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Evaluation of the Antifungal Efficacy of *Acacia Niloticia, Ocimum Tenuiflorum* incorporated into Short Term Soft Denture Liner: An *In Vitro* Study

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Abstract

Background and Objectives: Soft liner materials, though widely used in prosthodontics, have some physical and microbiological disadvantages. One such problem is the colonization of the denture surface by *Candida albicans*, thereby causing denture stomatitis. This study aims to evaluate and compare the inhibitory efficacy of *Acacia, Ocimum,* and Nystatin.

Methodology: A total of 75 specimens were prepared by adding *Ocimum, Acacia,* or Nystatin to a commercially available soft liner in increasing order of concentrations. 5 ml of Sabouraud Dextrose broth (SDB) was poured into each test tube and autoclaved. The broth was inoculated with an entire loop of *C. albicans* for 24 hours at 37ºC. Discs were placed in the test tube and incubated for 24 hours at room temperature. The broth was removed with a sterile pipette after incubation. Discs were rinsed with sterile water to remove unattached *C. albicans* and sonicated in sterile water to remove surface organisms. The attached *C. albicans* were measured by inoculating on Sabouraud Dextrose agar (SDA).

Results: Nystatin showed higher antifungal activity against *C. albicans* compared to *Acacia nilotica* and *Ocimum tenuiflorum*, and it was statistically significant. *A. nilotica* showed better antifungal activity than *O. tenuiflorum*, which was statistically significant. The higher concentration of these agents exhibited better antifungal activity, and the difference was statistically significant.

Conclusion: Nystatin showed the best inhibitory effect on *C. albicans*, followed by *A.nilotica* and *O. tenuiflorum.* The Inhibitory effect increased statistically on increasing the concentration of these additives.

Keywords: Denture stomatitis, *Candida albicans*, Antifungal efficacy of *Acacia, Ocimum*

Introduction

Denture soft liners are often used as adjuncts in prosthodontics for managing traumatized mucosa. These soft liners have certain microbiological and physical disadvantages. One such problem is the colonization of tissue surface of the denture by *Candida albicans*

and other microorganisms, which can cause denture stomatitis. Incidence of fungal growth was found to be 85% and 44% respectively, in patients wearing mandibular and maxillary dentures with soft liners.[1,2]

Microorganisms first adhere to the surface of the lining, and then they penetrate the material. This action restricts the efficacy of conventional cleaners commonly used by patients. These shortcomings have led to the incorporation of antifungal agents or antimicrobial agents into soft liners with varied degrees of success.[3]

Acacia nilotica is a source of various phytoconstituents like alkaloids, polyphenolic compounds, tannins, and flavonoids, which have therapeutic effects. *Ocimum sanctum L*, commonly known as holy basil (Tulsi), is one of the most important sources of medicine and drugs with many secondary metabolites.[4,5]

The natural plant extracts used as antimicrobial agents are safe, have no side effects, and are economical and environmentally friendly. The study aimed to evaluate the inhibitory efficacy of *A.nilotica, O. tenuiflorum* incorporated into short-term soft denture liner on fungal growth.

Materials and Methods

This *in vitro* study was conducted in the Department of Prosthodontics at M R Ambedkar Dental College and Hospital, Bangalore, Karnataka. A total of 75 samples were divided into 25 each of *A. nilotica, O. tenuiflorum,* and Nystatin.

Preparation of denture liner samples

Leaf samples were washed thoroughly 2-3 times under running tap water followed by sterile water and air-dried (Figure 1). They were then powdered and stored in air-tight containers. Aqueous extract was prepared by soaking 30 g of powdered plant material in 100 ml of distilled water for 72 hours in the dark at room temperature. Ethanol was used as an organic solvent, and the mixture was filtered and concentrated using Whatman filter paper. Ethanol was evaporated entirely to obtain the extract, and the residue was stored dry in sterile containers. The soft liner was mixed with one of the three antifungal agents and placed in a mold with a diameter of 10mm and a depth of 2mm. Specimens were prepared by adding *Ocimum, Acacia* and Nystatin in the increasing order of concentrations (6.25%, 12%, 25%, 50%, 100%). 1 part of additive and 1 part of tissue conditioner powder were taken to prepare master stock, which was considered 100% of the additive. The serial dilutions were made to get 100%, 50%, 25%, 12.5%, and 6.25% of the additive (Figure 2).

