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Original Investigation

An Evaluation of Antifungal effectiveness of Henna and Turmeric against *Candida albicans* adhered to Acrylic resin: An invitro analysis for Prevention of Denture stomatitis

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ABSTRACT

INTRODUCTION: *Candida albicans* has been known as the most common etiologic agent to cause denture stomatitis. Due to increasing resistance of this organism towards antifungal agents, plants with medicinal value are being used as alternatives. Thus this study was done to evaluate the antifungal efficacy of medicinal plants such as henna and turmeric against *Candida albicans* attached to acrylic denture resin by incorporating them into the resin. **MATERIALS AND METHODS:** 252 acrylic strips were prepared of Polymethyl methacrylate-based heat polymerizing denture base resin by compression molding technique and divided into 7 groups consisting of 36 samples each. The first group was prepared with only polymer and monomer and used as control. The remaining groups were divided according to the concentrations of henna and turmeric used. The concentrations of henna used were 0.5% (H1), 4% (H2) and 10% (H3) and of turmeric were 0.1% (T1), 3% (T2) and 7% (T3). The acrylic samples were exposed to *Candida albicans* by adhesion-based microbiological method. The amount of *Candida* adhered to acrylic samples was evaluated by two methods: slide count and plate count method. **RESULTS:** In both the methods used, H3, T2 and T3 showed significant antifungal effect. However when their antifungal effect was compared within the subgroups, no significant difference was found. **CONCLUSIONS:** Adding 10% henna, 3% and 7% turmeric can inhibit the growth of *Candida albicans* on the acrylic resin surface.

Keywords: Candida albicans, henna, PMMA-based heat polymerizing denture base resin, turmeric

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INTRODUCTION

The edentulous patients most commonly require rehabilitation with complete denture that may be tissue or implant supported, generally fabricated with polymethyl methacrylate (PMMA) acrylic resin [1]. It is susceptible to microbial colonization in the oral environment and when there is an immune suppression, the normal oral microbes can cause infection [2]. Elderly population do not or cannot follow the necessary denture-hygiene practices which cause microbial colonization leading to negative oral health impacts such as denture stomatitis and many more [3]. Previous reports demonstrated that *C. albicans* was responsible for about 54 – 74% of denture stomatitis cases [4]. *Candida* has the ability to adhere and proliferate upon both soft and hard tissue surfaces within the oral cavity, by formation of biofilm [5]. Many approaches to overcoming fungal adhesion to a denture base and attainment of antimicrobial denture have been established through surface modification of acrylic surface using different coatings [6,7] or by incorporating an antifungal additive into the denture base material [1,8]. The inappropriate use of antifungals by patients have made certain strains of fungi resistant to current antifungal therapy [9]. Thus use of medicinal plants is increasing day by day [10]. Some medicinal plants have also been added to the denture base as an antifungal agent to prevent adhesion of *Candida* to denture base like henna [11], neem [12] and black seeds [13]. Due to limited number of studies of such medicinal plants against *C. albicans*,

this study was designed to determine antifungal efficacy of henna and turmeric, both of which have proven antimicrobial properties [14,15], against *C. albicans* when incorporated into denture base resin as a possible method for prevention of *Candida* associated denture stomatitis.

MATERIALS AND METHODS

Study design and setting

This quantitative observational study was conducted in department of Prosthodontics and maxillofacial prosthetics at UCMS College of Dental Surgery from 1st November 2018 to 30th October 2020.

