Antifungal effect of green tea extracts on oral Candida species: An in vitro study

M. G. Madhura¹, R. D. Shweta¹, B. Veerendra Kumar¹, D. Savithri², S. Gajalakshmi¹, B. V. Soumya¹

¹Department of Oral and Maxillofacial Pathology, DAPM RV Dental College & Hospital, Bengaluru, Karnataka, India, ²Department of General Pathology and Microbiology, DAPM RV Dental College & Hospital, Bengaluru, Karnataka, India

Abstract

Background: Candida is one of the commensals housing the normal oral flora. Oral candidiasis is a common opportunistic infection caused by Candida species (Candida albicans being the most common one). Green tea is well-known for its various health benefits, and the literature reveals ongoing studies on the action mechanisms of green tea extract, in vitro and in vivo.

Aims and Objectives: The present study was undertaken to assess antifungal effect of green tea extracts; if proven, could it be used in future as an adjunctive treatment modality for treating candidiasis.

Materials and Methods: About 15 healthy volunteers with coated tongue were included for the study. Oral sample, from dorsum of the tongue was collected by commercially available pre-sterilized swabs. The material was inoculated into Sabouraud’s Dextrose Agar; confirmed for the presence of Candida species. The sensitivity test was carried out on a lawn culture with 25% and 50% green tea extracts at 24 h and 48 h intervals. The antifungal activity was assessed. Distilled water was used as a control. Student’s paired t-test was used to assess the difference in inhibition effect between 25% and 50% of green tea extract solution.

Results: Statistically significant increase in the zone of inhibition was noticed at both 25% and 50% concentration of green tea extracts when compared to control group, which did not show any zone of inhibition.

Conclusion: Having shown antifungal effects, the green tea extracts may be considered in future as a therapeutic adjunct.

Keywords
Antifungal activity, epigallocatechin-gallate, green tea extracts, polyphenols

Introduction

Candida species (Candida spp.) contribute to the indigenous microbial flora of oral cavity in humans.¹ One or more species of Candida (Candida tropicalis, Candida albicans, Candida krusei, Candida parapsilosis, Candida guilliermondii, Candida glabrata, and others) are responsible for oral candidiasis, which in turn is described as the "the disease of the diseased."²⁻⁴ Weakening of the host immune responses is responsible for the transition of harmless commensal fungi (Candida spp.) to pathogenic microorganisms.⁵⁻⁶

Oral candidiasis is often treated by antifungal agents, nystatin being more popular.² The commonly used antifungal drugs are expensive and are associated with undesirable side effects; the most emergent problem being drug resistance.⁶ Use of natural extracts as adjuncts for treating candidiasis is gaining popularity. One such natural product is green tea extract. Use of green tea may have a spectrum of health benefits in different ailments. The health benefits of green tea consumption on cardiovascular diseases, cancer, inflammatory disorders, and other ailments are under investigation.⁷⁻⁹ In addition, the anti-bacterial, antiviral, and antifungal effects of green tea are also being studied.¹⁰⁻¹² Many of the health promoting effects of green tea are credited to its polyphenol content and catechins.¹³,¹⁴ Therefore, the present study was aimed at investigating the antifungal effects of green tea extracts using the active ingredients: 90% polyphenol and 40% epigallocatechin-gallate (EGCG) on oral Candida species in vitro.

Objectives
1. To account for the inhibitory effect (if any) of green tea extract: 90% polyphenol and 40% EGCG toward Candida species obtained from oral samples.
2. To measure the zone of inhibition (if any) for 25% and 50% active ingredients of green tea and distilled water (control) after 24 h and after 48 h.

Materials and Methods

About 15 healthy volunteers between age group of 60-80 years with coated tongue were included for the study. Patients with any premalignant lesions and conditions, malignancies and patients on recent antimicrobial therapy were excluded from the study. Informed written consent from the volunteers was obtained. Ethical Clearance from Institutional Review Board was obtained. Oral sample, from dorsum of the tongue was collected from the participants of the study using commercially available pre-sterilized swabs.

