of 60 completely edentulous old denture wearers were included in the study. These elderly denture wearer patients were divided into two age groups namely; Group A Clotrimazole and Group B Morinda citrifolia. The two surfaces from which the swabs were collected were the tissue fitting denture surface and the palatal mucosa.

Further the number of candida colonies were identified and counted using CHROMagar. This sample collection and microbiological assessment was done in the same way after usage of Morinda citrifolia and Clotrimazole.

The resultant data was tabulated and then subjected to statistical analysis to draw conclusion from experimental data. Mann-Whitney U test was done to collectively compare the test group pair-wise. Comparison in between the pre usage and post usage of Morinda citrifolia and Clotrimazole was done using Wilcoxon matched pairs test

The results showed that the Clotrimazole showed more reduction in candida species. Among the candida species *candida albicans* showed a greater reduction in colony count than *candida glabrata* and tropicalis. The study also shows a reduction in the colony count of multispecies candida by using Morinda citrifolia product but more so seen with *candida albicans.* Thus, this natural herbal product may be effective in reducing the colonization of the oral cavity with candida in elderly denture wearers.

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ANNEXURE I

ETHICAL CLEARANCE

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

Sl. No. : 1327

CERTIFICATE

This is to Certify that the synopsis titled

COMPARATIVE EVALUATION OF PREVALENCE OF CANDIDA SPECIES AND ANTI-CANDIDA EFFECT OF MORINDA CITRIFOLIA [NONI] AMONG COMPLETE DENTURE WEARERS : AN IN-VIVO STUDY *Submitted by*

Dr. DIVYA JOSHI *P. G. Student / Staff, Guided by* DR. RAMESH NAYAKAR *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 and Ethical Committee
KLEVK Institute of Dental Sciences
BELAGAVI.

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

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

ANNEXURE II

PATIENT INSTRUCTION PERFORMA

Department of Prosthodontics and Crown & Bridge.

KLE V.K Institute of Dental Sciences, Belagavi.

"TO EVALUATE THE ANTI CANDIDA EFFECT OF CLOTRIMAZOLE AND MORINDA CITRIFOLIA (NONI) ON THE PREVELANCE OF ORAL CANDIDA IN DENTURE WEARERS" AN IN VIVO STUDY.

My name is Dr. Divya Joshi. I am Post Graduate student in the Dept. of Prosthodontics & Crown & Bridge KLE V.K. Institute of Dental Sciences, KAHER, Belagavi. I am doing this study to evaluate the anti-candida effect of Clotrimazole and Morinda citrifolia (Noni) among the complete denture wearers.

Patient is instructed to apply Clotrimazole and Morinda citrifolia (Noni) apply twice daily for 14 days. Patient asked to report on the 15^{th} day. Patient is asked to apply Noni with sterile swabs on intaglio surface of denture and palatal mucosa. Patient is asked to wear denture in closed contact with the mucosa until consumption of next meal.

The patients are informed that the herbal products have no side effects, and side effects related to Clotrimazole is explained to the patient.

The patient is asked not to consume any caffeine related products like tea and coffee for at least 1 hour after the application of Noni.

In case of any discomfort the patient is asked to contact the doctor.

Signature of the Participant _____ Date _____

Signature of Investigator _____ Date _____

रुग्णसूचनापरफॉर्मे

प्रोस्थोडोंटिक्सआणिक्राउनआणिब्रिजविभाग.

केएलईव्ही. के. दंतविज्ञानसंस्था, बेलागवी.

“संपूर्ण कवळी धारण करणार्यां मध्ये कॅन्डिडाप्रजातींच्या प्रचाराचे तुलनात्मकमूल्यांकन आणि क्लोट्रिमाझोलआणि मोरिंडा सिट्रीफोलिया (नोनी) च्या□ँटी-कॅन्डिडा प्रभावाचे तुलनात्मकमूल्यांकन.”

माझे नाव डॉ दिव्या जोशी आहे. मी प्रोस्थोडॉन्टिक्स आणि क्राउन आणि ब्रिज विभागातील पदव्युत्तर विद्यार्थी आहे. वळी धारण करणाऱ्या मध्ये क्लोट्रिमाझोल आणि मोरिंडा सिट्रीफोलिया (नोनी) च्या □ँटि कॅन्डिडा प्रभावाचे मूल्यां कन करण्यासाठी मी हा □ भ्यास करतआहे.

क्लोट्रिमाझोल आणि मोरिंडा सिट्रीफोलिया (नोनी) 14 दिवसांसाठी दर रोज दोन दा लावण्याची सूचना रुग्णाला दिलीजाईल. 15 व्यादिवशी रुग्णांना परत बोलविले जाईल. पेशंटला टाळुला वकवळीच्या आतल्या बाजईला निर्जंतु की करण केलेल्या स्वाब्ससह नोनी लावण्यास सांगितले जाईल. पुढच्या जेवणा पर्यंत म्यूकोसाच्या बंद संपर्का तरुग्णाला कवळी घालण्यास सांगितले जाते.

