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Comparative assessment of antibacterial efficacy of aqueous extract of commercially available black, green, and lemon tea: an *in vitro* study

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ABSTRACT

Objectives: The objective of this study is to determine and compare antibacterial efficacy of aqueous extracts of black, green, and lemon tea of a commercially available brand.

Methods: The well-diffusion method was used to evaluate the antibacterial efficacy of commercially available black tea, green tea, and lemon tea at three different concentrations (1.5 g, 5 g, and 7.5 g) against *Streptococcus mutans* and *Lactobacillus acidophilus*. After incubation in appropriate culture medium, diameter of zone of inhibition was measured to assess the antibacterial efficacy of tea.

Results: Maximum zone of inhibition was found with lemon tea (27 mm) followed by green tea (26 mm) and black tea (13 mm) against *S. mutans* and *L. acidophilus*. Zone of inhibition was highest at 7.5 g concentration (1 and half tea spoon) for lemon tea followed by green tea and black tea. Results were statistically analyzed with the analysis of variance (ANOVA). For pairwise intergroup multiple comparisons, bonferroni test was applied. The difference between black tea, green tea, and lemon tea were statistically significant ($P < 0.001$) at 5% of level of significance.

Conclusion: Lemon tea at 7.5 g concentration was more effective followed by green tea and black tea against *S. mutans* and *L. acidophilus*.

Keywords: *Lactobacillus acidophilus, Streptococcus mutans*, tea

Introduction

Maintenance of optimal oral health is critical to enjoy the quality of life. Oral health-related quality of life is an integral part of general health and well-being and is recognized by the WHO as an important segment of the Global Oral Health Program (WHO, 2003).[1] Habit is an action performed repeatedly on a regular basis. An upsurge of industrialization, urbanization, economic development, and market globalization occurring in most countries around the world is associated with changes in the diet and lifestyle of individuals. Lifestyle is defined in terms of diet-pattern, social class, total income, education level, habits, culture, and environment. Tea consumption is also one of the most common habits.

Tea is one of the most commonly consumed beverages across the world[2-5] after water,[6,7] originated in China, that has conquered the world's taste over the last 2000 years.[3] It is the second most commonly consumed beverage in the world.[3] Its first use is believed to be about 5,000 years ago and has remained popular as the most pleasurable and efficacious beverage in the world. Tea drinking has been gaining further acceptance now due to its natural health benefiting properties. It is an infusion made by steeping processed leaves, buds, or twigs of tea bush, *Camellia sinensis*, in hot water for several minutes, after which it is drunk.[2] It can be prepared as a drink, which can have many systemic health effects or an "extract" can be made from the leaves to use as medicine.[8] They grow as small bushy plants about 3-4 feet high.[8] Tea leaves are picked three to four times between spring and fall of each year.[8] Bangladesh is the highest tea (black tea) producer in the world followed by India (black tea), Japan (green tea), and China (different sorts of tea).[9] The per capita mean consumption of tea in the world has been reported to be 120 ml/day.[3] In India, the per capita consumption of tea annually is 706g.[3] Antibacterial property of tea was first reported from Japan using Japanese tea against various diarrheal pathogens.[6] For the first time in 1989, it was demonstrated that Japanese green tea has an inhibition effect on *Streptococcus mutans*.[10] In 1958, a British botanist J. R. Sealy classified all plants in the genus *Camellia* and tea was given the name it has today.[11] The heritage drink has withstood the test of time, and it

may well be the drink of the future. The journey of the beverage through the passage of time has been glorious and fascinating.

As tea contains various trace elements, the attention to the biological functioning of tea beverages has rose during the last few years.[12] Three commonly used and commercially available tea varieties were selected for this study. Black tea is consumed all over the world and, in India, it is the most favored one.[3] Black tea and green tea have been well researched on its antibacterial efficacy against oral micro biota. On lemon tea, relatively less research has been done, as it is less consumed. This study was therefore undertaken to assess whether aqueous extracts of three types of tea (black, green, and lemon tea) have any inhibitory effect on the *S. mutans* and *Lactobacillus acidophilus* (cariogenic bacteria).

