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Effect of *Curcuma longa* extract on *Candida albicans* adhesion to heat cure acrylic resin denture material: An *in-vitro* study

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Abstract

Objective: Adherence of Candida species to oral surfaces is regarded as a prerequisite for successful colonisation of the oral cavity. Acrylic dentures act as reservoirs and increases the risk of Candida colonisation, as denture stomatitis is the frequently reported complication among denture wearers. Commercial denture cleansers have been studied but the use of natural products is greatly advantageous. Curcumin, the major active compound of *Curcuma longa* (turmeric) noted for its potent antifungal action has been shown *in-vitro* to inhibit adherence of Candida species to human buccal epithelial cells. Hence, to know the effect of curcumin on Candidal adherence to acrylic surfaces, this study was undertaken.

Methods: 15 square blocks (1 cm x 1 cm x 2 mm) of heat cure acrylic resin were prepared, autoclaved and incubated in a suspension of *Candida albicans* (600x105 CFU/ml). A set of 5 were transferred to test tubes containing 2 ml of 0.1%, 0.2% and 0.3% curcumin solution and left for 8 h. Each block was imprinted onto separate SDA agar plate and incubated for 24 h at 37 ºC. Then the CFU's were counted and statistically analyzed.

Results: 0.3% of curcumin solution was found to be effective against *Candida albicans* adherence onto heat cure acrylic denture material with a statistically significant p value < 0.05.

Conclusion: Curcumin proved to be effective, thus paving the way to be used as an effective and a natural denture cleanser.

Keywords: Curcumin, *Candida albicans*, Heat cure acrylic resin, Denture cleanser

Introduction

Denture stomatitis is a most common chronic inflammatory condition that affects denture wearers. Microbial plaque on tissue surface of dentures acts as a significant co-factor in its pathogenesis [1]. Although bacteria and other yeasts could be pathogens in few cases; it has been proven that *Candida albicans* is the primary microbial factor in denture stomatitis [2, 3]. Because the adhesion of microorganisms to a surface is prerequisite for the colonization at that surface, the denture functions as a reservoir of infection. There are many studies regarding the adhesion mechanisms of *Candida albicans* to denture base materials as well as factors affecting these mechanisms [4, 5].

Numerous studies have shown that several Candida species possess a multitude of virulence mechanisms leading to successful colonization and infection of the host when suitable conditions occur. The recognition that Candida is an important pathogen has led to many laboratory studies evaluating their virulence attributes in an attempt to clarify the pathogenesis of the disease. The progress made in understanding some of these features, such as the mechanisms that result in adherence to surfaces, cell surface hydrophobicity, and saliva is very impressive though yet in many aspects inconclusive [6].

Traditional treatment modalities of denture stomatitis include the use of antifungal agents and modification of the prosthesis to receive a denture liner and the most widely used antifungal agent is nystatin [7, 8].

The use of natural products as disinfectants or denture cleansers is greatly advantageous over using systemic approach by antibiotics or local approach with synthetic products or some oral antibiotics. Al-Haroni (2008) [9] showed that orally directed therapies against bacteria is superior to the use of broad spectrum antibiotics. The advantages of using natural products as denture cleansers include: safety and bio-compatibility, less chances of developing bacterial resistance, effective fungicidal and bactericidal agents, and has anti-tumor, anti-oxidant, anti-inflammatory properties together with stimulating the immune system. Alkaline peroxide cleansers are the most commonly used which causes maximum discoloration [10].

Turmeric is a spice which is obtained from the rhizomes of plant *Curcuma longa*, a member of the family Zingaberaceae. Turmeric consists of 3-5% curcuminoids, which include

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mainly curcumin (diferuloyl methane), demethoxycurcumin and bisdemethoxycurcumin. Curcumin is the most important fraction which is responsible for the biological activities of turmeric. Because of its biological activities, a large number of studies have been presented on curcumin. According to these studies, curcumin exhibits anti inflammatory, antioxidant, anti carcinogenic, antiviral, antimicrobial activity. Besides these, curcumin has a variety of potentially therapeutic properties, such as anti neoplastic, anti apoptotic, anti angiogenic, cytotoxic, immunomodulatory and antithrombotic, wound healing anti diabetogenic, antistressor and antilithogenic actions [11, 12]. The purpose of this study was to conduct an in-vitro experiment to evaluate the antifungal action of varying concentrations 0.1%, 0.2% and 0.3% of *Curcuma longa* extract on *Candida albicans* adhered to heat cure acrylic resin blocks.

