



Efficacy of Natural oils as Denture Cleansers against *Candida albicans* - An In Vitro Study

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ABSTRACT

The objective of this study was to evaluate the antifungal activities of some natural oils on opportunistic fungal species like *Candida albicans* to disinfect dentures. Two methods were followed in this study which were: Evaluation of the action of the disinfectant when coated on the denture bases contaminated with candida suspension. Evaluation of the action of the standardized concentration of the disinfectant when directly added to the broth. Sesame oil is shown to have greater properties than sun flower oil but both natural oils have lesser effect than chlorhexidine ($p=0.003$). It is found that sesame oil has a more potent action against *Candida albicans* as compared to sunflower oil but is not as effective as chlorhexidine in their action in the concentrations studied.

Keywords: Sesame oil, Sun flower oil, *Candida albicans*, Dentureresin, antifungal.

INTRODUCTION

A Complete denture is defined as a dental prosthesis, which replaces the entire dentition and associated structures of the maxilla and mandible¹. A complete denture restores the aesthetic, phonetic and masticatory functions of the individual. A denture placed in the oral environment acquires a bio film on its surface which makes it susceptible for infections. Patients who wear dentures often present a variety of symptoms and abnormal intraoral findings. The advanced age of the average denture wearer and the nature of the denture bearing mucosa appear to influence the nature of the problems. Superimposed infection with candidal organisms and traumatic lesions are the most commonly encountered abnormalities². Denture stomatitis had been reported in 11-67% of complete denture wearers³. Denture biofilm is an important factor in in the pathogenesis of denture stomatitis. *Candida* species, notably *Candida albicans*, is the major fungal pathogen in humans. It is a dimorphic fungus capable of causing superficial mucosal infections, as well as systemic infections, in immune compromised individuals. The factors responsible for its pathogenesis are still not fully understood and increasing resistance to commonly used antifungal agents necessitates the search for new formulations. Many plant extracts and essential oils have biological activity both in vitro and in vivo, which has justified research on traditional medicine focused on the characterization of their antimicrobial activity⁴. The antimicrobial activity shown by plant oils is mainly due to a number of phenolic and terpenoid compounds, which have antibacterial or antifungal activity⁵. In addition, it is expected that plant compounds with target sites other than those currently used by antimicrobials will be active against drug-resistant microbial pathogens. Yet, the information available regarding plants (particularly

medicinal plants) that are active against this microorganism has, until recently, not resulted in effective formulations for human use.

Candida albicans found in the biofilm has been reported as an important agent for the installation and maintenance of denture stomatitis⁶. The prevalence of *Candida albicans* in the denture is significantly higher than that in mucosa⁷. In healthy individuals it has a prevalence rate of 45-65% with a higher in children and young adults. In denture wearers the prevalence of candida increases to 60-100% due to the fact that dentures decrease the flow of oxygen and saliva to the underlying tissue producing a local acidic and anaerobic micro environment that favors yeast overgrowth⁸.

Candida species are yeasts and within the oral cavity. It is one of the main causative organisms of denture- induced stomatitis which is primarily due its ability to adhere and form biofilms on oral cavity tissues and denture surfaces as well as due to its resistance to anti-fungal agents. This biofilm grows extensively on acrylic resin denture material and its effective removal is a significant challenge by both chemical and mechanical methods⁹. Dentures can be cleaned mechanically, chemically or through a combination of both these methods. Mechanical methods are comprised of brushing and ultrasonic treatment though the use of ultrasonic cleansers is limited due to the lack of information and discouraging cost. Brushing is simple inexpensive and effective method when used meticulously in removing denture biofilm. However, abrasive action could result in the wear of the denture base and relining materials. Another disadvantage of the mechanical methods is among the physically challenged or geriatric denture wearers. So efficient chemical denture cleansers might be an important alternative to mechanical cleansing.



