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IN VITRO ANTICANDIDAL EFFECT OF THREE NATURAL ESSENTIAL OILS AGAINST *CANDIDA ALBICANS* BIOFILM FORMED ON ACRYLIC RESIN BLOCKS

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ABSTRACT

Purpose: *Denture stomatitis due to Candida infection is very common among type 2 diabetic subjects. This can affect 10 to 75% of denture wearers. The present study was opted to assess the anticandidal effect ofnatural oilsthat can be used as a treatment modality to prevent denture stomatitis.* ***Aim:*** *The aim of this study was to evaluate the in vitro anticandidal effect of three natural essential oils- coconut oil,sesame oil and clove oil against Candida albicans biofilm formed on acrylic resin blocks.* ***Materials and method:*** *C. albicans isolated from type 2 diabetes mellitus patient with denture stomatitis was used for the present study.Initially the anticandidal efficacy of the natural essential oils was assessed by agar diffusion assay and broth micro dilution assay. The anticandidal effect of the three natural essential oils was further assayed on the C.albicans biofilm formed in vitro on acrylic resin blocks.* ***Results:*** *Clove oil exhibited significant anticandidal activity against C.albicans biofilm on acrylic resin blocks. While, Coconutoil and sesame oil did not show anticandidal effect.* ***Conclusion:*** *Clove oil can be used as a herbal alternative to prevent the C.albicans biofilm formation on acrylic resin dentures.*

KEY WORDS

Denture stomatitis,Candida albicans, Diabetes mellitus,Natural essential oils.

Introduction:

Edentulism is a debilitating and irreversible condition which is described as the "final marker of disease burden for oral health" [1] The prevalence of edentulism in India varies from 60% to 69% among age group 25 years and above [2]. By 2025, India may have highest elderlypopulation, resulting in edentulism beinga rampant problem [3].Dentures arethe Prosthetic devices made of acrylic, used to replace missing teeth. The dentures are either partial or full dentures which are supported by the surrounding soft and hard tissues of the oral cavity.

Denture stomatitis is the inflammatory process of the oral palatal mucosa that bears a complete or partial removable denture [4,5]. It is characterized by chronic pain, inflammation of mucosal area covered by dentures and halitosis. The most common etiology of denture stomatitis is infection by *Candida albicans* [6]. Several studies suggest that denture stomatitis can affect as many as 10-75% of individuals who wear dentures [7, 8, 9, 10, 11]

Many species of Candida are endosymbionts in animal hosts including human [12]. Although varied Candida species colonize the oral cavity, *C. albicans* is the principle pathogenic contributor to denture stomatitis due to its capability to produce enzymes that enables adherence of the organism to the host or the prosthesis forming a biofilm [13].*C.albicans*is an innocuous, normal commensal in the oral cavity which transforms into opportunistic pathogenwhen the host immunity is reduced. Other than the ill-fitting denture, numerous systemic factors play an important role in denture stomatitis such as type 2 diabetes mellitus, , Human Immunodeficiency Virus (HIV),nutritional factors likeanemia, vitamin deficiency, reduced pH and reduced flow of saliva.

Candidal infection is more common in patients with type 2 diabetes mellitus due to decreased neutrophil action resulting in the reduced immunity. This microenvironment favors adhesion, colonization andinvasion of the Candida into the host cells. Candidal adherence to the Palatal Epithelial Cells is more in patients with NIDDM[14,15], due to the accumulation of Advanced Glycation end products (AGEs)[16], which increases the receptors for *C. albicans* binding .The conversion of candida from commensal to pathogen involves cascade of events and secretion of enzymes. Following invasion, candida secretes extracellular enzymes such as Aspartyl proteases [17] that cause the release of pro inflammatory mediators such as IL-β which triggers the inflammation.

The pain and discomfort associated with denture stomatitis encourages the patients to discontinue the use of dentures and this in turn causes residual ridge resorption and alveolar bone loss.General preventive measuresadvised to denture wearers includemaintenance of good oral hygiene, regular cleansingof dentures to prevent the accumulation of plaque, overnight removal and soaking of dentures in denture cleansers preferably 0.2% chlorhexidinesolution.Traditional treatment modality is the topical application of antifungal agents such as nystatin or fluconazole, which arenonpalatable for most of the patients. In severe recurrent cases and in immuncompromised individuals oral antifungal drugs are prescribed. Antifungal treatment may pose danger of recurrent fungal infections with resistant strains.

Hence this study was aimed toevaluate the antifungal effect of natural essential oils on *C. albicans* biofilm.

Materials and Methods

This invitro study was conducted at Sree Balaji dental college and hospital, Pallikaranai, Chennai -100.

C.albicanswere isolated from patients with type 2 diabetes mellitus and denture stomatitis from the department of prosthodontics after getting informed consent. This study was approved by institutional ethical committee.

