



Antifungal Activity of Green Tea and Ginger Extract on heat Polymerised Polymethylmethacrylate Denture Base Resin Material

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ABSTRACT

Introduction: An increased prevalence of candidiasis is well documented and also *Candida albicans* has been shown to play an important role in oral candidiasis. The study aimed to compare the antifungal activity of extracts of green tea, ginger, a mixture of green tea and ginger against laboratory (ATCC 90028) and clinical strains of *Candida albicans*². The study aimed to compare the antifungal activity of extracts of green tea, ginger, a mixture of green tea and ginger against laboratory (ATCC 90028) and clinical strains of *Candida albicans*.

Materials and methods: Green tea leaves and ginger were collected and sun-dried for 3 days, and ground to powder. The alcoholic extract was prepared using a Soxhlet apparatus. The concentrated extract was used to prepare serial dilutions into which incubated heat Polymethylmethacrylate (PMMA) specimens were immersed, which was used in testing antimicrobial efficacy. The study was carried out using well diffusion technique. The zone of inhibition was measured for each sample.

Results: Laboratory strains have shown about 16 to 18 mm of a zone of inhibition at a concentration of 100 mg/mL. For clinical strains, Ketoconazole and denture cleansing tablet have shown considerable inhibition as compared to green tea and ginger extract.

Conclusion: Indiscriminate use of such plant material by either incorporation, immersion or mouthwash is not going to reduce Candidal activity. There is a need to estimate the specific biological activity of medicinal plants for better application in medical field.

Keywords: *Candida albicans*, Clinical strains, Laboratory strains, PMMA.

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INTRODUCTION

Candidiasis is associated in one way or another with the formation of *Candida* biofilms on the inert surfaces or biological surfaces.¹ An increased prevalence of candidiasis is well documented and also *Candida albicans* has been shown to play an important role in oral candidiasis, denture stomatitis, and severe periodontitis. Candidiasis is a very common oral mucosal lesion, affecting approximately 50% of denture wearers. Recently, there is an increased interest to use natural antimicrobial compounds.²

Herbal medicines are still widely used in many parts of the world especially in areas where people do not have access to modern medicines. Green tea was selected for the study because it has been used as both a beverage and a medicine in most of Asia. The ability of tea plant extract to inhibit the growth of fungal strains is an indication of its antifungal property.³ The *Zingiberaceous* plants are characterized by their tuberous or non-tuberous rhizomes, which have strong aromatic and medicinal properties. Since ginger is easily available and well-tolerated, it can be incorporated into medications for topical antifungal therapy.⁴ However, some reports suggest that clinical isolates (*Candida albicans*) appear to be more resistant to the common anticandidal drugs when compared to the laboratory strains.⁵ The present study aimed to test the antifungal effect of green tea leaves extract and ginger extract on laboratory strain (ATCC 90028) as well as clinical isolates of *Candida albicans* and to compare with the commercially available denture cleansing tablets and a standard drug.

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MATERIALS AND METHODS

Preparation of Extract

Commercially available green tea leaves (*Assamica agro*) were obtained and made it into a fine powder. Ginger (local shop) was cut into pieces, dried under sunlight for three days and was powdered. About 100 gm of both the powders were subjected to Soxhlet extraction by using 99.9% ethyl alcohol for about 72 hours each. The extract was dried under reduced pressure, stored at 4° C for further analysis.