Chemical methods include soaking in commercial (peroxides, acids, mouth washes and enzymes) or household (hypochlorides, sodium chloride vinegar). These solutions are simple to employ and can easily reach undercuts of the denture base, acrylic resins surface roughness remains unchanged and less susceptible to bio film accumulation¹⁰

Chemical denture cleansers can be classified as:

According To Type

Creams, pastes, gels and solutions or even tablets that are made to clean dentures. Soaking dentures in the cleaning solution varies from a few minutes to overnight depending on the manufacturer's instructions whereas denture cleansing creams, pastes or gels are brushed on the denture after it is removed from the mouth and then rinsed off.

According to the mode of action

The most commonly used cleansers are represented by the group of alkaline peroxides.

Oxidizing (bleaching) agents

Alkaline perborate, sodium perborate or potassium monopersulfate. These compounds remove staining and kill the bacteria harbored on a denture's surface.

Reducing Solutions- Sodium hypochlorite

Effervescing agents - Perborate, carbonate or citric acid. Effervescing agents provide for the rapid disintegration of the product and also create a mechanical cleansing action.

Chelating agents - EDTA. This type of compound helps to remove the tartar that has accumulated on a denture's surface.

Detergents-Sodium polyphosphate. These compounds assist in cleansing the denture.

Additional compounds -Dye a marker that provides a color change when the cleansing process has been completed. Flavorings and fragrances.

Enzymes – Protease, amylase

Disinfectants- Potassium permanganate, glutaraldehyde

Natural oils-Sesame oil, Sunflower oil

Oil pulling with sunflower and sesame oil has both historical and present significance in India for prevention of tooth decay, oral malodor, bleeding gums, etc^{11, 14}.

It has been claimed that they activate enzymes and draw toxins out of blood¹².

It has been hypothesized that sesamin and fatty acid components of sesame oil are involved in its antifungal activity¹³.

Sesame oil is known to inhibit the growth of both the mycelial as well as yeast forms of *Candida albicans*. Sunflower oil also inhibits the growth but to a lesser

extent than sesame oil³.

Sesame oil and sunflower oil have been proven to be safe and biocompatible materials. This study therefore aims to study the effectiveness of natural oils as denture disinfectants and compare the action of sesame oil with that of sunflower oil.

METHODOLOGY

Two methods were followed in this study which were-

Evaluation of the action of the disinfectant when coated on the denture bases contaminated with candida suspension.

Evaluation of the action of the standardized concentration of the disinfectant when directly added to the broth.

Sample fabrication

A total of 40 heat-polymerized acrylic denture strips were obtained from a wax pattern with the dimension of 5x1cm. The wax pattern was invested with dental stone (type III gypsum) in a metallic flask. After the setting of dental stone, the wax was removed and heat-polymerized acrylic resin was mixed according to the manufacturer's recommendation and packed into the mold at the dough stage. The metal flask was then closed and subjected to a short curing cycle. On completion of curing cycle, the flask was allowed to completely cool before opening and the denture sample was obtained. The denture sheets were cut into strips of 5x1cm dimension. The cameo surface of the strips were sandpapered and polished (wet and dry). On completion of processing, the strips were packed and autoclaved.

Preparation of suspension

The specimens for each group were immersed for 8hrs in the tested oils (sun flower oil, sesame oil) after they had been infected with *Candida albicans* and incubated for 48hr. The bacteriological procedure were accomplished by using the standardized Candidal cell suspensions (600x10⁶ CFU/ml), that is equal to McFarland standard bacteriological solution tube no.2. The procedure involve preparing the McFarland Standard Bacteriological Solution (tube No.2) that composed of 0.2 ml. Barium Chloride of 1% and 9.8 ml. H₂SO₄ of 1%,, then put 1 ml. of the prepared bacterial suspension in a screw capped bottles then immerse one acrylic specimen in each one, then incubated for 24hrs at 37°C, where after incubation we take 0.01 ml. of the bacterial suspension and plated on Sabouroid agar for counting of *C. albicans* colonies after incubated for 24hrs at 37 °C.