After sample collection, the swab was taken in a sterile test tube, to the Department of Microbiology. Using an inoculating loop, the material from the swab was inoculated into Sabouraud’s Dextrose Agar. After inoculation, the culture plate was incubated at 37°C for 24 h. After 24 h, the culture media were checked for the presence of colonies [Figure 1]. One colony was selected, and a smear was prepared and was stained with Gram’s stain to confirm the presence of Candida species [Figure 2].

After establishing the presence of Candida species, the colony was taken and mixed with peptone water in a sterile test tube, and the suspension was compared with 0.5 McFerland standard units. The suspension was incubated at 37°C for 4-6 h to obtain the organism in log phase of growth. With the help of sterile swab the incubated suspension was spread over the culture plate for the lawn culture; to this, 3 wells were punched and 50 µl of 25% green tea extract, 50% green tea extract and distilled water (as control) were added [Figure 3] and incubated at 37°C for 48 h. The antifungal activity was assessed by measuring the diameter of the area (in mm) around the wells where the growth was inhibited.

The above-mentioned protocol was followed for all 15 samples. Strict aseptic precautions were maintained.

The data were tabulated in a master chart for further analysis and interpretation. (Note: 25% and 50% of the active ingredients [90% polyphenols and 40% EGCG] were prepared by mixing the powder in 100 ml of distilled water [W/V], as per manufacturer’s instructions).

Student paired $t$-test was used to assess the difference in inhibition effect between 25% and 50% of green tea extract solution at 2 different time points.

Results

The study comprised 3 groups; two groups with intervention at 25% and 50% green tea extracts and the third group was the control group (distilled water).

The means of the zones of inhibition of Candida after 24 h of incubation at 25% and 50% concentration were 13.87 ± 1.51 mm and 19.6 ± 1.50 mm, respectively. The means of the zones of inhibition of Candida after 48 h of incubation at 25% and 50% concentration were 16.67 ± 0.82 mm and 21.93 ±1.67 mm, respectively [Table 1]. Statistically significant increase in the zone of inhibition was noticed at both 25% and 50% concentration when compared to control group, which did not show any zone of inhibition.

Discussion

Candida species are found in the oral cavity in both health and disease states. The popular antifungal agents available are with the considerable amount of side effects.\cite{1,2} This has led to the emergence of various herbal alternatives.

The association between consumption of green tea and human health has long been appreciated. The chemical composition of green tea appears to be quite complex; proteins, amino acids, carbohydrates, lipids, vitamins, xanthic bases,
Antifungal effect of green tea on oral Candida species

Madhura, et al.

The health benefits of green tea are attributed to polyphenols, comprising flavanols, flavonoids, and others. Green tea extracts are found in liquid or powder forms, with varying concentrations of polyphenols. The majority of the polyphenols found in green tea are flavanols, which are also called as catechins. Four different types of catechins are found in green tea: Epicatechin, epigallocatechin, epicatechin-3-gallate, and EGCG.\(^{[15,16]}\)

Various experiments have demonstrated health benefits of green tea catechins against a variety of ailments.\(^{[3,6-12]}\)

Review of English literature has revealed few studies carried out to assess the antifungal effects of green tea active ingredients. There is only one reported study on antifungal effects of green tea extract done on oral samples.

Hence, the present study was aimed at checking the antifungal effects of green tea extracts on oral samples and further to propose to consider green tea extract as an adjunctive and or alternative therapeutic modality.

Oral samples from 15 participants of the study constituted the study material. Dorsum of the tongue was the oral site for collecting the sample with pre-sterilized swabs. The material from the swab was grown in Sabouraud’s Dextrose Agar. After confirming the Candida species using Gram’s stain, the effect of 25% and 50% of the active ingredients (90% polyphenols and 40% EGCG) was checked on lawn culture plates, after 24 h and after 48 h. Distilled water was used as a control.