Figure 1: Leaf samples

Figure 2: Soft liner discs (5 x 1mm)

The specimens were divided into three groups of 25 each for *O. tenuiflorum, A. nilotica* and Nystatin. Each group was further subdivided into five subgroups based on the concentration of the additive: 100%, 50%, 25%, 12.5% and 6.25%.

Preparation for inoculum

Figure 3: *C. albicans* loop inoculation

A standard ATCC-approved *C. albicans* strain was collected. *C. albicans* suspension of about 10^7CFU/ml, equal to 0.5 McFarland standards, was prepared

by diluting a small amount of inoculum in a normal saline test tube. The suspension was diluted many times by adding more normal saline (NaCl) to reduce the density until 0.5 McFarland was obtained. 3 ml of Sabouraud's broth was filled into each of the 75 test tubes and autoclaved. The broth in each test tube was inoculated with an entire loop of *C. albicans* from the prepared suspension and incubated for 24 hours at 37ºC. The discs were placed in a test tube once the growth of *C.albicans* was noticed and then were incubated for 24 hours at room temperature. After incubation, broth was extracted using a sterile pipette. Sterile water was used to rinse the discs to wash away the unattached *C. albicans*. The surface organisms were eliminated by sonication in sterile water.

Assessment of Candidal Growth

Agar plates were prepared, and the test organisms attached to the discs were spread over the surface of the solidified agar. The agar plates were then incubated for 24 hours at 37ºC (Figure 3). This was followed by counting the number of *C. albicans* present on SDA as CFU using a colony counting machine.

Statistical analysis

Statistical analysis was done using SPSS v.22 software IBM Corporation. A descriptive analysis of the data was presented as frequency and mean. Mann-Whitney U test was used to compare the intergroup mean scores at different concentrations between the subjects. Kruskal-Wallis test was used to compare the mean ranks between the three groups. The level of significance was set at $P<0.05$.

Results

Nystatin showed the highest antifungal activity against *C. albicans*, followed by *A. nilotica* and *O. tenuiflorum* at different concentrations. The mean CFU ranks of the various *A. nilotica* and Nystatin concentrations were 7.00 and 2.50, respectively (Table 1). The Mann-Whitney U test revealed a significant difference in efficacy ($P < 0.05$).

The mean CFU ranks of the various *O. tenuiflorum* and Nystatin concentrations were 8.00 and 3.00, respectively (Table 2). Mann-Whitney U test was employed to compare the efficacy, and the difference was statistically significant ($P<0.05$). The mean CFU ranks of the various *O. tenuiflorum* and *A. nilotica* concentrations were 8.00 and 3.00 respectively (Table 3). To compare the efficacy, the Mann-Whitney U test revealed a significant difference in efficacy ($P<0.05$).

Table 1: Comparison of antifungal efficacy between *Acacia nilotica* and Nystatin

Concentrations	*Groups*	Mean rank	U	Z	*P*
100	*Acacia Nilotica*	7.00	0.000	-2.570	0.01*
	Nystatin	2.50			
50	*Acacia Nilotica*	7.00	0.000	-2.502	0.01*
	Nystatin	2.50			
25	*Acacia Nilotica*	7.00	0.000	-2.470	0.01*
	Nystatin	2.50			
12.5	*Acacia Nilotica*	7.00	0.000	-2.491	0.01*
	Nystatin	2.50			
6.25	*Acacia Nilotica*	7.00	0.000	-2.502	0.01*
	Nystatin	2.50			

Table 2: Comparison of antifungal efficacy between *Ocimum Tenuiflorum* and Nystatin

Concentrations	*Groups*	Mean rank	U	Z	*P*
100	*Ocimum Tenuiflo-rum*	8.00	0.000	-2.795	0.005*
	Nystatin	3.00			
50	*Ocimum Tenuiflo-rum*	8.00	0.000	-2.703	0.007*
	Nystatin	3.00			