Preparation of disinfectants

Preparation of disinfectant: The chemicals and sterile distilled water were mixed in a sterile disposable container and transferred using 5mL syringe.



Incubation

In a sterile disposable container, 50 ml of sterile artificial saliva and Candida suspension are added and checked for demonstrable amount of organism.

Strips were placed using sterile forceps and the containers are incubated overnight to form the biofilm.

Evaluating results

The strips were rinsed in clean drinking water and placed in the disinfectant solution for 8 hours. Swabs are taken from the strips and inoculated in SDA and incubated overnight at 37 degrees Celsius. After this the growth is evaluated.

Broth culture verification

The disinfectant material is taken in a standardized concentration in 5 cuvettes of 1ml each, the candidal suspension which was made with turbidity matching 0.5 McFarland standard is taken and 10 microliter of the suspension is added to disinfectants taken in cuvette. It was allowed to react for 6 hours at room temperature. After the 6 hour period 10 microliter of this preparation was transferred to sabourauds dextrose agar and incubated for 12 hours at 37 degrees Celsius. The test was done along with a positive and a negative control.

The results were then verified.

RESULTS

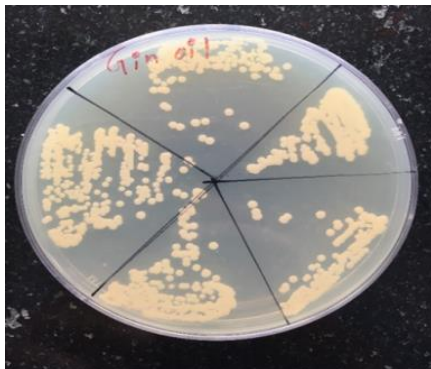


Figure 1: Sesame oil sample

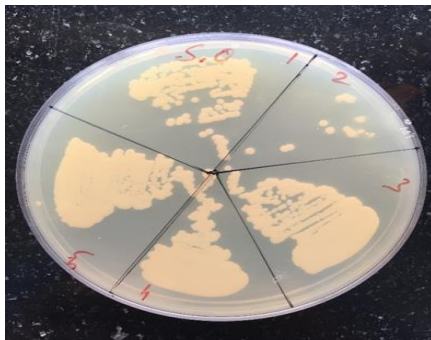


Figure 2: Sunflower oil sample

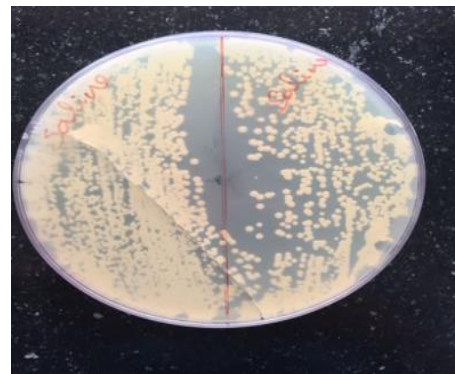


Figure 3: Positive control

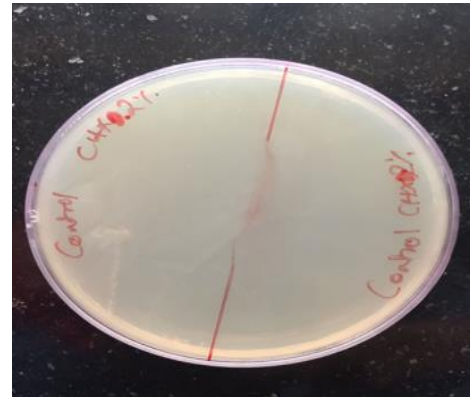


Figure 4: Negative control



Figure 5: Denture strips 4 x 1mm



Figure 6: Contamination of denture strips in candida suspension

Table 1: The table below denotes the results of the first method of testing the growth of *Candida albicans* on the denture strips contaminated with Candida suspension.