Participants, sample size and sampling technique:

Two hundred fifty two (252) acrylic strips were prepared of PMMA-based heat polymerizing denture base resin (Coltene/Whaledent GmbH & Co KG) by conventional compression molding technique using metal strips of 65mmX10mmX2.5mm dimension (according to ADA Specification no. 12 for denture base polymers at room temperature) for preparation of split mold . The total specimens were divided into 7 groups of 36 samples each out of which the first group was used as control prepared with only polymer and monomer and the remaining groups were prepared with incorporation of different concentrations of antifungal agents, henna (Godrej Consumer Products Limited) and turmeric in the acrylic resin mixture during the packing stage **(Table 1).**

Table 1 | Grouping of samples

Groups	Concentration
Control	Standard polymer and monomer ratio (2:1 by weight)
Henna	H1: 0.5% (0.1gm by weight)
	H2: 4 % (0.8gm by weight)
	H3: 10% (2gm by weight)
Turmeric	T1: 0.1% (0.02gm by weight)
	T2: 3% (0.6gm by weight)
	T3: 7% (1.4gm by weight)

Study procedure

Preparation of specimens

For the control group 20 gm polymer was mixed with 10 gm monomer. The required amount of antifungal agents were weighed and immersed in 10 gram of predetermined volume of monomer. Then the required mass of polymer was added to the mix to obtain 2:1 ratio by weight.

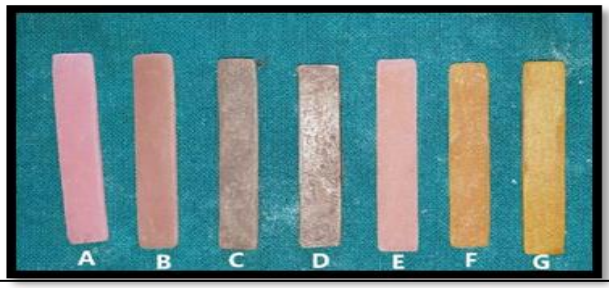
For H1, 0.5% by weight (0.1 gram) was added to 19.9 gram of polymer. For H2, 4% by weight (0.8gram) was added to 19.2 gram of polymer. For H3, 10% by weight (2 gram) was added to 18 gram of polymer.

For T1, 0.1% by weight (0.02 gram) was mixed with 19.98 gram of polymer. For T2, 3% by weight (0.6 gram) was added to 19.4 gram of polymer. For T3, 7% by weight (1.4 gram) was added to 18.6 gram of polymer. The mixture was polymerized by conventional compression molding technique using short curing cycle.

Finishing of the acrylic strips

The plaster traces and excess resin was removed with tungsten carbide bur and stone bur to specific dimension but polishing was not done as the strips are supposed to represent the mucosal side of a real denture **(Figure 1)**. The specimens were stored in distilled water at 37^{0}C for 48 hours (according to ADA specification no 12 / ISO: 1567-1981(ISO 6887-1986) for denture base polymers at room temperature).

Figure 1 | Acrylic strips with different concentrations of henna and turmeric:
(A) Control, (B) H1, (C) H2, (D) H3, (E) T1,(F)T2 (G) T3



Sterilization of the equipment

Sterilization was done in autoclave at 121^{0}C, 15 psi for 20 minutes and hot air oven at 170^{0}C for 1 hour. The inoculation procedure of *Candida* cells was done in laminar air flow chamber. All the microbiological procedures were done maintaining an aseptic environment.

Preparation of cell suspension of *Candida albicans* ATCC 10231

C. albicans strain ATCC 10231 was used. These fungal agents were cultured on Sabouraud Dextrose agar (SDA) at 37^{0}C for 48 hours aerobically and the inoculums of these fungal agents were adjusted to 1.5× 10^8 CFU/mL (colony forming unit/mL) in the Sabouraud Dextrose Broth (SDB) according to the 0.5 McFarland test standard turbidometrically. The culture was maintained at 4-6^{0}C during the experimental period in a refrigerator.

Exposing acrylic specimens to *Candida albicans*

C. albicans cells were suspended by inoculating loop in 15ml of sterile distilled water placed in test tubes and

compared with the standard suspension according to turbidity into which acrylic strips were immersed **(Figure 2).** Then they were incubated at 37^{0}C for 90 minutes for the adhesion phase in an incubator. The specimens were washed with sterile distilled water twice to remove non-adhesive cells and then immersed in 15 ml of SDB. All the test tubes were aerobically incubated at 37^{0}C for 24 hours for the formation of biofilm.