रुग्णांना सांगितले जाईल की हर्बल उत्पाद नांवर कोणतेही दुष्परिणा महोत नाहीत आणि क्लोत्रिमाझोल शी संबंधित दुष्परिणा मरुग्णांमना समजावून सांगितले जातील.

.नोनी लावल्या नंतर १ काता सापर्यंत चहा व कॅफीचे सेवण न करण्यास सांगितले जाईल.

कोणती ही □ स्वस्थ ता □ सल्यास रुग्णाला डॉक्टरांशी संपर्क साधण्यास सांगितले जाईल

सहभागींचीस्वाक्षरी _____ तारीख _____

□ न्वेषकांचीस्वाक्षरी _____ तारीख _____

ರೋಗಿಯಸೂಚನಾಕಾರಯಕಷಮತೆ

ಪರೊಸಟೊಡಾಂಟಿಕಸಮತತುಕರೌನಮತತುಸೇತುವೆಇಲಾಖೆ.

ಕೆಎಲ್ಇವಿ.ಕೆಇನಸಟಿಟಿಯೂಟಲಫಡೆಂಟಲಸೈನಸಸ, ಬೆಳಗವಿ

ಸಂಪೂರಣದಂತಪದರವಯಧರಿಸುವವರಲ್ಲಿಕೆಯಾಂಡೆಡಾಪರಭೇದಗಳಹರಡು
ವಿಕೆಮತತುಕಲೋಟರಿಮಜೋಲ್ಮತತುಮೊರಿಂಡಾಸೆಟರಿಫೋಲಿಯಾ
(ನೋನಿ)
ಯವಿರೋಧಿಕಯಾಂಡೆಡಾಪರಿಣಾಮದತುಲನಾತಮಕಮೌಲ್ಯಮಾಪನ.
"ಆನ್ವಿವೋಸಟಡೆ

ನನನಹೆಸರುಡಾ. ದಿವಯಾಜೋಶಿ.
ನಾನುಪರೊಸಟೊಡಾಂಟಿಕಸಮತತುಕರೌನಮತತುಸೇತುವೆವಿಭಾಗದಲ್ಲಿಸ
ನಾತಕೋತತರವಿದಯಾರ್ಥಿಕೆ.ಎಲ್.ಇವಿ.ಕೆ.
ಇನಸಟಿಟಿಯೂಟಲಫಡೆಂಟಲಸೈನಸಸ, ಕಹೇರ, ಬೆಳಗವಿ.
ಸಂಪೂರಣದಂತಪದರವಯಧರಿಸಿದವರಲ್ಲಿಕಲೋಟರಿಮಜೋಲ್ಮತತುಮೊ
ರಿಂಡಾಸೆಟರಿಫೋಲಿಯಾ (ನೋನಿ)
ಯಆಂಟಿಕಾಂಡೆಡಾಪರಿಣಾಮವನ್ನುಮೌಲ್ಯಮಾಪನಮಾಡಲುನಾನುಈಅ
ಧಯಯನವನ್ನುಮಾಡುತತಿದದೇನೆ

ರೋಗಿಗೆಕಲೋಟರಿಮಜೋಲ್ಮತತುಮೊರಿಂಡಾಸೆಟರಿಫೋಲಿಯಾ
(ನೋನಿ) 14
ದಿನಗಳವರೆಗೆಪರತಿದಿನಎರಡುಬಾರಿಅರಜಿಸಲ್ಲಿಸಲುಸೂಚಿಸಲಾಗಿದೆ.
ರೋಗಿಯು 15 ನೇದಿನವರದಿಮಾಡಲುಕೇಳಿಕೊಂಡರು.
ದಂತವೈದಯಮತತುಪಯಾಲಾಟಲ್ಲಲೋಳೆಪರೆಯಇಂಟಾಗಲಿಯೊಮೇ
ಲ್ಮೈಯಲ್ಲಿರೋಗಿಯನ್ನುಬರಡಾದಸವಿಯಾಚ
ಗಳೊಂದಿಗೆಅನ್ವಯಿಸಲುರೋಗಿಯನ್ನುಕೇಳಲಾಗುತತದೆ. ಮುಂದಿನ
.ಟವನ್ನುಸೇವಿಸುವವರೆಗೆಲೋಳೆಪರೆಯಂದಿಗೆಮುಚಚಿದಸಂಪರಕದಲ
ಲಿದಂತಪದರವಯವನ್ನುಧರಿಸಲುರೋಗಿಯನ್ನುಕೇಳಲಾಗುತತದೆ

ಗಿಡಮೂಲಿಕೆಔತಪನ್ನಗಳಿಗೆಯಾವುದೇಅಡಡಪರಿಣಾಮಗಳಿಲ್ಲಎಂದುರೋ
ಗಿಗಳಿಗೆತಿಳಿಸಲಾಗುತತದೆಮತತುಕಲೋಟರಿಮಜೋಲ್
ಗೆಸಂಬಂಧಿಸಿದಅಡಡಪರಿಣಾಮಗಳನ್ನುರೋಗಿಗೆವಿವರಿಸಲಾಗುತತದೆ.