Denture strips	sesame oil	sunflower oil	chx 0.2% (Negative control)	Normal saline (Positive control)
1	+	+	-	+
2	-	+	-	+
3	-	+	-	+
4	-	+	-	+
5	-	+	-	+
6	-	+	-	+
7	+	+	-	+
8	-	+	-	+
9	-	+	-	+
10	+	+	-	+

These results correlated with the broth verification method.

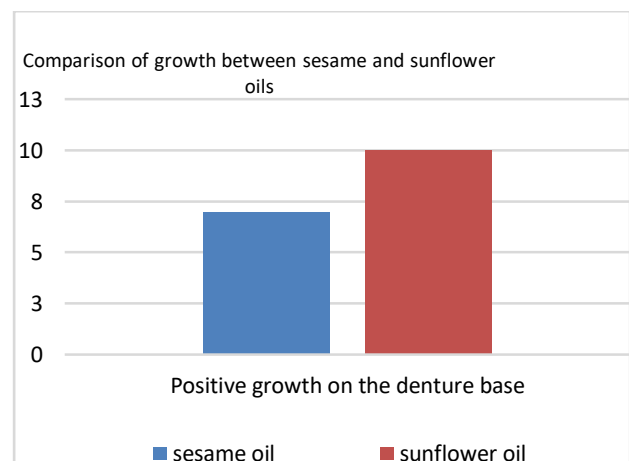
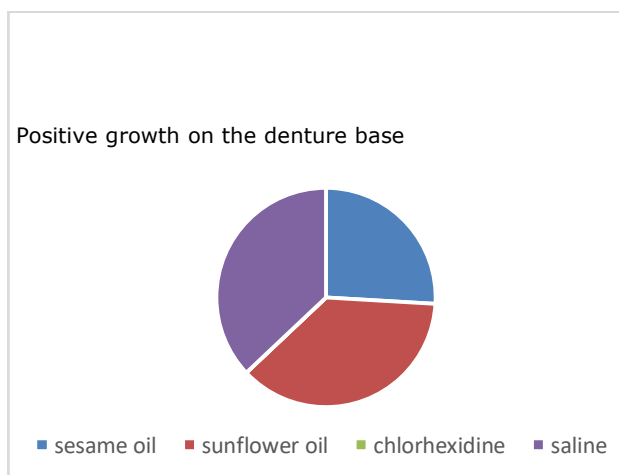
Analysis and Interpretation

Table 2: Distribution of study population according to oils and controls

	Positive		Negative		Total	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
Sesame oil	3	30.0	7	70.0	10	100%
Sunflower oil	10	100.0	-	-	10	100%
Chlorhexidine	-	-	10	100.0	10	100%

From the Frequencies table that 30% of the denture bases did not develop growth of candida and showed beneficial results and 70% of the denture bases developed growth of candid and showed negative results on use of sesame oil, whereas 100% of the denture bases showed growth of candid on using sunflower oil. Finally, 100% of the denture bases showed no growth of candid on using chlorhexidine. In the sense that the study was designed to evaluate the change in the proportion of positive and negative growth among patient’s usage of natural oils and controls for mouth, it appears that chlorhexidine is more powerful than the natural oils.

The above diagram shows the positive growth on the denture base, 37% of positive growth for saline and sunflower oil and only 26% of the patients said positive growth for the usage of sesame oil.



From the above bar diagram sunflower oil has 100% positive growth, whereas sesame oil has 70% positive growth.

COCHRAN’S Q TEST

The non-parametric Cochran’s Q test for related categories where the response is binary. Cochran’s Q is used for testing k= 2 or more matched sets, where a binary response (e.g. 0 or 1) is recorded from each

category within each subject. Cochran's Q tests the null hypothesis that the proportion of "successes" is the same in all groups versus the alternative that the proportion is different in at least one of the groups.

Hypothesis

H_0 : There is no significance different between the sesame oil and sunflower oil.

H_1 : There is significance different between the sesame oil and sunflower oil.