Statistically significant increase in the zone of inhibition was noticed at both 25% and 50% concentration of active ingredients. The control group did not reveal any zone of inhibition.

In one reported study, the minimum inhibitory concentration of green tea leaf extract on C. albicans growth was at 12.5% and minimum bactericidal concentration was at 25%.\(^{[17]}\) In the present study, the inhibitory effect toward Candida species was present at both 25% and 50% concentration of the active ingredients, at 24 h and at 48 h.

In another study (Hirasawa, 2004), the susceptibility of C. albicans to catechins and the synergism of combination of catechins and antimycotics were evaluated, and the results showed that EGCG enhanced the effects of antimycotics. The authors have proposed that catechins could be used with antimycotics, as a combined treatment which may help to avoid the side effects of antimycotic drugs.\(^{[1]}\)

Thus, green tea extract may be used as an adjunct to treat candidal infections.

A study by Taley et al. revealed that all the studied species (Aspergillus niger, Mucor piriformis, Alternaria alternata [Fr.], C. albicans, and others) were found to be sensitive to green tea leaves extract.\(^{[18]}\)

The present study showed that the zone of inhibition was seen at 25% and at 50% concentration at both 24 h and at 48 h. Distilled water which was taken as a control did not show any inhibition for the growth of Candida. Maximum inhibition was at 50% concentration at 48 h incubation.

The present study was a preliminary attempt to check for antifungal effects of green tea extract. Therefore, the sample considered in this study was from healthy individuals in whom the Candida species would mainly present as commensal yeasts. It is necessary to find out whether green tea extract has similar effects even in disease states because of the anticipated confounding factors such as the acquisition of other virulence factors such as phenotypic switching, morphologic transition, and host factors.

**Conclusion**

Green tea extracts- imparting a spectrum of health benefits also possess antifungal effect which could be made use as an alternative medicine or as an adjunct to conventional therapy for the treatment of oral candidiasis.

---

Table 1: Student paired \(t\)-test

<table>
<thead>
<tr>
<th>Time period</th>
<th>Concentration</th>
<th>N</th>
<th>Mean±SD</th>
<th>SEM</th>
<th>Mean difference</th>
<th>(T)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>After 24 h</td>
<td>25</td>
<td>15</td>
<td>13.87±1.51</td>
<td>0.39</td>
<td>−5.733</td>
<td>−9.235</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>15</td>
<td>19.6±1.50</td>
<td>0.39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 28 h</td>
<td>25</td>
<td>15</td>
<td>16.67±0.82</td>
<td>0.21</td>
<td>−5.267</td>
<td>−11.6479</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>15</td>
<td>21.93±1.67</td>
<td>0.43</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N: Study group, SD: Standard deviation, SEM: Standard error of mean, *Statistically significant
Having shown antifungal effects, the green tea extracts may be considered in future as a therapeutic adjunct.

As the present study was conducted in vitro, the duration of contact of the active ingredients in the biological environment (in vivo) is not clear and further study is recommended to standardize the dosage and to study all the effects of green tea extract.

**Scope for Future Research**

- Quantitative and qualitative assessment of catechins are influenced by various preparation techniques, as catechins are relatively unstable. Therefore, more representative models with specific and sensitive methods, along with good predictive biomarkers may enhance our understanding of how green tea interacts with exogenous factors and with endogenous systems.
- The development of biomarkers for assessing green tea consumption and also molecular markers to know its biological effects will definitely add a significant quantum of knowledge to this field.
- Well-designed observational studies and intervention trials are awaited for drawing definitive conclusions.

**Acknowledgments**

The authors would like to acknowledge Dr. Vinitha M, Professor and Head, Department of Microbiology, Bangalore Institute of Dental Sciences, Bengaluru for giving her valuable inputs.

**References**
