

How to Cite:

Chellappa, L. R., & Doraikannan, S. (2022). Assessment and comparison of the antimicrobial activity and antioxidant potential of commercially available probiotic infused green tea, slim tea and green coffee. *International Journal of Health Sciences*, 6(S1), 2487–2498.
<https://doi.org/10.53730/ijhs.v6nS1.5315>

Assessment and Comparison of the Antimicrobial Activity and Antioxidant Potential of Commercially Available Probiotic Infused Green Tea, Slim Tea and Green Coffee

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Abstract---Aim: The aim of the study is to assess and compare the antimicrobial and antioxidant activity of commercially available probiotic infused green tea, green coffee and slim tea. Materials and methods: Synthesis of probiotic medium is done by 2 gm of each sample was taken in 1 flask of 15 ml peptone water and mixed well. Then it is inoculated in MRS agar plate and it is sealed in an anaerobic jar for 48 hours. Antimicrobial activity is tested by agar well diffusion method against *S.mutans* and the zone of inhibition is measured. Antioxidant activity is measured with DPPH and % of inhibition is measured. Results: In the antimicrobial activity, in all the samples the activity increased with increasing concentration, but the maximum was seen in slim tea. In antioxidant activity, all the samples showed antioxidant activity although green tea showed more antioxidant activity than green coffee and slim tea. Conclusion: In our study, *Bacillus coagulans* incorporated green tea, green coffee and slim tea exhibited both antimicrobial and antioxidant properties. Since there was a good antimicrobial activity against *S. mutans*, these can be incorporated in mouthwashes as a potential antibacterial agent for oral infections and as an antioxidant carrier in the local drug delivery.

Keywords---probiotic, probiotic tea, green tea, antimicrobial activity, DPPH, *S.mutans*.

Introduction

Probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host”(1). Lactobacillus, Bifidobacterium, Enterococcus, Streptococcus, Bacillus and fungal strains belonging to Saccharomyces species are the most commonly reported microorganisms as probiotics for animal and human consumption (2) The intervention of certain probiotic strains have shown promise in improving clinical conditions of acute and antibiotic-associated diarrhoea, irritable bowel syndrome, inflammatory bowel disease, and also atopic dermatitis(3). However, most of the non-spore forming probiotics (Lactobacillus and Bifidobacterium) cannot be stored at ambient temperature for long shelf life and lose their viability during storage(4). Several methods like microencapsulation and refrigeration during storage and retail distribution are followed to retain the probiotic viability of these probiotic products So, it can be a major burden for manufacturers to provide cost effective probiotic ingredients (5)

The consumption of tea and coffee is very common in the world but its consumption can vary from person to person in accordance with type , frequency, temperature and strength. Tea and coffee are widely consumed mostly in four forms– green tea, black tea, regular coffee and black coffee. Green tea is a ‘non-fermented’ tea which contains more catechins compared to black tea or oolong tea(6). Literature has reported that green tea has many medicinal properties such as antioxidant, anti angiogenesis and anti proliferative properties that are potentially relevant to prevention and treatment of various forms of cancer (7). In the recent times, probiotic foods have gained a lot of attention by health practioners and consumers which have led to significant increase in production of probiotic food production and incorporation into dairy products, beverages and infant formulas (8). The delivery of probiotic food as a carrier is an important strategy to health benefit. These foods or the food processing must not affect the probiotic potential of the food and also it should alter the taste of the food for worse (9)

So the aim of the present study is to assess and compare the probiotic activity and anti-oxidant potential of commercially available probiotic infused tea, green tea and green coffee in India.

Materials and Methods

Samples used :

Sample 1 : Probiotic Green tea - Green tea extract , stevia, Bacillus coagulans SNZ 1969 (temperature acid resistant)

Sample 2 : Probiotic Green coffee – Green coffee extract, natural coffee flavor, Bacillus coagulans SNZ 1969 (temperature acid resistant)

Sample 3 : Probiotic Slim tea - Green tea extract , Garcinia, extract , stevia, Bacillus coagulans SNZ 1969 (temperature acid resistant)

Synthesis of probiotic medium: 2 gm of each sample was taken in 2 tubes of 15 ml peptone water and mixed well. Then it is kept in a shaker at 250rpm for 24 hours. Then it is inoculated in MRS agar plate and it is sealed in an anaerobic jar for 48 hours.