Invitro biofilm formation of _C.albicans_ on acrylic resin block:

C. albicans isolated from type 2 diabetes patient with denture stomatitis was used for the present study. Overnight broth culture of *C. albicans* was prepared in RPMI 1640 medium. The turbidity was adjusted to McFarland standard 0.5 as to obtain a cell density of 1× 10^8cfu/ml. [18] Heat cured acrylic denture resin used to make dentures were prepared and were cut into blocks of size, 1cm×1cm×2mm. The acrylic blocks were immersed in sterile distilled water in bijou bottles and sterilized by autoclaving at 121°C for 15 mins. The sterilized acrylic blocks (1 block/well) were placed in the wells of the 24 well sterile tissue culture plate (Zellkulter plate, Germany). Two ml of *C.Albicans* broth culture was added to each well and the plates were incubated aerobically at 35°C for 48 hours for biofilm formation on the acrylic surface. After incubation, the acrylic blocks were washed thrice with sterile distilled water to remove the planktonic cells.

Minimum InhibitoryConcentration (MIC):

Three natural essential oils - coconut oil, sesame oil and clove oil were used for the study. Sterile saline was used as the control. Minimum Inhibitory Concentration (MIC)of the three essential oils was assessed by Broth micro dilution method. Briefly, the biofilm on acrylic dentures were immersed in wells containing varying concentrations (50%, 37.5%, 25% and 12.5% v/v) of the essential oils and incubated at 35°C for 18 hours. Following incubation, the acrylic blocks were washed thrice with sterile distilled water to remove the planktonic cells. The treated acrylic blocks were vortexed to mechanically dislodge the candidal biofilm in 1 ml of sterile RPMI 1640 medium. Ten microliters of this suspension was inoculated onto SDA plates and were incubated at 35°C for 18 hours. After overnight incubation, the plates were examined for the viability of *C. albicans* and the number of cfu/ml was calculated. The assay was performed in triplicate. MIC was calculated as the lowest concentration of the essential oil that resulted in 99% growth reduction compared to the control.

Result:

Among the three natural essential oils tested for the antifungal activity only clove oil showed complete inhibition of fungal growth at a concentration of 12.5 % (Table 1). Sesame oil and coconut oil did not exhibit anticandidal activity. The colony count was >1,00,000 CFU/mL irrespective of the concentration of the essential oil used.

Table 1: CANDIDAL GROWTH FOLLOWING OVERNIGHT IMMERSION IN NATURAL OILS.

Particulars	50%dilution (CFU/mL)	37.5% dilution (CFU/mL)	25% dilution (CFU/mL)	12.5% dilution (CFU/mL)
Control	+++	+++	+++	+++
Clove oil	---	----	---	---
Sesame oil	+++	+++	+++	+++
Coconut oil	+++	+++	+++	+++

NOTE: +++ - COLONY COUNT MORE THAN 1,00,000/mL; ---- - NO COLONY GROWTH.

Discussion:

Patients with type 2 diabetes mellitusare highly susceptible to oral candidiasis and denture stomatitis compared to those without diabetes [19,20,21]. Among varied herbal products, natural oils are promising therapeutic agents for oral fungal infections. Natural oils are complex mixtures of volatile compounds obtained from plants. The primary compounds include terpenes and terpenoids. Secondary compounds are the aromatic compounds like eugenol, tyrosol etc. Sesame oil contains non-glyceride components such as sesamin, sesamonal. These compounds possess antioxidant and antifungal properties against a wide range of pathogens, including *C. Albicans* and dermatophytes[22,23,24,25].

Earlier studies have demonstrated the antimicrobial effect of sesame oil, supporting the habit of 'oil pulling' in traditional Indian system of alternative medicine [26,27], however the mechanism by which oil pullingeduces oral microbial flora is not clearly understood. It is believed that the emulsification effect due to salivary bicarbonates [28] and viscosity of the oil decreases the adhesion of microorganisms to the oral cavity. The results of the present study with regard to sesame oil is not in agreement with the previous reports [29,30]

Hierholzer and Kabara[31]in 1982, first reported the antimicrobial activity of coconut oil. The antifungal and anticandidal activity of coconut oil has been studied by many researchers [32,33]. The fatty acids in the coconut oil -caprylicacid, capricacid, lauric Acid are found to be effective against the Candida, andcapric acid was found to have effective antifungal action in shortest time [34]. However, in our study coconut oil did not reveal anticandidal activity which was well in concurrence with Agarwal*et al.,* 2010[35]

In the present study, clove oil is found to possess excellent anticandidal effect compared to the other two oils. The antifungal effect of clove oil may be due to the active compound eugenol. Eugenol is a phenyl propanoid[36] and it has anti-inflammatory property. The phenyl propanoid eugenol is found to have greater antifungal especially anticandidal activity as it inhibits the ergosterol synthesis needed for the synthesis of fungal cell membrane [37]. It also contains variety of flavonoids, including kaempferol and rhamnetin, which has anti-inflammatory and antioxidant properties. The anticandidal effect of clove oil is proven in earlier studies also [39, 34].

