Comparative Evaluation of Antifungal Activity of Green Coffee and Green Tea Extract against *Candida albicans*: An *In Vitro* Study

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**Abstract**

**Aim and objective:** To compare the antifungal efficacy of green tea and green coffee extracts against *Candida albicans*.

**Materials and methods:** Green tea and green coffee were obtained from the authorized dealers. The extracts were prepared and antifungal efficacy of the extract was carried out on *C. albicans* MTCC 227 using zone of inhibition and minimum fungicidal concentration (MFC) methods.

**Results:** The study comprises two groups, green tea extract and green coffee extract. The zone of inhibition was done for both the groups at 10, 25, 50, 100, 150, and 200 mg/mL. Green coffee showed a higher zone of inhibition at all the concentrations gradually from 50 C as compared to green tea. Green coffee showed a 50% reduction of *C. albicans* at 160 mg/mL and a 90% reduction at 200 mg/mL. On the other hand, green tea showed a 50% reduction at 200 mg/mL.

**Conclusion:** In the present study, green coffee was shown to have lower inhibitory concentration and higher zone of inhibition when compared to green tea suggesting that the antifungal efficacy of green coffee was better than green tea.

**Clinical significance:** Recently, many studies have shown the prominent role of *Candida* species especially *C. albicans* in the pathology of periodontal disease. Till now, there have been no studies that have included antifungal agents as adjuncts to periodontal therapy targeting *C. albicans*. Moreover, commercially available antifungal drugs come with many side effects. Green coffee and green tea being natural agents with antimicrobial, antioxidant, antifungal, antiviral, anti-inflammatory, and anticancer potential can definitely prove to be a viable option to the general population. Hence, opening the venue to formulate drugs in the form of mouthwashes, gels, and local drug delivery agents which can be used as adjuncts to periodontal therapy.

**Keywords:** Antifungal agents, *Candida albicans*, Chronic periodontitis, Coffee, Tea.

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**Introduction**

The oral disease that frequently affects the tooth and its supporting structures is periodontal disease. The periodontal disease can be limited to gingiva, i.e., gingivitis or it can affect the other supporting structures like periodontal ligament and bone resulting in periodontitis.

It is a well-established fact that *Porphyromonas gingivalis* is the keystone periodontal pathogen.¹ Other microorganisms which have shown to play a role include *Tannerella forsythia*, *Fusobacterium nucleatum*, and *Actinobacillus actinomycetemcomitans*.² Recently, systematic review and few studies have confirmed the role of fungus in particular *Candida albicans* in the etiology of periodontal disease; hence, paving the way to include antifungal agents as an adjunct to scaling and root planing.³

Numerous commercially available mouthwashes with antifungal effects are available but their cost and side effects remain its limitation.⁴ Commercially consumed natural substances worldwide are green tea and green coffee. These natural products have a lot of health benefits which include antibacterial, antifungal, and antiviral properties. The antifungal activity of green tea is credited to its polyphenol content and catechins.⁵ And for green coffee, it is caused by the chemical compounds in Arabica beans which include classes of compounds such as caffeine, phenol, alkaloids, flavonoids, and saponins.⁶

Many studies have evaluated and compared the antibacterial efficacy of green tea and green coffee.⁷⁻⁸

**Materials and Methods**

**Preparation of Green Tea and Green Coffee Extract**

Green coffee (*Coffea arabica*) (Fig. 1A) and green tea (*Camellia sinensis*) (Fig. 1B) were purchased from Nuherbs Organics and then 25 g of green tea and green coffee samples were taken separately,
cleaned, and grounded in a mortar and pestle. The extraction was carried out by a Soxhlet extraction method. The fine powder of green coffee (Fig. 2A) and green tea (Fig. 2B) was packed tightly in the Soxhlet extractor separately. A 250 mL of methanol was used as a solvent for extraction. This process was carried out for 6 hours. The extract was then filtered and re-extracted using a rotator evaporator, evaporated to dryness under reduced pressure at 60°C to get the solid product. The product was then stored in 1 mL centrifuge tubes for further analysis.