Test Statistics	
N	10
Cochran's Q	10.000 ^a
Df	1
Asymp. Sig.	.002
a. 2 is treated as a success	

We found that there exists a significant difference in usage among the two kinds of oils, sesame oil and sunflower oil. We surveyed ($\chi^2(1) = 10.000$, $p = 0.002$). This indicates that negative growth of the method is higher.

FRIEDMAN TEST

Friedman test is a test for comparing three or more related samples and which makes no assumptions about the underlying distribution of the data. The data is set out in a table comparing rows and columns.

Hypothesis

H_0 : There is no significance different between the oils and chlorhexidine.

H_1 : There is significance different between the oils and chlorhexidine.

Ranks	
	Mean Rank
Chlorhexidine	1.05
Oil	1.95

The Friedman test compares the mean ranks between the related groups and indicates how the groups differ. From the above mean rank table, mean rank of chlorhexidine is 1.05 and mean rank of both oils is 1.95. So, there is a marked difference between the natural oils and chlorhexidine. This further shows that the efficacy of the natural oils is not superior to that of chlorhexidine.

Test Statistics ^a	
N	10
Chi-Square	9.000
df	1
Asymp. Sig.	.003
Friedman test	

The Friedman test, which evaluated differences in medians among two method, is significant ($1, N = 10$) = 9.000, $p = 0.003$. This indicates that there is a significant difference among the two methods –natural oil and chlorhexidine.

DISCUSSION

Biofilm-forming clinical isolates of *C. albicans* showed different Sensitivity to the tested oils. Plant oils used as cooking and flavoring agents are increasingly claimed to have broad spectrum antimicrobial activity. Selected oils have been suggested to have potent antimicrobial activity against skin infections, insect bites, colds, flu, sinus congestion, asthma, bronchitis, pneumonia, tuberculosis and cholera, probably due to their phenolic, alcoholic and terpenoid constituents^{15, 16}. However,azole antifungal agents and their derivatives continue to dominate as the drugs of choice for treating *Candida* infections as either topical applications or oral drugs^{17,18}. Plant oils could find use as anti-*Candida* agents against azole-resistant strains. Most of the oils used in this study have a long history of use in food, confectionery and as components of perfume¹⁹.

However, before they are considered for use as topical preparations, a careful exploration of their undesirable effects needs to be undertaken. Efforts are being made to discover new antifungal agents from sources like microorganisms, animals and plants, which are either less toxic or not toxic at all. Use of plant extracts has a long history in Indian scenario for treatment of different ailments. Plants have been found to have rich source of antibacterial, antioxidant and antifungal activities. We found that there exists a significant difference in usage among the two kinds of oils, sesame oil and sunflower oil. We surveyed ($\chi^2(1) = 10.000$, $p = 0.002$). This indicates that negative growth of the method is higher.

Several studies using plant essential oils have been carried out against *Candida* isolated from areas other than oral cavity^{20,21}.

From the above mean rank table, mean rank of chlorhexidine is 1.05 and mean rank of both oils is 1.95. So, there is a marked difference between the natural oils and chlorhexidine. This further shows that the efficacy of the natural oils is not superior to that of chlorhexidine.

No studies are reported in the literature regarding their effect against oral species of *Candida* in a denture wearer. Hence this study was undertaken with the aim of evaluating the effect of two different plant essential oils on oral isolates of *Candida* retrieved from a denture wearing patients.

Denture stomatitis is a very common type of infection of the oral mucosa found in 60 to 65% of the denture wearing population^{22, 23}. Various reasons have been cited by the authors for this prevalence. Denture hygiene, trauma and allergy to the denture base materials are the commonest causes. In addition, when the immune status

of the patient is lowered due to systemic diseases or due to immunosuppressive therapy, the otherwise normal oral commensal *Candida* turns pathogenic and causes denture stomatitis. In diabetes mellitus, the patients have been found to be more susceptible to oral candidiasis than patients without diabetes^{24, 25}. If the patient is a denture wearer, it has been found to favor the growth of *Candida* to cause candidiasis²⁶. In a study by Sanita et al, they said that *Candida* species were more prevalent in patients with denture stomatitis in both, diabetic and non diabetic conditions. Diabetes mellitus has been a risk factor for the development of DS²⁷.