Figure 1: Rhizomes of *Curcuma longa*

Figure 3: Preparation of 0.1%, 0.2% and 0.3% of Curcumin solution

Figure 6: Incubation of acrylic strips in Candidal suspension

Figure 2: Soxhlet apparatus for the extraction of curcumin

Figure 4: Materials used for the preparation of heat cure acrylic blocks

Figure 7: Immersion of acyrlic strips in 0.1%, 0.2% and 0.3% of Curcumin solution for 8 h

Figure 5: Prepared blocks incubated in Candidal suspension

Figure 8: Counting of Candidal colonies on SDA plate by using colony counter

Materials and methods

The study protocol was divided into four parts:

- Preparation of *Curcuma longa* extract
- Preparation of heat cure acrylic resin blocks
- Preparation of Candidal suspension
- Adherence assay & effect of curcumin on *C. albicans*

Preparation of *Curcuma longa* extract

Fresh rhizomes were cleaned, washed with de-ionised water, sliced and dried in the sun for one week and again dried at 50 ºC in a hot air oven for 6 h. Dried rhizomes were cut in small pieces, powdered by electronic mill. 6 gm of sample were taken into a thimble and placed in a Soxhlet apparatus and 250 ml of ethyl alcohol was added as a solvent. After completion of extraction, the dark brown extract was then

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cooled, concentrated using rotary evaporator to get a crude dried extract, which was black orange in colour. This extract was made into solutions of three different concentrations, by dissolving 10 mg of curcumin in 100 ml = 0.1% (i), 20 mg of curcumin in 100ml = 0.2% (ii) and 30 mg of curcumin in 100 ml = 0.3% (iii).

Preparation of acrylic resin blocks

A specially designed metal mold was fabricated and was used for obtaining wax blocks of uniform dimensions of 1cm x 1cm with a thickness of 2mm which later were processed into heat cure acrylic resin blocks by the following conventional methods namely,

- ✓ Flasking of wax sample
- ✓ Wax elimination
- ✓ Packing
- ✓ Acrylization
- ✓ Deflasking

15 samples were prepared following the same procedure and were sterilized by autoclaving

Preparation of Candidal suspension

The procedure was accomplished according to Kazazoglu (2003) [13] by using the standardized Candidal cell suspensions (600×106 CFU/ml), that is equal to Mac-farland standard solution tube no. 2 which is composed of 0.2 ml barium chloride of 1% and 9.8 ml H_2SO_4 of 1%. Prepare new culture of pure *C. albicans* (so it will be fresh and in the active face), mix loop full *C. albicans* for several times with sterile distilled water to prepare a Candidal suspension matching MacFarland standard solution tube no. 2 by using UV spectrophotometer [10].

Methodology

15 acrylic blocks were placed in 1 ml of Candidal suspension (1 sample per 1 ml) and incubated for 24 h at 37 ºC. These 15 acrylic samples were divided into three groups (5 samples in each). These were immersed in three different concentrations viz., 0.1%, 0.2% and 0.3% of curcumin solution for 8 h.

After 8 h, the acrylic blocks were taken out using sterile forceps and imprinted on SDA agar culture plate and incubated for 24 h at 37 ºC, for *Candidal* growth. After 24 h soft cream-coloured colonies with yeasty odour demonstrating *Candida albicans* was noted. The colonies were counted using a colony counter.