**Microorganism Preparation**

*Candida albicans* (MTCC 227) was obtained from the Dextrose Laboratories, Bengaluru, India and was used for the study. The culture was grown in Sabouraud dextrose broth before the experiment.

**Zone of Inhibition**

The antifungal activity was evaluated by well diffusion technique and the experiment was performed in triplicates. The plates were allowed to dry after autoclaving and 6 mm wells were punctured on the surface of the agar plate. The agar plates were seeded with 100 μL of the inoculums and spread evenly over the plate with a sterile glass spreader. Each sample (10, 25, 50, 100, 150, and 200 mg) was added to separate wells in the culture plates and incubated at 30°C for 24 hours. After 24 hours of incubation, diameter of the zone of inhibition was measured to the nearest millimeter using a Vernier caliper (Fig. 3)

**Minimum Fungicidal Concentration**

Susceptibility was tested by a spectrophotometric method using the dye MTT [3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyl tetrazolium bromide] in triplicates. Microtitration plates were prepared and incubated for 24 hours at 35°C, with concentrations of 100–200 mg/mL. Then, 25 μL of MTT was added at the above-mentioned concentrations to each well. Incubation was done for 30 minutes at room temperature and then gentle agitation. The overdose was measured spectrophotometrically with a microplate reader at 540 nm.
Antifungal Efficacy of Green Tea and Green Coffee

Statistical Analysis
An independent sample t-test was used to compare the zones of inhibition and MFC between the two groups. Friedman test was used to do the intragroup comparison at different concentrations.

Results
The study comprises two groups: green tea extract and green coffee extract.

Intergroup Comparison
Zone of Inhibition
The zone of inhibition was done for both the groups at 10, 25, 50, 100, 150, and 200 mg/mL. Both the groups started showing antifungal efficacy at 50 mg/mL. The concentrations from 50 to 200 mg/mL were taken for statistical analysis.

As shown in Table 1, the zone of inhibition was higher with green coffee at all the concentrations gradually from 50 C (11.10 ± 0.14) to 200 C (18.75 ± 0.07) as compared to green tea (Fig. 3). When the independent sample t-test was applied to compare the zone of inhibition between the groups at different concentrations, it was highly significant at 50 C (p = 0.002) and 100 C (p = 0.005) it was very highly significant and at 200 C (p = 0.000).

Minimum Fungicidal Concentration
To determine the lowest concentration of green tea and green coffee that can inhibit the growth of fungus, a minimum fungicidal concentration (MFC) test was performed. An independent sample t-test was applied to compare the MFC between the groups. Minimum fungicidal concentration was checked for both the groups taking a MFC of 100 mg/mL. Microtitration plates were prepared and incubated for 24 hours at 35°C with a concentration between 100 mg/mL and 200 mg/mL. Green coffee showed inhibitory concentration, i.e., IC50 at 160 mg/mL and IC90 value at 200 mg/mL, whereas green tea showed IC50 value at 200 mg/mL which was statistically significant (p = 0.045) (Table 2).

Table 1: Comparison of the zone of inhibition between the groups using independent sample t-test

| mg/mL | Groups            | Minimum | Maximum | Mean   | Std. deviation | Mean diff | p value |
|-------|-------------------|---------|---------|--------|----------------|-----------|---------|
| 50 C  | Green coffee      | 11.0    | 11.2    | 11.10  | 0.14           | 2.1       | 0.002*  |
|       | Green tea         | 9.0     | 9.0     | 9.00   | 0.00           |           |         |
| 100 C | Green coffee      | 11.5    | 11.8    | 11.65  | 0.21           | 2.15      | 0.005*  |
|       | Green tea         | 9.5     | 9.5     | 9.50   | 0.00           |           |         |
| 150 C | Green coffee      | 14.9    | 15.4    | 15.15  | 0.35           | 3.5       | 0.007** |
|       | Green tea         | 11.5    | 11.8    | 11.65  | 0.21           |           |         |
| 200 C | Green coffee      | 18.7    | 18.8    | 18.75  | 0.07           | 6.45      | 0.00**  |
|       | Green tea         | 12.3    | 12.3    | 12.30  | 0.00           |           |         |

