Antifungal Efficacy and the Mechanical Properties of Soft Liners against *Candida albicans* after the Incorporation of Garlic and Neem: An *In vitro* Study

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**INTRODUCTION**

Early in the second century AD, Galen[^1] described oral *Candida* as *aphthas albus*, and these infections are opportunistic and called as “the disease of the diseased.”[^2] Denture-induced stomatitis is found in about 65% of denture wearers particularly, the patients who are immunocompromised, elderly, or the patients undergoing immunosuppressive therapy.[^3] Several researchers have reported that causes are multifactorial for the denture-induced stomatitis such as lack of oral hygiene status, allergy for the denture base material, trauma from occlusion, existing fungal infections, dietary deficiency, and hematological disorders. Microporosity and roughness in the denture surface will lead to adherence of *Candida albicans* and formation of the colonies on the fitting surface of the denture.[^4] These microorganisms have to be removed either by mechanical or chemical cleansing; however, in some situations, these cannot be completely removed from the denture surfaces.[^5] Poly methyl methacrylate (PMMA) is the most common material used in the fabrication of removable complete/partial denture prosthesis. Constructing smoother tissue surface and well-polished denture may reduce the adherence of *C. albicans* cells when compared to rough surfaces of the denture.[^5] For that, soft lining materials modified by neem or garlic incorporation.

**Objectives:** To evaluate the *in vitro* growth inhibition of *Candida albicans*, in the soft-liner material and Shore A hardness from resin-based denture soft lining materials modified by neem or garlic incorporation.

**Materials and Methods:** Resin discs were prepared with poly methyl methacrylate (PMMA) and soft liners incorporated with varying concentrations of neem or garlic. For antifungal activity, resin discs were placed on agar plates inoculated with *C. albicans* and were evaluated after 2, 4, and 7 days using the streaking method. The hardness of the PMMA was evaluated with the use of Shore A at 2, 4, and 7 days. Data were statistically processed by SPSS software (IBM Company, Chicago, USA) using Kruskal–Wallis test, and *post hoc* comparisons were done using Dunn’s test. *P* < 0.05 was considered statistically significant.

**Results:** Neem and garlic added to PMMA soft liner had an inhibitory effect on *C. albicans*. Both the neem and garlic when added showed positive results against *C. albicans* when compared to the control group. The soft liner hardness increased statistically by time but not for the different plant extract concentrations.

**Conclusions:** Within the limitations of this *in vitro* study, it was found that neem and garlic can be used as an additive to tissue conditioner to reduce the adherence of *C. albicans* without significantly affecting the hardness of the heat-polymerized acrylic resin.

**KEYWORDS: Antifungal property and tissue conditioners, Candida albicans, garlic, neem**

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materials are applied over the tissue surfaces of the denture to provide equal distribution of the functional load during occlusion and also a cushioning effect between the hard denture base and the tissues to improve the tissue health. However, studies have reported that soft lining materials are also prone to adhesion of C. albicans.\(^8\)

Denture-induced stomatitis are treated with antifungal drugs such as nystatin and fluconazole.\(^9\) Although systemic dosage of antifungals may be effective against mucosal lesions, they are not effective for Candida-infested denture fitting surface.\(^1\) Furthermore, these drugs are found to have toxic effect on human beings if it is overused. Hence, alternative antifungal agents extracted from the plants are being researched for the use of treating oral candidosis.

India is considered to be a rich emporium of medicated plants, fundamentally utilized as a part of preventive and healing medicine.\(^9\) Neem (Azadirachta indica) is found to have antifungal effect and antimicrobial effect.\(^10-12\) Garlic is another indigenous to Asian region and utilized as a topping, particularly in a large portion of the Asian cooking since it acts as an antioxidant\(^14\) (antimicrobial, antibacterial, antiviral, antifungal, and antiprotozoal properties) and also have beneficial properties on the cardiovascular and immune systems.\(^13,15,16\) However, only few or no studies have been reported on the use of neem and garlic as an adjunct for the use of treating denture-induced stomatitis.

The purpose of this study was to determine whether addition of neem and garlic extracts to the soft liner would inhibit the growth of C. albicans and also to evaluate the hardness of the soft lining material after the incorporation of neem and garlic.

**Materials and Methods**

The study was carried out for 10 days in total, which includes the sterilization and the observation of the Candida growth in the samples and also the mechanical testing of the acrylic sample for hardness. Institutional review board no SRPOC/2017/950 was obtained for this study.