Different treatment modalities have been proposed for Candida-associated denture stomatitis. However, optimal or suboptimal levels of topical antifungal agents and systemic therapy can induce side effects, resistance and recurrence. Thus, new therapeutic strategies are necessary and the natural products with the promising results can play an important role in the prevention and treatment of Candida associated denture stomatitis.

The advantage of using natural products as denture cleansers include- safety and biocompatibility, have minimum chance to develop antimicrobial resistance, effective as fungicidal and bactericidal agents, and has antitumor, anti-oxidant, anti-inflammatory effect and also stimulate the immune system in addition to its low cost and easy availability.

Conclusion:

Within the limitations of this study, it could be concluded that clove oil can be an alternative denture cleanser to prevent *C.albicans* bio film formation.

Bibliography:

1. J. Cunha-Cruz, P. P. Hujoel, P. Nadanovsky.Secular trends in socio-economic disparities in edentulism: USA, 1972–2001, Journal of Dental Research 2007;86(2):131-136.
2. Shah N, Pandey RM, Duggal R, Mattur IP, Rajan K. Oral health in India: a report of the multicentre study ministry of health, Gov. of India. WHO: 2007.

3. Dubey RK, Gupta DK, ShettyP.Current status of Edentulousness in India: Systematic Review. Chhattisgarh Journal of Health Sciences, 2013;1(1).
4. Dhir G, Berzins DW, Dhuru VB, Peria-thamby AR, DentinoA.Physical proper-ties of denture base resins potentially resistant to Candida adhesion. J Prosthodont.2007;16(6):465-72.
5. Karaagaclioglu L, Can G, Yilmaz B, Ayhan N, Semiz O, Levent H. The adherence of Candida albicans to acrylic resin reinforced with different fibers. J Mat Sci.,2008; 19(2):959-63.
6. Gendreau L, Loewy ZG, Epidemiology and etiology of denture stomatitis,JProsthodont 2011, 20:251-260.
7. Kebe K, Morii K, Matsuda Kk, Hata K, Nokubi T. Journal of OralRehabilitation 2006;33:36–42.
8. Liu X, Hua H. Oral Manifestation of Chronic MucocutaneousCandidiasis: Seven Case Reports. J oral Pathol Med 2007; 36:528-32.
9. Pereira-cenci T, Del belcury AA, Crielaard W, Ten cateJM.Development of Candida-associated Denture Stomatitis: NewInsights. J Appl Oral Sci. 2008; 16:86-945.
10. Williams D, Lewis M. Pathogenesis and treatment of oral candidosis.Journal of Oral Microbiology 2011:3.
11. Emami E, Taraf H, Grandmont P, Gauthier G, Koninck L, LamarcheC,de.Souza R. The association of Denture Stomatitis and Partial RemovableDental Prostheses: A Systematic Review. Int J Prosthodont 2012; 25:113-9.
12. Ryan KJ, Ray CG. Sherris Medical Microbiology, 4th edition; McGraw Hill2004.
13. Salerno C, Pascale M, Contaldo M, Esposito V, Busciolano M, Milillo L, Guida A, Petruzzi M, SerpicoR.Candida-associated denture stomatitis.Med Oral Patol Oral Cir Bucal. 2011;16(2):139-43.
14. Darwazeham,lameypj, samaranayake.L.P, et al .The relationship betweencolonization, secretor status and*in-vitro*adhesion of Candida albicanstobuccal epithelial cells from diabetics. JMed Microbiol1990; 33: 43-9,
15. Dorocka-Bobkowska B, Budtz-Jörgensen E, Włoch S. Non-insulin-dependent diabetes mellitus as a risk factor for denture stomatitis. J Oral Pathol Med. 1996 Sep;25(8):411-5.
16. Brownlee M, Cerami A, Vlassara H. Advanced glycosylation and products intissue and the biochemical basis of diabetic complications. I VenEngl J Med1988; 318: 1315-21.
17. Vinayabhat,SharmaSM,Veenashetty, et al The extracellular enzymes of Candida albicans and their role in the development of denture stomatitis- a review,JIIADS2011; vol.2(1).
18. McFarland, J., J.Amer.Med.Assoc. 14:1176, 1907.
19. Belazi M, Velegraki A, Fleva A, Gidarakou I, Papanaum L, Baka D, et al.Candidal overgrowth in diabetic patients: Potential predisposingfactors. Mycoses 2005; 48:192-6.
20. Khosravi AR, Yarahmadi S, Baiat M, Shokri H, Pourkabireh M. Factorsaffecting the prevalence of yeasts in the oral cavity of patients withdiabetes mellitus. J Mycol Med 2008; 18:83-8
21. Guggenheimer J1, Moore PA, Rossie K, Myers D, Mongelluzzo MB, Block HM, Weyant R, Orchard T. Insulin-dependent diabetes mellitus and oral soft tissue pathologies: II. Prevalence and characteristics of Candida and Candidal lesions.
22. Palmeira de Oliveira A, Salgueiro L, Palmeira de Oliveira R, Martinez deOliveira J, PinaVaz C, Queiroz JA, Rodrigues AG: Anti-Candida activity ofessential oils. Mini-Reviews in Medicinal Chemistry 2009, 9:1292-1305.
23. Pauli A: Anticandidal low molecular compounds from higher plants withspecial reference to compounds from essential oils. Med Res Rev 2006,26:223-268.
24. Petra Beatriz Navas Hernández. Volatile Aroma Components of Cold Pressed Virgin Oils from Several Venezuelan Seeds.Research and Reviews: Journal of Food and Dairy Technology. 2013.Vol1(2).13-17.
25. B.S.NarasingaRao .Nonglyceride components of edible oils and fats.1.Chemistry and distribution.Food and Nutrition Bulletin 2001; vol. 22(1):81-86.
26. Sroisiri Thawe boon, Jurai Naka parksin, Boonyanit Thaweboo. Effect of Oil-Pulling on Oral Microorganisms in Biofilm Models.Asia Journal of Public Health, 2011. Vol.2 (2)
27. Anand T., Pothiraj C., Gopinath R. M. and Kayalvizhi B.: Antibacterial activity of sesame oil against dental caries causing bacteria. African J Microbiol Re-search.2008; Vol. (2) pp.063-066.
28. Asokan S, Rathan J, Muthu MS, Rathna,P V, Emmadi P, Raghuraman C.Effect of oil pulling on Streptococcus mutans count in plaque and saliva using Dentocult SM strip mutans test: A randomized, controlled, triple-blind study. J Indian SocPedodPrev Dent 2008; 26: 12-7.
29. Shittulaj, Bankolem.a., Ahmed t., Bankolem.n., Shittur.k., Saaluc.l., Ashiru O.A, Antibacterial and Antifungal Activities of Essential Oils of Crude Extracts of Sesame Radiatum against Some Common Pathogenic Microrganisms. Iranian Journal of Pharmacology & Therapeutics,2007. Vol.6:165-170.
30. Ogawa T, Nishio J, Okada S Effect of Edible Sesame Oil on Growth of Clinical Isolates of Candida albicans. Biological research for nursing 2013 Sep 19;16(3):335-343.
31. Hierholzer JC, Kabara JJ. In vitro effects ofmonolaurin compounds on enveloped RNA and DNA viruses. J Food Safety 1982; vol.4: 1-12.