The culture is sub-cultured twice and centrifuged and the supernatant was stored for further study

Antimicrobial activity

Antibacterial activity of the supernatant strains against oral pathogen was determined using the agar-well diffusion method with some modifications of the protocol indicated by Chellappa et al. (10) (*Streptococcus mutans*). The selected LAB isolates were inoculated from slants to fresh MRS broth containing 1% glucose and incubated overnight at 37°C overnight active culture broth of each isolate was centrifuged separately at 5000 rpm for 10 min at 4° cell-free supernatant from each separate culture was collected as a crude extract for the antagonistic study against selected oral pathogen. Pure cultures of oral pathogen were inoculated from slants to brain heart infusion broth. After 24-hour incubation at 37°C, a volume of 100 µl of inoculum of each indicator bacteria was swabbed evenly over the surface of nutrient agar plates with a sterile cotton swab. plates were allowed to dry, and a sterile cork borer (diameter 5 mm) was used to cut uniform wells in the agar. Each well was filled with 100 µl culture-free filtrate obtained from each of the acid-bile-tolerant LAB isolates. After incubation at 37°C for 24 to 48 hours, the plates were observed for a zone of inhibition (ZOI) around the well.)The diameter of the inhibition zone was measured by calipers in millimeters, and a clear zone of 1 mm or more was considered positive inhibition. Experiment was carried out in triplicates, and the activity was reported as the diameter of ZOI ± SD.

Antioxidant activity

The 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) free radical scavenging activity of the chocolate samples was determined to assess its antioxidant potential. Various concentrations (10-50 µg/ml) of inoculated culture were mixed with 1 ml of 0.1 mM DPPH in methanol solution and 450 µl of 50 mM Tris-HCl buffer (pH 7.4), and incubated for 30 min. After incubation, the reduction in the number of DPPH free radicals was measured based on the absorbance at 517 nm. Ascorbic acid was used as the standard controls and the percent (%) inhibition was calculated from the following equation:

$$\% \text{ Inhibition} = \frac{[\text{Absorbance of control} - \text{Absorbance of test sample}]}{\text{Absorbance of control}} \times 100$$

Absorbance of control

Results

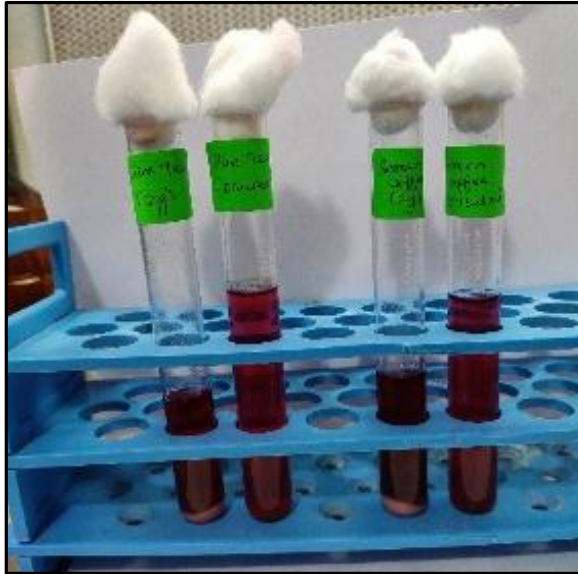


Figure 1 : Preparation of green tea, green coffee and slim tea extract with peptone water