25	*Ocimum Tenuiflo-rum*	8.00	0.000	-2.660	0.008*
	Nystatin	3.00			
12.5	*Ocimum Tenuiflo-rum*	8.00	0.000	-2.652	0.008*
	Nystatin	3.00			
6.25	*Ocimum Tenuiflo-rum*	8.00	0.000	-2.652	0.008*
	Nystatin	3.00			

Table 3: Comparison antifungal efficacy between *Acacia nilotica* and *Ocimum Tenuiflorum*

Concentrations	*Groups*	Mean rank	U	Z	P
100	*Acacia Nilotica*	3.00	0.000	-2.627	0.009*
	Ocimum Tenuiflo-rum	8.00			
50	*Acacia Nilotica*	3.00	0.000	-2.627	0.009*
	Ocimum Tenuiflo-rum	8.00			
25	*Acacia nilotica*	3.00	0.000	-2.214	0.02*
	Ocimum Tenuiflo-rum	8.00			
12.5	*Acacia Nilotica*	3.00	0.000	-2.530	0.01*
	Ocimum Tenuiflo-rum	8.00			
6.25	*Acacia Nilotica*	3.00	0.000	-2.619	0.009*
	Ocimum Tenuiflo-rum	8.00			

Discussion

Flavonoids in the flower, fruit, and leaves are the key constituents responsible for antimicrobial properties. They act by inhibiting cytoplasmic membrane function, inhibiting attachment and biofilm formation, and altering membrane permeability.[4] *O. sanctum* L, commonly known as holy basil (Tulsi), is an important source of medicine and drugs with many secondary metabolites. It shows antimicrobial, antidiabetic, adaptogenic, antispasmodic, anticancerous, antifungal, antifertility, hepatoprotective, analgesic, cardio protective, antiemetic and diaphoretic properties.[5]

The denture liner samples that were incorporated with nystatin showed the highest mean inhibitory efficacy, followed by *A. nilotica* and *O. sanctum* on *C. albicans*. This study agrees with the study conducted by Mohammed, who concluded that incorporating nystatin into tissue conditioners is beneficial with slight or no consequences on the physical properties of tissue conditioners.[6]

The inhibitory effect on *C. albicans* increased statistically by increasing these additives' concentration. However, Douglas and Walker stated that incorporating only a fraction of the conventional total dose of nystatin is necessary.[7] This study agrees with a study conducted by Mithun in which *Acacia* showed a significant reduction in *C. albicans* colonization.[8] Yusra conducted a study to assess the efficacy of the *A. nilotica* extract as a disinfectant on additional silicone impressions. A bactericidal effect was seen with an optimum 75 mg/ml concentration.[9] *Acacia* bark decoction was used to treat sore throats and ulcers. *Acacia* leaves were used to treat bleeding gums and its bark was used in toothpaste as a cleanser.[10] When the antifungal inhibitory efficacy of Group 2 and Group 3 was compared, Group 3 showed the best inhibitory efficacy.

Jai Mehta conducted a study to compare the antimicrobial efficacy of *O. sanctum* and *Punica granatum* extracts as herbal denture cleansers in geriatric denture wearers, and an *O. sanctum* extract solution was found to be slightly more effective than *P. granatum*.[11] In a study conducted by Aamir *et al., Ocimum* showed statistically significant antifungal activity.[12] Gopalkrishna *et al.* found that antifungal activity in *C. albicans* increased with an increase in the concentration of *O. sanctum*.[13]

However, this study has a few limitations. The longevity of the drug effectiveness was not evaluated, as inhibitory

efficacy was evaluated after an incubation period of only 24 hours. Moreover, as *in vitro* results cannot be extrapolated *in vivo*, further investigation is needed by launching *in vivo* clinical trials using a larger sample size. Further studies are required to determine the half-life of the antimicrobial agents after they are mixed with denture liner and the rate of release of these antimicrobial agents from it.[14]

Conclusion

Nystatin had the best inhibitory effect on *Candida albicans* compared to *Acacia nilotica* and *Ocimum tenuiflorum*. *A. nilotica* showed a better inhibitory effect on *C. albicans* compared to *O. tenuiflorum*. The inhibitory effect increased statistically on increasing the concentration of these additives. Adding *A. nilotica, Ocimum*, and Nystatin into the soft denture liner can help in decreasing the incidence of denture stomatitis.

Source(s) of support

Nil

Conflict of Interest

Nil

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