32. Amitkumar et al. Antifungal Activity of Some Natural Essential Oils against CandidaSpecies Isolated from Blood Stream Infection JKIMSU,2012, Vol. 1 (1) 61-66.
33. Ogbolu DO, Oni AA, Daini OA, Oloko AP. In vitro antimicrobial properties of coconut oil on Candida species in Ibadan, Nigeria journal of medicinal food,2007 ;10(2):384-7.
34. BergssonG ,Arnfinnsson J, Steingrímsson O, Thormar H. In vitro killing of Candida albicans by fatty acids and monoglycerides. Antimicrob Agents Chemother.2001 ;45(11):3209-12.
35. Vishnu Agarwal, Priyanka Lal, Vikas Pruthi. Effect of Plant Oils on Candida albicansJ .Microbiol. Immunol Infect 2010;43(5):447–451.
36. Chaieb K, Hajlaoui H, Zmantar T, Kahla-Nakbi AB, Rouabhia M, Mahdouani K, BakhroufA. Phytother Res. 2007;21(6):501-6.
37. Eugénia Pinto,Luís Vale-Silva ,Carlos Cavaleiro, Lígia Salgueiro. Antifungal activity of the clove essential oil from Syzygiumaromaticumon Candida, Aspergillus and dermatop hytespecies. J Med Microbiol.2009 ;vol. 58 (11): 1454-1462.
38. N. Ahmad, M.K. Alam, A. Shehbaz, A. Khan, A. Mannan, S. Rashid Hakim, D. Bisht, M. Owais. Antimicrobial activity of clove oil and its potential in the treatment of vaginal candidiasis. Journal of drug targeting 2005, Vol. 13(10) , 555-561.

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