Figure 2 : Anaerobic jar kept after incubating the samples with MRS agar

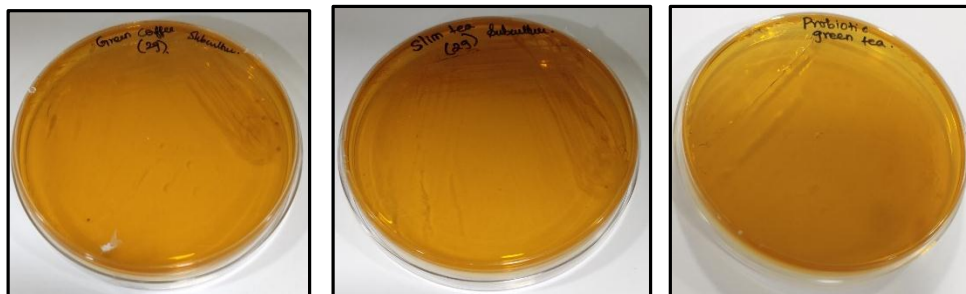


Figure 3 : Subcultures of the 3 samples



Figure 4 : Collection of supernatants after centrifugation



Figure 5 : Antimicrobial activity of the samples against S.mutans

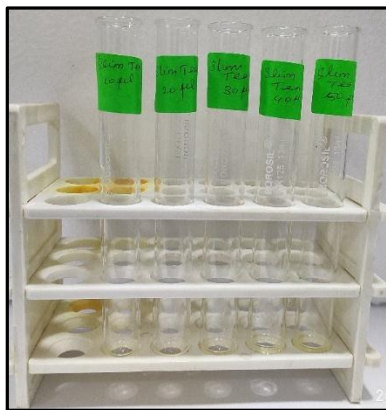


Figure 6 : Antioxidant activity of slim tea

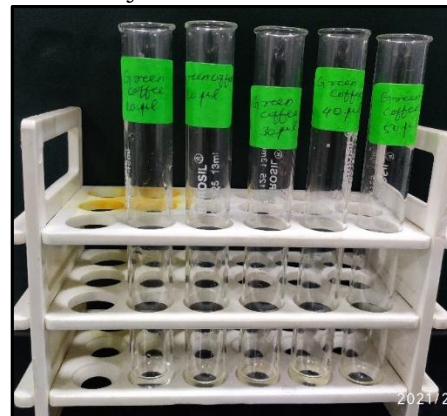
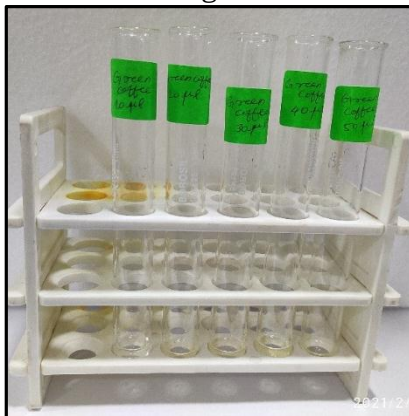
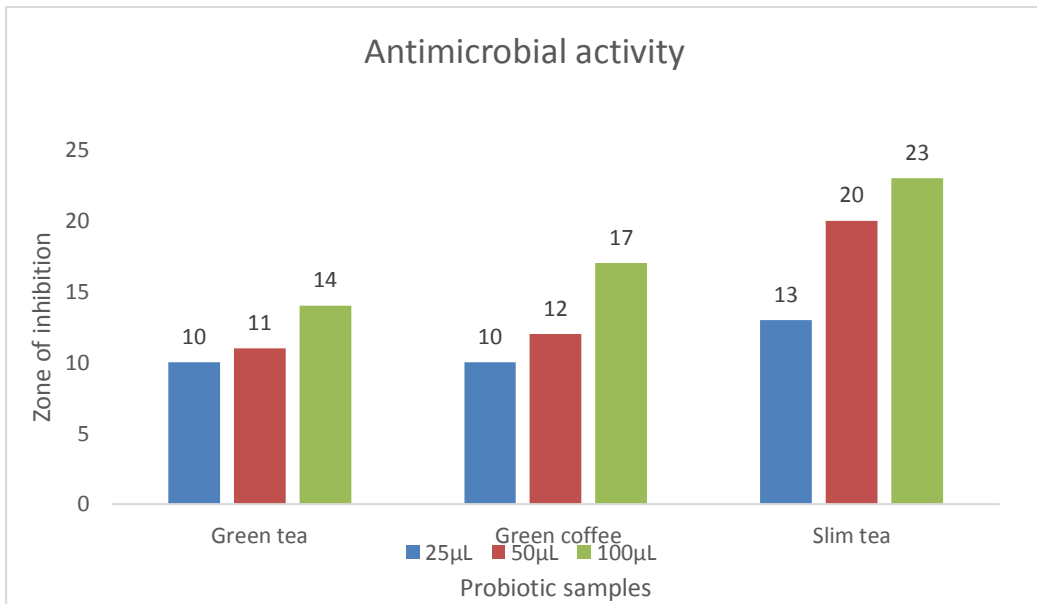