Preparation of the Acrylic Samples

The heat-polymerized PMMA resin (Vertex RS, Denti-mex, Netherlands) blocks were prepared using a stone mold. Heat polymerized PMMA was mixed in the polymer to monomer ratio of 3:1 by volume in a porcelain jar. When the material reached the dough stage, it was packed in the flask and kept under pressure of 2000 Mpa for bench curing of 30 minutes. Once the bench curing was done, the flasks were clamped and polymerized using a short curing cycle of 74° C for two hours and then 100° C for one hour. After the flasks were cooled, the specimens were deflasked, and the excess material was trimmed using tungsten carbide bur. Eighteen PMMA samples (5 mm × 5 mm × 1 mm) were cut out from the resin blocks with the diamond disc (Axis Dental, Kerr Corporation, USA). The test surfaces of the samples were not polished so as to give an accurate impression of the tissue surface of the dentures and it was grouped as follows:

- T1-Ginger extract
- T2-Green tea extract

Microbiological Study Procedure

The antifungal activity was carried out by well diffusion method. For heat cure specimens spread plate technique was used. The clinical strains were obtained from Dr Joshi's central clinical microbiology laboratory, Vashi, Navi Mumbai. Forty-eight hours culture of *Candida albicans* grown on Sabrauds Dextrose Agar was taken. Inoculums were prepared by adjusting the cell density to 2×10^8 CFU/mL in brain heart infusion (BHI) broth, using 0.5 McFarland standard. Ten ml BHI was taken in the required number of sterile test tubes. Autoclaved sterilized acrylic sheets were added in the test tubes and were Infected with 100 µl of inoculum. Incubated at 37° C for 24 hours. The acrylic sheets were washed three times with 5 mL of phosphate buffered saline (PBS). Ten mL of the test agent at the desired concentration was taken and expose the acrylic sheet for 10 minutes. Control tube has D/W instead of the

test agent. The acrylic sheets were washed 3 times with 5 mL of PBS and suspended in 10 mL of sterile D/W. Twenty-five µl of Neat and 1:10 dilution on phosphate buffered saline (SDA) plates and were spread properly using a sterile cotton swab. Incubated at 37° C for 24 hours. The colonies grown on the SDA plates were counted and multiplied with the respective dilution factor to get the colony forming units (CFU)/mL. The positive and negative controls were compared.

RESULTS

For Laboratory Strains

When ginger and green tea extract were tested at concentrations of 50 mg/mL, 80 mg/mL, and 100 mg/mL and compared with ketoconazole and denture cleansing tablet as positive control and distilled water as negative control—both the extracts have shown about 16 to 18 mm of zone of inhibition at a concentration of 100 mg/mL as seen in Figures 1 and 2.



Fig. 1: Zone of inhibition for green tea extract



Fig. 2: Zone of inhibition for ginger extract

For Clinical Strains

When ginger and green tea extract were tested at concentrations of 50 mg/mL, 80 mg/mL, and 100 mg/mL and compared with ketoconazole and denture cleansing tablet as a positive control and distilled water as negative control:

- Ketoconazole and denture cleansing tablet have shown considerable inhibition whereas ginger extract, green tea extract and a combination (as seen in Figs 3 to 5) have shown a very little zone of inhibition of about 4 to 6 mm at 100 mg/mL.
- Negligible inhibition was recorded at 50 mg/mL and 80 mg/mL.

When the heat cure polymethyl methacrylate denture base material was examined there was no antimicrobial activity seen, when tested as a cleansing agent.