*p < 0.01 = highly significant
**p < 0.001 = very highly significant

Table 2: Comparison of the MFC levels between the groups using independent sample t-test

| Groups    | Minimum | Maximum | Mean  | Std. deviation | Mean diff | p value |
|-----------|---------|---------|-------|----------------|-----------|---------|
| Green coffee | 0.188   | 0.872   | 0.502 | 0.278          | −0.184    | 0.045*  |
| Green tea  | 0.567   | 0.797   | 0.686 | 0.070          |           |         |

*p < 0.05 = significant

Figs 3A and B: (A) Zone of inhibition with green coffee; (B) Zone of inhibition with green tea
Intragroup Comparison
Intragroup comparison of a zone of inhibition for both green coffee and green tea at varying concentrations did not show a statistically significant difference ($p = 0.18$) (Table 3).

Discussion
This in vitro study was aimed to compare the antifungal efficacy of green tea and green coffee on C. albicans at different concentrations. Presence of a susceptible host along with bacterial plaque is a necessity for the development of periodontal disease. Some individuals consider periodontopathogenic bacteria as “required but not sufficient” to cause periodontal disease. However, no disease process results from a single isolated cause or event. 5

Periodontopathogenic bacteria involved in periodontal disease are P. gingivalis, T. forsythia, and A. actinomycetemcomitans. 2

The role of opportunistic fungus C. albicans among other species of its genus in periodontal disease have been confirmed by various studies. 3 Hence, in the present study, C. albicans culture was used.

Recently, many natural products have been shown to have antibacterial, antiviral, and antifungal effects. 10 Two such natural products with these health benefits are green tea and green coffee. Many studies have been done to check and compare the antibacterial efficacy of these against periodontal pathogens. 10 Few studies have also checked their antifungal efficacy individually.

To the best of our knowledge, there are no studies done to date which has compared the antifungal efficacy of green tea and green coffee. Hence, the present study was undertaken to compare their antifungal efficacy against C. albicans.

Coffee arabica and Coffee Robusta (Coffea canephora) are the two types of coffee beans available. In the present study, C. arabica was used. These beans when compared to robusta are easy to find and are cost-effective making them the most popular coffee bean in the world. Studies have shown that coffee beans have therapeutic effects such as anti-inflammatory, antifungal, and antibacterial, they are widely used in the community, their side effects are relatively lower than synthetic drugs and they can be easily obtained. 10 Chlorogenic acid (CGA) is a polyphenolic natural compound. Structurally, it is an ester of caffeic acid with the 3-hydroxyl group of quinic acid. It has been reported to possess many health benefits including antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, chemopreventive, and other biological activities. 11

The antifungal activity in green tea has been attributed to EGCG (Epigallocatechin gallate) and ECG which is the most abundant catechin in tea 12 and the antifungal activity of green coffee is due to the chemical compounds such as caffeine, phenol, alkaloids, flavonoids, and saponins. 10

Table 3: Comparison of the zone of inhibition within the group among different concentrations using Friedman test

| Groups                      | 25°C | 50°C | 100°C | 150°C | 200°C |
|-----------------------------|------|------|-------|-------|-------|
| Green Coffee               |      |      |       |       |       |
| Min.                        | 8.00 | 8.00 | 8.00  | 8.00  | 8.00  |
| Z                           |      |      |       |       |       |
| p value                     |      |      |       |       |       |
| Max.                        | 0.092| 0.092| 0.092 | 0.092 | 0.092 |
| Green Tea                  |      |      |       |       |       |
| Min.                        | 8.00 | 8.00 | 8.00  | 8.00  | 8.00  |
| Z                           |      |      |       |       |       |
| p value                     |      |      |       |       |       |
| Max.                        | 0.092| 0.092| 0.092 | 0.092 | 0.092 |

While comparing both the groups for their antifungal effect, green coffee showed a higher zone of inhibition at 200 mg/dL when compared to green tea. The higher the zone of inhibition, the higher
is the antifungal activity. So, therefore, our study showed that green coffee had better antifungal activity than green tea.