In a study by Emami et al, they found that the patient who is wearing a removable denture whether partial or complete is at a risk of developing DS²⁸.

Candida albicans has been found to be the most prevalent species in *Candida*-related denture stomatitis. Under normal circumstances, it is a common oral commensal not causing any pathosis². However, during favorable conditions it transforms itself into a virulent pathogen using various mechanisms. Sesame oil inhibited the growth of both mycelial and yeast forms. Safflower oil also inhibited the growth of both forms of *C. albicans* but to a lesser extent than sesame oil. The ability to inhibit the growth of the mycelial form correlated with sesame oil concentration. Roasting influenced growth inhibition ability and high roasted sesame oil most effectively inhibited the yeast form. The growth inhibitory effect differed among the isolates. We can hypothesize that the sesamin and fatty acid components of sesame oil are involved in its antifungal activity¹².

CONCLUSION

It has been found in this study that sesame oil has a more potent action against *Candida albicans* as compared to sunflower oil but is not as effective as chlorhexidine in their action in the concentrations studied.

The results also encourage further examination of the efficacy of plant oils at greater concentrations and intensive experimental procedures against other forms of systemic and superficial fungal infections, but also the exploration of their broad spectrum effects against other pathogenic manifestations, including malignancies, in the coming years.

REFERENCES

- Nallaswamy D. Textbook of prosthodontics. New Delhi: Jaypee Brothers Medical Publishers. 2003, 266-7.
- Agarwal V, Lal P, Pruthi V. Effect of plant oils on *Candida albicans*. Journal of Microbiology, Immunology and Infection. 43(5), 2010 Oct 1, 447-51.
- Bhat V, Sharma SM, Shetty V, Shastry CS, Rao V, Shenoy SM, Saha S, Balaji S. SCREENING OF SELECTED PLANT ESSENTIAL OILS FOR THEIR ANTIFUNGAL ACTIVITY AGAINST CANDIDA SPECIES ISOLATED FROM DENTURE STOMATITIS PATIENTS. Nitte University Journal of Health Science. 4(1), 2014 Mar 1.
- Dorey JL, Blasberg B, MacEntee MI, Conklin RJ. Oral mucosal disorders in denture wearers. The Journal of prosthetic dentistry. 53(2), 1985 Feb 1, 210-3.
- Arendorf TM, Walker DM. Denture stomatitis: a review. Journal of oral rehabilitation. 14(3), 1987 May 1, 217-27.
- Neppelenbroek, K. H. (2015). The importance of daily removal of the denture biofilm for oral and systemic diseases prevention. Journal of Applied Oral Science, 23(6), 547–548.
- Nanetti A, Stancari F, Ferri M, Mazzoni A. Relationship between *Candida albicans* and denture stomatitis: a clinical and microbiological study. The new microbiologica. 16(3), 1993 Jul, 287-91.
- Gleiznys A, Zdanavičienė E, Žilinskas J. *Candida albicans* importance to denture wearers. A literature review. Stomatologija. 17(2), 2015, 54-66.
- Faot F, Cavalcanti YW, e Bertolini MD, de Rezende Pinto L, da Silva WJ, Cury AA. Efficacy of citric acid denture cleanser on the *Candida albicans* biofilm formed on poly (methyl methacrylate): effects on residual biofilm and recolonization process. BMC oral health. 14(1), 2014 Jun 23, 77.
- Yadav R, Yadav VS, Garg S, Mittal S, Garg R. Effectiveness of different denture cleansing methods on removal of biofilms formed in vivo. Journal of Cranio-Maxillary Diseases. 2(1), 2013 Jan 1, 22.
- Thaweboon S, Nakaparksin J, Thaweboon B. Effect of oil-pulling on oral microorganisms in biofilm models. Asia J Public Health. 2(2), 2011, 62-6.
- Ogawa T, Nishio J, Okada S. Effect of edible sesame oil on growth of clinical isolates of *Candida albicans*. Biological research for nursing. 16(3), 2014 Jul, 335-43.
- Al – Rafidain Dent J Vol. 12, No2, 2012.
- Lakshmi T, Rajendran R, Krishnan V. Perspectives of oil pulling therapy in dental practice. Dental Hypotheses. 4(4), 2013 Oct 1, 131.
- Ellepola AN, Samaranayake LP. Adjunctive use of chlorhexidine in oral candidoses: a review. Oral diseases. 7(1), 2001 Jan 1, 11-7.
- Deans SG. Evaluation of antimicrobial activity of essential (volatile) oils. In: Linskens HF, Jackson JF, eds. Modern Methods of Plant Analysis. New Series Essential Oils and Waxes, volume 12. Berlin:SpringerVerlag, 1991, 309–18.
- Shuford JA, Piper KE, Steckelberg JM, Patel R. In vitro biofilm characterization and activity of antifungal agents alone and in combination against sessile and planktonic clinical *Candida albicans* isolates. Diag Microbiol Infect Dis 57, 2007, 277–81.
- Rex JH, Rinaldi MG, Pfaller MA. Resistance of *Candida* species to fluconazole. Antimicrob Agents Chemother 39, 1995, 1–8.
- Mukherjee PK, Chandra J, Kuhn DM, Ghannoum MA. Mechanism of fluconazole resistance in *Candida albicans* bio films: phase-specific role of efflux pumps and membrane sterols. Infect Immun 71, 2003, 4333–40.