Evaluation

The acrylic strips were removed from the test tubes. The test tubes were vibrated using a vortex mixer for 10 minutes and then centrifuged at 3000 rpm for 5 minutes to get concentrated bullet of *Candida*. 100µl of the sample was serially diluted. Two methods of evaluation were used to calculate the amount of *Candida albicans* adhered to each acrylic resin specimens as follows:

1. Slide count Samples were placed on Hemocytometer after adding 20µL of Crystal violet to 60µL of each diluted sample where *Candida* cells appeared purple in colour **(Figure 3)** and were evaluated under magnification of 40X in compound light microscope **(Figure-4)**. *Candida* cells were counted in two squares out of the four main squares of the chamber and multiplied by 2 and corrected for the dilution factor.

2. Inoculated Plate count Plate count was done by Miles and Misra method. Briefly, 5µL of the diluted sample was spread on half of a petridish containing SDA with sterile cotton swab and then incubated at 37^{0}C for 24 hrs. Colonies of *Candida* were counted and corrected for the dilution factor **(Figure-5).**

Statistical analysis and data management

IBM SPSS version 19.0 was used for the statistical data analysis. The level of significance was fixed at 5%. One-way analysis of variance (ANOVA) was performed to find the significant difference in *Candida* growth between the groups. The Post-Hoc Tukey HSD test was used to find the pair-wise significance of *Candida* growth between all groups and subgroups. If the p value was <0.05, it was considered statistically significant.

Ethical consideration

This study was approved by Institutional Review Committee of UCMS-TH with reference number (UCMS/IRC/195/18).

Figure 2 | Immersion of acrylic strips in *Candida* cells suspension

Figure 3 | Stained sample in hemocytometer

Figure 4 | *Candida* cells seen in compound light microscope at 40X

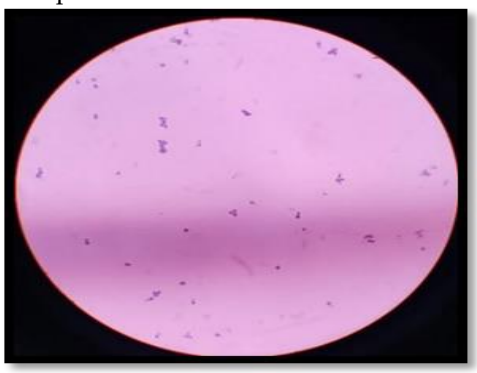


Figure 5 | Colonies of *Candida* cells in SDA

RESULTS

In Table 2 presents mean ± standard deviation of all groups for two methods. For turmeric group, mean *Candida* growth was lower compared to control with increase in percentage. However for henna group, mean of all henna subgroups was lower than that of control but mean of H2 was greater than H1. In slide count method, mean of all test groups was lower than that of control and it was in decreasing order with the increase in percentage for both henna and turmeric group.Table 3, 4 and 5 presents Post Hoc Tukey HSD Test of significance for *Candida* growth differences among the mean of different groups, within subgroups of henna and within subgroups of turmeric.

By both the methods, significant difference in mean *Candida* growth in control was found with from H3, T2 and T3 as shown by table 3. By both the methods, the mean difference of all subgroups of turmeric and all subgroups of henna was not statistically significant as shown by table 4 and 5.

Table 2 | Mean ± standard deviation of all groups for two methods

Group	Plate count Mean ± SD	Slide count Mean ± SD
Control	23436.44±6776.18	60053.33±18049.56
H1	19176.89±8807.63	54414.22±18658.82
H2	20899.56±6153.30	43633.78±18189.90
H3	17870.22±7679.87	30218.86±20210.03
T1	17468.44±7282.18	46734.22±22483.28
T2	11204.89±5667.63	43804.44±18202.28
T3	10694.67±6638.32	41457.78±25024.33

Table 3 | Post Hoc Tukey HSD Test of significance for *Candida* growth differences among the mean of different groups for two methods