As both the groups started showing their antifungal activity at 50 mg/mL and the highest antifungal activity was seen at the range of 100–200 mg/mL. The MFC was evaluated between 100 mg/mL and 200 mg/mL. Green coffee showed a 50% (IC50) reduction of C. albicans at 160 mg/mL and 90% (IC90) reduction at 200 mg/mL. On the other hand, green tea showed a 50% reduction at 200 mg/mL. Lower the inhibitory concentration higher is the antifungal activity thus, in the present study green coffee showed lower inhibitory concentration when compared to green tea suggesting the antifungal efficacy of green coffee was better than green tea.

An in vitro study done by Arora and Ohlan evaluated the antifungal activity of tea and coffee against wood-rotting fungi which concluded that both tea and coffee do exhibit antifungal properties.23 After an immense literature search and to the best of our knowledge, this is the first study that compares in vitro the antifungal efficacy of green tea and green coffee against C. albicans. Hence, there are no studies available to discuss our results from the present study.

Alkaloids and polyphenols are the main constituents of tea and coffee. Out of the alkaloids, caffeine is the main constituent and the in vitro study done by Arora and Ohlan showed that at 0.3% concentration of caffeine, >50% showed total growth inhibition. Coffee has more concentration of caffeine than tea24 which could be one of the main reasons why in the present study green coffee showed better antifungal efficacy than green tea.

Intriguing comparison has shown no statistical difference in antifungal activity at different concentrations of green tea and green coffee; however, the intragroup comparison has shown the presence of antifungal activity at different concentrations which were statistically significant.

As periodontitis is a major oral health concern, the antifungal efficacy of green tea and green coffee paves the way to evaluate them as a therapeutic antifungal agent whose efficacy as an adjunct to non-surgical therapy can be checked in vivo on the patients at risk for periodontitis and the antifungal potential of these natural products can prove to be beneficial in conditions like denture stomatitis, oral thrush, and other oral forms of candidiasis.21

**Clinical Significance**

Recently, many studies have shown the prominent role of Candida species especially C. albicans in the pathology of periodontal disease.3 Till now, there have been no studies that have included antifungal agents as adjuncts to periodontal therapy targeting C. albicans. Moreover, commercially available antifungal drugs come with many side effects. Green coffee and green tea being natural agents with antimicrobial, antioxidant, antifungal, antiviral, anti-inflammatory, and anticancer potential25 can definitely prove to be a viable option to general population. Hence, opening the venue to formulate drugs in the form of mouthwashes, gels, and local drug delivery agents which can be used as adjuncts to periodontal therapy.

**Limitations**

This study was initially planned to be an ex vivo study using plaque samples from periodontal pockets, but due to the present pandemic situation, it was changed to an in vitro study. The in vitro MFC though is not a true indicator of the activity of the drug at the locus of infection, it can serve as surrogate markers attempting to quantify the drug activity.7

**Conclusion**

The present in vitro study shows that green coffee has better antifungal activity than green tea against C. albicans. Further studies have to be undertaken to check their antifungal efficacy in vivo in plaque samples isolated from patients diagnosed with periodontitis. And determining their concentrations in gingival crevicular fluid and saliva might help us to know the ideal antifungal dose which can be used as an adjunct to treat periodontitis.

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**Author’s Contribution**

All authors were involved in data collection, drafting the manuscript, revising the manuscript, and had approved for the submission of the manuscript.

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