Results and discussion

Of the three concentrations i.e., 0.1%, 0.2% and 0.3% used, 0.3% of curcumin solution found to be effective in bringing down the colony forming units of *Candida albicans*. The P value is <0.05 which implies it is statistically significant.

There is good evidence that adherence by a micro-organism to a surface is an important prerequisite for the permanent colonization or infection of a site exposed to a constant flow of fluid. This is certainly true in the unique environment of the mouth, where the continuous flushing effect of saliva is a very powerful defence mechanism. Although there is an extensive body of information on bacterial adhesion, investigations on Candidal adherence are comparatively limited. Nevertheless, a rapidly expanding literature on Candidal adherence attests to the potential significance of understanding the behaviour of this ubiquitous yeast and the pathogenesis of infections, which it causes in the human host. Candidal adhesion to epithelial cells has been investigated in order to define parameters relevant to the pathogenesis of oral, gastrointestinal, vaginal and urinary candidiasis [14-17]. Further, the attachment of the organism to fibrin, fibrin-platelet matrices and to vascular endothelial cells has also been examined to elucidate initial events leading to Candidal endocarditis and hematogenously disseminated infection [18, 19]. There is also a growing body of data on the adhesion of *Candida* to inert non-biological surfaces such as denture prostheses, intra-vascular and urinary catheters, and prosthetic cardiac valves [20, 21].

Edentulousness is not a disease entity in itself, but rather a consequence of pathology. Increasing incidence of edentulousness over the recent years has questioned the adequacy of dental treatment. Yet, the mainstay for the management of the edentulous state till date remains to be an acrylic complete denture. Treatment of these individuals with complete dentures not only rehabilitates them functionally but also esthetically and psychologically. However, prosthetic rehabilitation of the aged has been of great concern. The difficulties that arise not only be attributed to denture construction, but also to associated problems with continuous denture wearing. Denture stomatitis is a very frequently seen condition, occurs secondary to Candidal infection. Denture stomatitis is a term applied to an inflammation of the denture bearing mucosa, which may affect as many as two-thirds of denture wearers. Its incidence has been reported to occur among 11 to 67% of the denture wearers. *Candida albicans* has been demonstrated on the fitting surface of the dentures. This was attributed to the acidic pH prevalent under the fitting surface of the dentures which aided the proliferation of the fungi [22-25].

Colour stability is an important property of denture base acrylic resin. Colour changes indicate aging or damaged dental materials. However, according to a study by Hong et al (2009) [26], utilizing 3 types of denture base acrylic resins, it was inferred that the colour stability of denture base resins is influenced by the type of denture cleanser used. The least discoloration was found with acid type denture cleansers; whereas the most commonly used alkaline peroxide cleansers caused maximum discoloration. Difficulty in cleaning resilient denture liners remains a material disadvantage. Another study by Yoji et al (2000) concluded

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that increasing time of interaction between the denture and cleanser beyond a certain extent did not improve the efficacy of the cleanser [27]. Renata et al (2003) concluded in their study that denture cleansers used in clinical practice resulted in increased weight changes in denture liners [28]. Problems have occurred with both proper and improper use of these products, in past. The ingredient, persulfate which is known to cause allergic reactions is used in most denture cleansers as part of the cleaning and bleaching process [29-31].

Substances and extracts isolated from different natural resources especially plants have always been a rich arsenal for controlling the fungal infections and spoilage. Due to extensive traditional use of turmeric in food products, various researches have been done in order to study the turmeric and curcumin with the aspect of controlling fungal pathogens [32].

Various studies have proved the antifungal action of curcumin. Antifungal activity of curcumin was evaluated on its inhibitory effect on the adhesion of Candida species to human buccal epithelial cells with MIC: 64 mg/dl curcumin. It was 32 fold more potent than fluconazole. The study of addition the turmeric powder in plant tissue culture showed that turmeric at the 0.8 and 1.0 g/L had appreciable inhibitory activity against fungal contaminations. The methanol extract of turmeric demonstrated antifungal activity against *Cryptococcus neoformans* and *Candida albicans* with MIC values of 128 and 256 µg/mL, respectively [33].