ಯಾವುದೇಅಸವಸಥತೆಇದದರೆರೋಗಿಯನ್ನುವೈದಯರನ್ನುಸಂಪರಕಿಸಲು
ಕೇಳಲಾಗುತತದೆ.

ನೋನಿಅನ್ವಯಿಸಿದನಂತರಕನಿಷಠ
ಗಂಟೆಚಹಾಮತತುಕಾಫಿಯಂತಹಯಾವುದೇಕೆಫೀನ್ಸಂಬಂಧಿತಔತಪನ್ನಗಳ
ನ್ನುಸೇವಿಸದಂತೆರೋಗಿಯನ್ನುಕೇಳಲಾಗುತತದೆ

ಭಾಗವಹಿಸುವವರಸಹಿ _____ ದಿನಾಂಕ _____

ತನಿಖಾಧಿಕಾರಿಯಸಹಿ _____ ದಿನಾಂಕ _____

CONSENT FORM

"TO EVALUATE THE ANTI CANDIDA EFFECT OF CLOTRIMAZOLE AND MORINDA CITRIFOLIA (NONI) ON THE PREVELANCE OF ORAL CANDIDA IN DENTURE WEARERS" AN IN VIVO STUDY.

I,_____ aged _____ have been informed about my involvement in the study.

I agree to give my personal details like name, age, sex, address, previous dental history and the required details for the study to the best of my knowledge.

I will cooperate with the dentist for my intra oral and /or extra oral examination.

I will follow the instruction given by the doctor during the study

I permit the operator to utilize the information given by me and results obtained from this study for presentation and publication.

I will not claim any returns for my cooperation in the study, even if it is being sponsored by an agency. I am participating with my own will and wish.

In my full consciousness and presence of mind, after understanding all the procedure in my vernacular language, I am willing and give my consent to participate in this study.

Patient's name:

Patient's signature:

Address:

Dentist's Name:

Dentist's signature:

ಒಪ್ಪಿಗೆ ಪತ್ರ

"ದಂತವೈದ್ಯರಲ್ಲಿ ಮೌಖಿಕ ಕಯಾಂಡಿಡಾದ ತಡೆಗಟ್ಟುವಿಕೆಯ ಮೇಲೆ ಕಲೋಟರೆಮಜೋಲ ಮತ್ತು ಮೊರೆಂಡಾ ಸೆಟರೆಫೋಲಿಯಾ (ನೋನಿ) ಯ ಆಂಟಿ ಕಯಾಂಡಿಡಾ ಪರಿಣಾಮವನ್ನು ಮೌಲ್ಯಮಾಪನ ಮಾಡಲು" ವಿವೋ ಅಧ್ಯಯನದಲ್ಲಿ.

ನಾನು, _____ ವಯಸ್ಸಿನ _____ ಅಧ್ಯಯನದಲ್ಲಿ ನನ್ನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಯ ಬಗ್ಗೆ ತಿಳಿಸಲಾಗಿದೆ.

ನನ್ನ ವೈಯಕ್ತಿಕ ವಿವರಗಳಾದ ಹೆಸರು, ವಯಸ್ಸು, ಲಿಂಗ, ವಿಳಾಸ, ಹೆಂಡತಿನ ಹಲ್ಲಿನ ಇತಿಹಾಸ ಮತ್ತು ಅಧ್ಯಯನಕ್ಕೆ ಅಗತ್ಯವಾದ ವಿವರಗಳನ್ನು ನನ್ನ ಜ್ಞಾನದ ಅತ್ಯುತ್ತಮವಾಗಿ ನೀಡಲು ನಾನು ಒಪ್ಪುತ್ತೇನೆ.

ನನ್ನ ಇಂಟರಾ ಮೌಖಿಕ ಮತ್ತು / ಅಥವಾ ಹೆಚ್ಚುವರಿ ಮೌಖಿಕ ಪರೀಕ್ಷೆಗೆ ನಾನು ದಂತವೈದ್ಯರೊಂದಿಗೆ ಸಹಕರಿಸುತ್ತೇನೆ.

ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ ವೈದ್ಯರು ನೀಡಿದ ಸೂಚನೆಯನ್ನು ನಾನು ಅನುಸರಿಸುತ್ತೇನೆ

ನಾನು ನೀಡಿದ ಮಾಹಿತಿ ಮತ್ತು ಈ ಅಧ್ಯಯನದಿಂದ ಪಡೆದ ಫಲಿತಾಂಶಗಳನ್ನು ಪ್ರಸ್ತುತಿ ಮತ್ತು ಪ್ರಕಟಣೆಗಾಗಿ ಬಳಸಿಕೊಳ್ಳಲು ಆಪರೇಟರ್‌ಗೆ ನಾನು ಅನುಮತಿ ನೀಡುತ್ತೇನೆ.

ಏಜೆನ್ಸಿಯಿಂದ ಪ್ರಾಯೋಜಿಸಲ್ಪಟ್ಟಿದ್ದರೂ ಸಹ, ಅಧ್ಯಯನದಲ್ಲಿ ನನ್ನ ಸಹಕಾರಕ್ಕಾಗಿ ನಾನು ಯಾವುದೇ ಆದಾಯವನ್ನು ಪಡೆಯುವುದಿಲ್ಲ. ನಾನು ನನ್ನ ಸ್ವಂತ ಇಚ್ will ಾಶಕ್ತಿ ಮತ್ತು ಆಶಯದೊಂದಿಗೆ ಭಾಗವಹಿಸುತ್ತಿದ್ದೇನೆ.

ನನ್ನ ಪೂರ್ಣ ಪ್ರಜ್ಞೆ ಮತ್ತು ಮನಸ್ಸಿನ ಉಪಸ್ಥಿತಿಯಲ್ಲಿ, ನನ್ನ ಸ್ಥಳೀಯ ಭಾಷೆಯಲ್ಲಿನ ಎಲ್ಲಾ ಕಾರ್ಯವಿಧಾನಗಳನ್ನು ಅರ್ಥಮಾಡಿಕೊಂಡ ನಂತರ, ನಾನು ಸಿದ್ಧನಾಗಿದ್ದೇನೆ ಮತ್ತು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನನ್ನ ಒಪ್ಪಿಗೆಯನ್ನು ನೀಡುತ್ತೇನೆ.

ರೋಗಿಯ ಹೆಸರು:

ರೋಗಿಯ ಸಹಿ:

ವಿಳಾಸ:

ದಂತವೈದ್ಯರ ಹೆಸರು:

ದಂತವೈದ್ಯರ ಸಹಿ:

संमतीफॉर्म

व्हेंटिओ अभ्यासातील "दंतचिकित्सकांमधील ओरिअल कंडीडा" च्या निवडीवर "क्लॉटरिमाझोल आणि मोरिंडा सिटीफोलि□ा (नोनी) चे संपूर्ण कंडीडा प्रभाव मूल्यांकन करण्यासाठी

दंतवैद्यांनी माझ्या भाषेत मला कार्यप्रक्रियास्पष्ट करून मला सांगितलेली आहे. फिक्स्ड पार्शि □ ल डेंचर बनविण्यासाठी नवीन रोटरीडायमंडबरचावापरहोणारआहे. हेबर नंतर सूक्ष्म जीव तपासणी आणि वैज्ञानिक पद्धतीने साफ करायला पाठविले जाणार. ह्याचे निकाल भविष्यात प्रकाशित होऊ शकता तह्याचे मला ज्ञान आहे.

मीवयमलाया□ भ्यासातमाझ्यासहभागाबद्दलसांगितलेआहे .

1. मीमाझ्यावैयक्तिकतपशीलांसहनाव _____, वय,_____ लिंग _____, पत्ता आणि □ भ्यासण्यासाठी लागणारे तपशील माझ्या चांगल्या ज्ञानास देण्यासाठी सहमत आहे .

2.मी दंतवैद्याला माझ्याकडून दिलेल्या माहितीचा वापर करण्यास परवानगी देतो आणि ह्या □ भ्यासातून प्राप्त झालेल्या परिणामांचे प्रस्तुतीआणि प्रकाशन हेतूसाठी वापर करण्याची परवानगी देतो.

3.मी ह्या □ भ्यासात माझ्या सहकार्यासाठी कोणत्या ही परताव्याचा दावा करणार नाही जरी तो कोणत्या ही संस्थेद्वारा प्रायोजित □ सेल. मी स्वतःच्या □च्छे सहसह भागी होतआहे.

4. मी दंतवैद्यांनी दिलेल्या निर्देशांचे पालन करिन.

माझ्या प्रादेशिक भाषेतील सर्व प्रक्रिया समझून घेतल्या नंतर माझ्या पूर्ण चेतनेने आणि मनाच्या उपस्थिती मध्ये, मी ह्या □ भ्यासात सहभागी होण्यासाठी माझी सहमती आणि संमती देत आहे.

तारीख: स्थान:

संमतीदाराची स्वाक्षरी साक्षीदाराची स्वाक्षरी