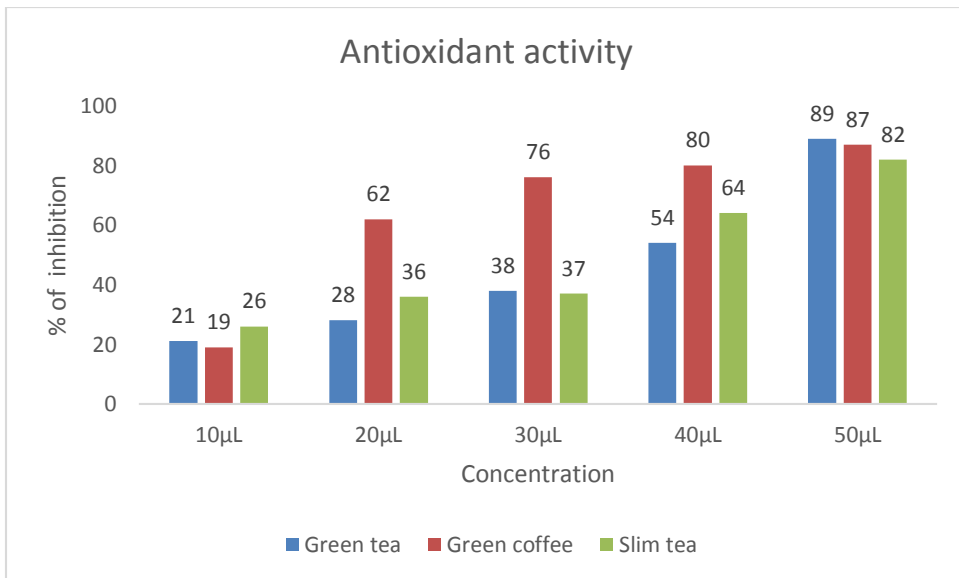
Figure 7 : Antioxidant activity of green coffee



Figure 8 : Antioxidant activity of green tea



Graph 1 : Antimicrobial activity of the samples against S.mutans



Graph 2: Antioxidant activity

The growth of the probiotic stains were confirmed by subcultures (figure 3). In the antimicrobial activity, green tea was found to have 10 mm, 11 mm and 14 mm zone of inhibition at 25 μ L, 50 μ L and 100 μ L respectively. Green coffee showed 10 mm, 12 mm and 17 mm zone of inhibition at 25 μ L, 50 μ L and 100 μ L respectively; and slim tea showed 13 mm, 20 mm and 23 mm zone of inhibition at 25 μ L, 50 μ L and 100 μ L respectively. Although all the samples the activity increased with increasing concentration, but the maximum was seen in slim tea (figure 5 and graph 1). In antioxidant activity, all the samples showed antioxidant activity with increasing concentration. although green tea showed more antioxidant activity than green coffee and slim tea at high concentrations (figure 6-8 and graph 2).

Discussion

Foods are now not only considered for taste but also for its nutritional values and ability to improve the health. So, the interest in food ingredients which contains valuable bioactive properties and lactic acid bacteria and bifidobacterial which possess antagonistic activity against pathogens (11). There are different mechanisms for control and inhibition of other microbes including nutrient competition, production of inhibitory compounds and immune stimulation. Amongst these, the bacteria which produces organic acids (lactic acid) to lower the pH are most important. Additionally, certain strains are also capable of producing bioactive molecules, such as ethanol, formic acid, fatty acids, hydrogen peroxide and bacteriocins, that have antimicrobial activity (12)

Green tea is made with unfermented leaves from the plant family *Camellia sinensis* L. These are rich in flavan-3-ols (epicatechin, catechin and galloylated derivatives), which has major health benefits. Other major polyphenols present in green tea are the flavonols, mainly derivatives of quercetin and kaempferol (principally glycosylated (13) Green tea also contains other compounds like tea pigments, amino acids, vitamins, carbohydrates, minerals and purine alkaloids (14). The beneficial effects of green tea have been attributed to all these compounds, but principally to phenolic compounds because of their high antioxidant properties (15) Literature says that green tea was found to have antimicrobial property against pathogens without damaging the probiotic bacteria. This selective activity towards microorganisms can promote a balanced intestinal microbiota, similar to the effects of yogurt intake (16).

In our study, all the probiotic samples exhibited antimicrobial activity against *S. mutans*. Similarly probiotic stains with different combinations have shown significant antimicrobial activity against several pathogens (13,17–19). In a study conducted by C. H. Jeong et al., the ABTS scavenging activity of the control yogurt, although lower than that of the GTP yogurt, was nevertheless around 50% (20). This can be attributed to the antioxidant properties of metabolites (e.g., polypeptides, peptides, and amino acids) produced during fermentation by LAB (21) Also this effect has been found in a previous study in which the scavenging activity of yogurt was increased due to production of peptides by probiotic bacteria. Therefore, the antioxidant activity of green tea yogurts is considered to derive from both the phenolic compounds present in green tea and the metabolites generated by LAB (22). In our study, all the probiotic samples

exhibited antioxidant activity with green tea being the highest. Similar findings were found in many studies (23–26)

Conclusion

In our study, *Bacillus coagulans* incorporated green tea, green coffee and slim tea exhibited both antimicrobial and antioxidant properties. Since there was a good antimicrobial activity against *S. mutans*, these can be incorporated in mouthwashes and as an antioxidant carrier in oral conditions like oral submucous fibrosis in the local drug delivery. Further studies should be done with different stains and in vivo methods.

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