**Specimen preparation**

*Preparation of acrylic discs*

Power sampling method was carried out to find out the number of samples to be included for the study and it has been concluded that 30 samples can be selected for this pilot study; hence, 30 acrylic discs measuring 12 mm in diameter and 6 mm in thickness were prepared using PMMA according to the manufacturer’s instructions [Table 1].

*Preparation of PEM discs*

30 wax discs were fabricated measuring 12 mm in diameter and 6 mm in thickness [Figures 3-5].

Three-part dental flask was used for flaking the fabricated wax patterns using dental stone. Reverse flaking technique was followed by wax elimination. Disc-shaped mold space was created where heat-activated PMMA resin was packed and cured according to the manufacturer’s instructions to obtain the acrylized discs [Figure 1].

Thirty acrylized discs were then grouped into three groups [Table 2]. These samples served as a base on which the soft liner to be tested was added later. One side of each sample was polished using tungsten carbide acrylic bur, silicon carbide papers, followed by pumice and rouge. The contradicting surface was left unpolished resembling the tissue surface of the denture.\(^15,17\) An index of the prepared specimens was made using polyvinyl siloxane material on which soft liner material was added. All these procedures were carried out in the laminar air-flow chamber to avoid contamination (aseptic environment), and also, the discs were disinfected by exposing them in ultraviolet light for 30 min [Table 2].

| Table 1: Materials used in the study |
|-------------------------------------|
| **Material**                        | **Manufacturer**                              |
| Heat cure poly methyl methacrylate  | DPI heat cure, DPI, Dental products of India, |
|                                     | Bombay Burmah Trading corporation, Ltd., India|
| Modeling wax no. 02                 | The Hindustan Dental Products, Hyderabad, India|
| Aquasil soft putty/regular set      | Dentsply                                    |
| (polyvinyl siloxane)                |                                            |
| Heat cure cold mold seal            | DPI heat cure, DPI, Dental products of India, |
|                                     | Bombay Burmah Trading corporation, Ltd., India|
| Dental stone                        | GC soft liner, 0104B236-0000, GC India       |

*Figure 1: Wax patterns*
Sterilization of the Specimen

The specimens were handled carefully which includes the utilization of nonsterile nonpowdered latex gloves for all phases of investing, dewaxing, packing, and retrieval of the specimens. Duplicates were handled with stainless-steel tweezers and were put away in sterile distilled water for 6–8 days and water was changed every 24 h.

Preparation of Neem and Garlic Powders

To obtain neem (A. indica) and garlic (Allium sativum) powder, crisp neem leaves were collected from the trees and garlic was bought from the open markets. The preparation of the powder was done at the Department of Pharmacology, Sri Ramachandra University, Porur, Chennai.

Preparation of Neem Extract

One kg of crisp, shade-dried leaves of A. indica grinded in 4 L of refined water and permitted to soak overnight. The suspension was centrifuged at 5000 rpm for 20 min and separated through a Whatman No. 1 channel paper. The supernatant liquid was permitted to dissipate in glass petri dishes under tube light to give heat and to anticipate clamminess so that no organism growth occurs. At the point when it is totally dry, the powder was gathered by scratching and was put away.

Preparation of Stock Solution – Neem and Garlic

Ten milligrams of the active extract was added to 10 ml of brain heart infusion (BHI) broth so that each ml contains 1 mg of the active ingredient of neem and garlic. Doubling dilution of the active ingredient was prepared (100%, 50%, 25%) using BHI broth and artificial saliva (Wet Mouth). To all the test tubes, 10 ml of 10^8 CFU (ATCC 90028) C. albicans strains were added. The tubes were incubated for 24 h at 37°F.

After 24 h, the tubes were checked for the presence of any growth by observing the turbidity, Gram staining, and by subculture on blood agar plates at 2nd, 4th, and 7th day. All the tubes which were clear, Gram stain of the broth showing no organisms, and subcultures showing no growth were recorded as no growth of any turbidity, negative for Gram staining and showed no colonies on subculturing into blood agar plates were taken as no growth or inhibition. While the tubes which were turbid, positive Gram staining and showed colonies on subculturing into blood agar plates were recorded for growth of colonies.