20. Javidnia K, Miri R, Assadollahi M, Gholami M, Ghaderi M. Screening of selected plants growing in Iran for antimicrobial activity. *Iranian Journal of Science & Technology, Transaction A*. 33, 2009, 329-333
21. Webb BC, Thomas CJ, Willcox MD, Harty DW, Knox KW. Candida associated denture stomatitis. Aetiology and management: a review. Part 2. Oral diseases caused by Candida species. *Aust Dent J*. 43, 1998, 160-6.
22. Salerno C, Pascale M, Contaldo M, Esposito V, Busciolano, Milillo L, Guida A, Petrucci M, Serpico R. Candida-associated denture stomatitis. *Oral Med and Pathol*. 2010.
23. Belazi M, Velegraki A, Fleva A, Gidarakou I, Papanau L, Baka D, et al. Candidal overgrowth in diabetic patients: Potential predisposing factors. *Mycoses* 48, 2005, 192-6.
24. Khosravi AR, Yarahmadi S, Baiat M, Shore H, Pourkabir M. Factors affecting the prevalence of yeasts in the oral cavity of patients with diabetes mellitus. *J Mycol Med* 18, 2008, 83-8.
25. Goncalves RH, Miranda ET, Zaia JE, Giannini MJ. Species diversity of yeast in oral colonization of insulin-treated diabetes mellitus patients. *Mycopathologia* 162, 2006, 83-9,
26. Li L, Redding S, Dongari-Bagtzoglou AD. Candida glabrata, an emerging oral opportunistic pathogen. *J Dent Res* 86, 2007, 204-15.
27. Sanita PV, Pavarina AC, Eunice TG, Silva MM, Mima EGO, Ribeiro DG, Vergani CE. Candida spp. prevalence in well controlled type 2 diabetic patients with denture stomatitis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 111, 2011, 726-33.
28. Emami E, Taraf H, Grandmont P, Gauthier G, Koninck L, Lamarche C, de Souza R. The association of Denture Stomatitis and Partial Removable dental Prostheses: A Systematic Review. *Int J Prosthodont* 25, 2012, 113-9.

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