Group	Plate count		Slide count	
	Mean difference	p-value	Mean difference	p-value
Control vs H1	4259.56	0.144	5639.11	0.901
Control vs H2	2536.89	0.731	16419.56*	0.012
Control vs H3	5566.22*	0.017	29834.47*	<0.001
Control vs T1	5968.00*	0.007	13319.11	0.082
Control vs T2	12231.56*	<0.001	16248.89*	0.014
Control vs T3	12741.78*	<0.001	18595.56*	0.002

Table 4 | Post Hoc Tukey HSD Test of significance for *Candida* growth differences among the mean of subgroups of henna for two methods

Group	Plate count		Slide count	
	Mean difference	p-value	Mean difference	p-value
H1vs H2	-1722.67	0.946	5639.11	0.901
H1 vsH3	1306.67	0.986	16419.56*	0.012
H2 vsH3	3029.333	0.537	29834.47*	<0.001

*. The mean difference is significant at p< 0.05 level.

Table 5 | Post Hoc Tukey HSD Test of significance for *Candida* growth difference among the mean of subgroups of turmeric for two methods

Group	Plate count		Slide count	
	Mean difference	p-value	Mean difference	p-value
T1 vs T2	6263.556*	0.004	2929.778	0.996
T1vs T3	6773.778*	0.001	5276.444	0.926
T2 vs T3	510.222	1.000	2346.667	0.999

***The mean difference is significant at p-value< 0.05 level.**

DISCUSSION

Incorporation of the antifungal agents in PMMA denture base resin at a concentration of <20% is in compliance with ISO 1567 requirement [16]. Concentration of filler greater than 20% will decrease the mechanical strength of the denture base [2]. In the present study, 3 concentrations of henna used were 0.5% (H1), 4% (H2) and 10% (H3). Similar study with similar percentages of henna was done by Nawasrah A, AlNimr A and Ali AA. [11], and found that significant difference in reducing the number of *Candida albicans* was with 1% and 10% henna. In the present study 0.5% concentration of henna was used to investigate if the antifungal efficacy could be found with even lower concentration. Also there was suboptimal effect by 5%, so a concentration of 4% was used in the present study. In the turmeric group, 3 concentrations of turmeric used were 0.1% (T1), 3% (T2) and 7% (T3). Similar concentrations of turmeric inhibited the growth of *Candida albicans* in studies done by Khan N et al.[17], and Murugesh J et al. [18]. The specimens obtained were trimmed to the specified dimension. Polishing was not done to represent the mucosal side of a real denture [19]. For the evaluation of attached cells, two methods were used, slide count and inoculated plate count by Miles and Misra method. The advantages of slide count method include practicality, ease and speed. Miles and Misra method is the standard, accurate culture-based test to quantify live organisms [11]. The values that are significant in both the studies have been considered. As evident from the results of the above mentioned tables, in both the methods used for evaluation of amount of *Candida* growth, H3 (10% henna), T2 (3% turmeric) and T3 (7% turmeric) showed significant antifungal effect in comparison with control as shown by table 3. The

result of this study is supported by a study done by Nawasrah A, AlNimr A and Ali AA [11] that the percentage used in H3 (10% henna) is effective in reduction of *Candida* adhesion and also there is no significant reduction of *Candida* growth by 5% henna. A study by Murugesh J et al [18] found that the concentration similar to T3 (7% turmeric) in the present study showed anticandidal effect. However when their antifungal effect was compared within the subgroups, the antifungal effect was found similar with no significant difference in mean *Candida* growth by the methods as evident from tables 4 and 5. The limitations of the study are that in vitro evaluation of antimicrobial agent following better oral simulation was not done. Stains that differentiate between live and dead organisms during the evaluation was not used.

CONCLUSIONS

Among the various percentages used of henna and turmeric, significant reduction in *Candida* adhered to acrylic resin was found with H3 (10% henna), T2 (3% turmeric) and T3 (7% turmeric). However, there was no significant difference in reduction of *Candida* among subgroups of henna and turmeric measured by both the methods.

ADDITIONAL INFORMATION AND DECLARATIONS

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Data Availability: Data will be available upon request to corresponding authors after valid reason.

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