Mechanical Testing

Shore A Durometer – the effect of incorporation of garlic and neem on the hardness property of the soft liner was evaluated by using Shore A durometer instrument after 2, 4, 7, and 10 days. For the mechanical testing purpose, specimens were prepared under control group and test group (with incorporation of neem and garlic powder).

During hardness testing, the specimen was supported by a glass slab, and indenter was allowed to penetrate the
specimen. Average of three different readings was taken as a testing value. The distance between the indenter and specimen surface was fixed by 20 mm, and contact time after penetration was 5 s. The reading was noted directly from the scale which represents the hardness value.

**Analysis Shore a Hardness**

Measurements were made according to ASTM D2240 using a digital Shore A durometer (Instrutherm, Sao Paulo, SP, and Brazil) based on a scale hardness tester (Wallace, Kingston, England). Six measurements were made on each side of all specimens.

**Results**

**Microbiological testing**

On subculturing on the agar plates, there were no colonies observed after 2nd, 4th, and 7th day with neem. However, with garlic, few colonies were observed on the 4th day after subculturing [Figure 2].

The hardness value was noted from the scale after 2nd, 4th, and 7th days [Table 3].

For comparison between the groups, Kruskal–Wallis test was used. *Post hoc* comparisons were done using Dunn’s test. *P* < 0.05 was considered to be significant. For comparison within the same group for different days, Friedman’s test was used. R version 3.3.0 software (IBM Company, Chicago, USA) was used for the analysis [Table 3].

Group 1 is control group, 2 and 3 being neem and garlic, respectively. On comparing the groups, there was no statistically significant change in hardness.

On day 4 and day 7, there was a statistically significant change in hardness observed between the groups.

On day 4: Group 2, i.e., neem group shows significant increase in hardness compared to the control group. The difference of hardness between the neem and garlic is not significant.

On day 7: Group 2, i.e., neem group shows significant increase in hardness compared to the control group. The difference of hardness between the neem and garlic is not significant [Figures 6 and 7].

**Discussion**

Dental prostheses made with acrylic (PMMA) will be coated with salivary proteins when inserted in the oral environment which in turn forms a pellicle. This pellicle alters the surface properties of PMMA and mediates subsequent adherence of microorganisms including *Candida* species in the denture surface (Edgerton and Levine, 1992). It has been reported that surface roughness

![Figure 2: Finished acrylic discs](image)

![Figure 3: Addition of the soft liner to the polyvinyl siloxane index](image)

| Serial number | Group | Day 2 | Day 4 | Day 7 |
|---------------|-------|-------|-------|-------|
|               |       | Mean  | Mean  | Mean  |
|               |       | SD    | SD    | SD    |
| 1             | 1     | 10.0  | 10.7  | 12.0  |
| 2             | 2     | 14.1  | 13.3  | 16.0  |
| 3             | 3     | 13.0  | 15.2  | 18.3  |

| *P*          |       | 0.015 |
|--------------|-------|-------|

SD=Standard deviation
is a vital property because of its impact on microbial adhesion.\textsuperscript{[21]} For this reason, if a soft-liner material incorporated with antifungal drugs, which has antifungal efficacy to be developed to interfere on the denture surface to avoid colonization and penetration by \textit{C. albicans} in the denture.\textsuperscript{[21]} However, it has been reported that antifungal drugs have some toxic effects on the patient if it is overused; hence, the plant extracts neem (\textit{A. indica}) and garlic (\textit{A. sativum}) which are naturally available and also possess antifungal property can be used for the same.\textsuperscript{[4,14,22-27]} This leads to the microbiological investigation conducted in this study, which confirms that the released concentrations of the antifungal agents (plant extracts) from the soft lining materials were able to induce an antifungal effect against \textit{C. albicans} on agar culture. Furthermore, the changes in any hardness of the denture base after the incorporation of the plant extracts were analyzed. The limitations of this particular study are decreased sample size and lack of oral environment, as this was an \textit{in vitro} study. Further studies have to be done \textit{in vivo} to confirm the results. The future prospects of the study can also include using these naturally available antifungal sources in drug delivery system.

**Conclusions**

Within the limits of this pilot study, it can be presumed that utilization of naturally occurring antifungal specialist agents such as neem and garlic can be utilized as an alternative for treating denture-induced stomatitis. However, further studies have to be conducted to find the pharmacological effects such as antibacterial, anticarcinogenic, and anti-inflammatory activities of the plant extracts for the use in the oral environment.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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