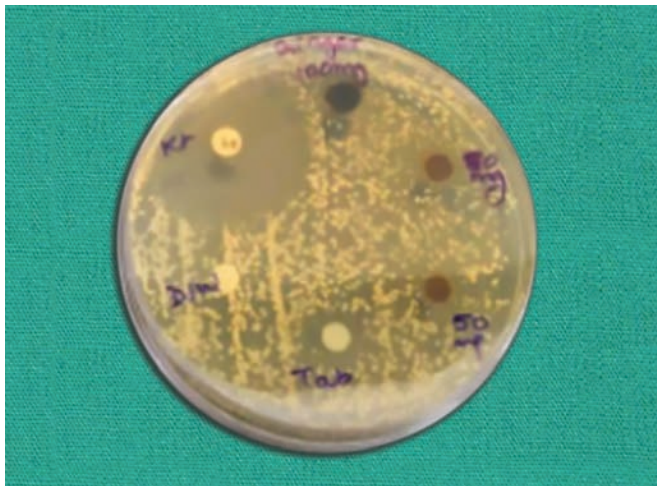


Fig. 3: Ginger extract

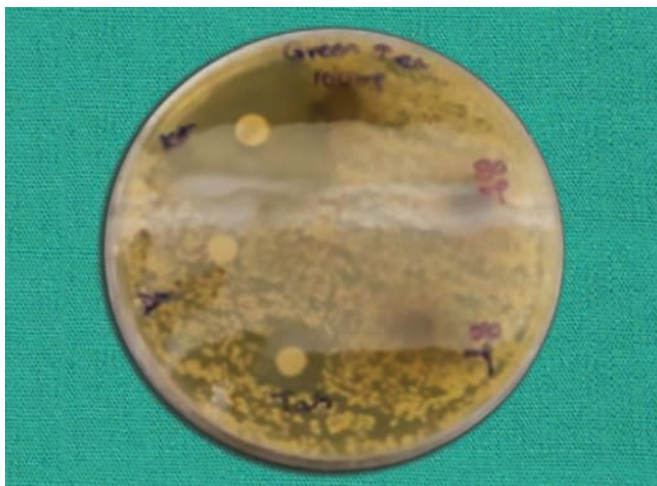


Fig. 4: Green tea extract

DISCUSSION

Candida albicans can grow, proliferate and co-exist with the host for a long time.

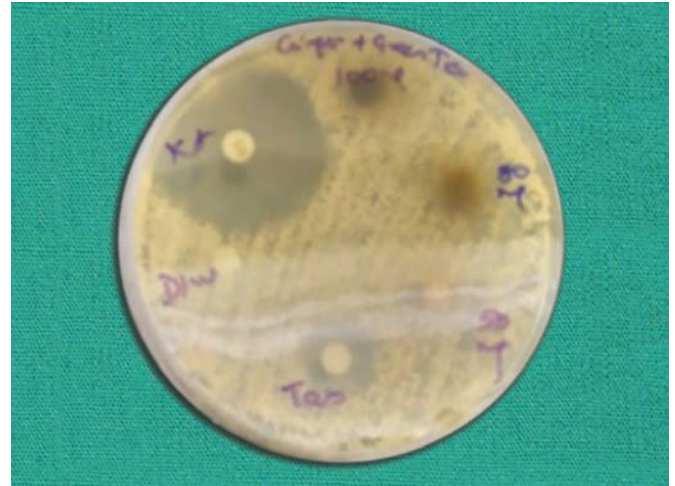


Fig. 5: Combination of green tea and ginger extract

Once *Candida* begins to co-exist, eradication is difficult, and it becomes harmful and highly virulent. In the present investigation, ginger and green tea extract have shown about 16 to 18 mm inhibition with ATCC strains and 4 to 6 mm in the clinical strains. In a study done by Aghazadeh et al.,⁶ only negligible amount of inhibition was noted on clinical isolates among *Candida albicans* which is similar to the results of the present study. These results are comparable with the results of Archana and Abraham,⁷ and Giriraju and Yunus.⁸ Also similar result was reported by Yassien.⁹

A study done by Kader et al.,¹⁰ a zone of inhibition of 9 mm was seen for clinical strains of *Candida albicans*. The difference observed could be attributed to variations in the environmental factors where the plant was grown, the concentration of active molecules present in them and also depends on the solvent system used to prepare extract.

CONCLUSION

Candida albicans (ATCC 90028) strains, 16 to 18 mm of a zone of inhibition was seen for ginger and green tea at a concentration of 100 mg/mL. For clinical strains, 4 to 6 mm of the zone of inhibition was seen. Indiscriminate use of such plant material by either incorporation, immersion or mouthwash is not going to reduce candidal activity and might even aggravate the condition. An attempt must be made to isolate and incorporate only the biologically active compounds to obtain anti-candidal activity.

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