Resistant strain development among the Candida species against existing antifungal drugs became a critical problem for therapeutic strategies. Thereby, finding new anti-Candida substances seems to be crucial. The study of curcumin against 14 strains of Candida including 4 ATCC strains and 10 clinical isolates showed that curcumin is a potent fungicide compound against Candida species with MIC values range from 250 to 2000 µg/mL [34]. In another study, anti-Candida activity of curcumin was demonstrated against 38 different strains of Candida including some fluconazole resistant strains and clinical isolates of *C. albicans*, *C. glabrata, C. krusei, C. tropicalis,* and *C. guilliermondii*. The MIC90 values for sensitive and resistant strains were 250–650 and 250–500 µg/mL, respectively. Intracellular acidification via inhibition of H^+ extrusion was identified as possible mechanism for cell death of Candida species [35]. The development of hyphae was proved to be inhibited by curcumin through targeting the global suppressor thymidine uptake 1 (TUP1) [36].

Table 1: The colony forming units (CFU) of *Candida albicans* grown on SDA plate taken from solutions of varying curcumin concentrations

Sample	0.1% Concentration I (CFU)	0.2% Concentration I (CFU)	0.3% Concentration I (CFU)
1	300	100	25
2	270	110	30
3	250	90	25
4	310	80	23
5	280	100	20

CFU – Colony Forming Unit

Lower numbers of CFU's are observed with 0.3% of curcumin solution compared to 0.1% and 0.2%

Table 2: The mean, number of samples and standard deviation for the antifungal action of these curcumin concentrations on heat cured acrylic resin denture base materials

Curcumin conc.	N	Mean	Std. deviation	Std. error	Minimum	Maximum
0.1%	5	282.0000	23.87467	10.67708	250.00	310.00
0.2%	5	96.0000	11.40175	5.09902	80.00	110.00
0.3%	5	24.6000	3.64692	1.63095	20.00	30.00
Total	15	134.2000	113.21105	29.23097	20.00	310.00

Mean of 0.1%, 0.2% and 0.3% curcumin solution are 282.0, 96.0 and 24.0 respectively.

Table 3: The one way analysis of variance (ANOVA) showed that at P<0.05 there were significant differences between treats

	Sum of squares	Deg. of freedom	Mean square	P-value
Between groups	176581.200	2	88290.600	<0.0001 VHS
Within groups	2853.200	12	237.767	
Total	179434.400	14		

The mean difference is significant at the 0.05 level.

A highly significant p value of <0.0001 was observed between the groups.

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Immersion cleansers are recommended to be used for minimum 20 min or even over night, when possible. Immersion was considered as an ideal method of cleaning for those patients who leave their dentures overnight. It was therefore decided to use an 8 h period of immersion for testing these products. Although many studies have evaluated the effect of denture cleansers and disinfectant solutions on initial *Candida* adherence to denture base materials, little attention has been paid to the effect of these denture cleansing agents on Candida associated mature biofilm, the cells of which are known to be more resistant to antimicrobial compounds and chemical cleansing. Nikawa et al (1999) in their review of *in vitro* and *in vivo* methods to evaluate the efficacy of denture cleansers concluded that chemical denture cleansers are not as efficacious in clinical use as in the *in vitro* assay [37]. The strong antifungal activity of *C. longa* rhizome and its low side effect, in addition to their low cost and availability in mostly every house were the main reasons to investigate and compare the yeast eradicating ability of various concentrations of curcumin from heat cure acrylic samples.

In our study 0.3% of curcumin was found to be effective against *C. albicans* adhesion onto heat cure acrylic resin dentures.

Conclusion

To the best of our knowledge, this is the first study to conduct in knowing the antifungal action of curcumin on *Candida albicans* adherence onto heat cure acrylic dentures. In our present study, 0.3% curcumin solution was found to be effective and can be used as a denture cleanser for overnight or 8 h immersion. Further studies need to be conducted to render curcumin as a potential denture cleanser.

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