Materials and Methods

The present study is an *in-vitro* study conducted at the Department of Microbiology, Annasaheb Chudaman Patil Medical College, Dhule, Maharashtra. Ethical clearance for the study was obtained from the Institutional Ethical Review Committee. Three different commercially available tea of single brand (one of the popular and commonly available - Tetley) were randomly selected taking into consideration their availability in the market. Recently, manufactured and packed commercially available three different tea was purchased from the local retail outlet. The study was conducted over a period of 10 days. The culture media used in the present study was blood agar for agar diffusion method while nutrient broth for bacterial isolates preservation. Freeze-dried strains of *S. mutans* (MTCC, 497) and *L. acidophilus* (MTCC, 10307) were obtained from the Institute of Microbial Technology, Chandigarh. Bactericidal activity was determined by agar well-diffusion method given by Norrel and Messley (1997).[2]

The above mentioned 2 different strains of organisms were first transferred to BHI broth and incubated for 24 h to check the viability. Then, these suspensions were smeared on blood agar plate. After 24 h of incubation at 37°C, 9-10 colonies were transferred to 10 ml brain heart infusion (BHI) broth for using it on the next day. These solutions were aseptically introduced and evenly spread on the blood agar plate (Lawn/ Carpet culture).[13] After removing excess solution, the inoculum was allowed to dry for a few minutes at room temperature with the lid closed. With the aid of a sterile 5mm metal borer, 4 equally spaced wells were bored in the agar plate aseptically.

Extract preparation

In the present study, different concentrations of tea were selected in grams. Commercially available tea bag contains 1.5 g, so this concentration was selected. According to the strength and choice of concentration of tea varying from one individual to another. Based on one tea spoon and one and half tea spoon, the second and third concentrations were set to 5 g and 7.5 g, respectively. Thus, above-mentioned concentrations were selected for the present study.

Medium

To overcome the problem of contamination due to regular water, distilled water was used to prepare aqueous extracts of tea. To avoid toxic effects of alcoholic medium on general as well as dental health,[14] aqueous medium was selected for this study. This mixture was filtered using whatman filter paper to obtain an extract.

As previous literature suggests, temperature to prepare tea taken was 73°C-83°C for 5.3-6.3 minutes.[15] All extracts were prepared by keeping boiling temperature and time, constant under aseptic conditions.

These extracts were allowed to cool at room temperature before antibacterial testing to avoid melting and mixing of agar with extracts. In one agar plate, 3 wells were prepared for 3 different concentrations of each tea. The above-mentioned concentrations of aqueous extracts of three different tea of 50 µl solution was poured in the respective prepared wells with the help of sterile and disposable dropper. Using this method, the experiment was done in triplicate for each type of tea extract and a total of 18 plates were prepared. After 24 h of incubation in incubator at 37°C, agar plates were observed for the zone of inhibition (areas without growth of test organisms). It was measured as the maximum width from the edge of the well to the periphery of the inhibition zone with the help of Vernier caliper.[3] Maximum zone of inhibition would determine the inhibition of bacterial growth in an agar plate, so maximum zone was measured. The principle investigator was calibrated in the Department of Microbiology and measured the maximum zone of inhibitions on all the agar plates.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS; version 16.0) was used for data analysis. Data were presented as mean ± standard deviation of mean zone of inhibiton. After testing, homogeneity of variances of data by Levene test. Data obtained was organized in an Excel sheet and subjected to statistical analysis. Analysis of variance (ANOVA) was used to find the significance of study parameters between three or more groups of samples and Bonferroni *post-hoc* test was used to compare mean zone of inhibition in between pairs of tea types for different concentration. The data exhibited a normal and homogeneous distribution; thus, zone of inhibition (in mm) was analyzed using mean of all the readings obtained and the level of significance at <0.05 was considered statistically significant.

Results

Commercially available black tea, green tea, and lemon tea were effective against test organisms (Tables 1 and 2).

Table 1: Mean and range of *S. mutans* zone of inhibition (mm) in each group for different concentrations

ANOVA test				
S. mutans	**Group**	**Mean±SD**	**F**	***P* value**
1.5 g concentration	1	0.0	759.250	<0.001
	2	17.7±0.6		
	3	19.0±1.0		
5 g concentration	1	4.3±3.8	53.956	<0.001
	2	19.7±0.6		
	3	21.7±0.6		
7.5 g concentration	1	11.3±1.5	173.727	<0.001
	2	25.0±1.0		
	3	26.7±0.6		

Group 1: Black tea, Group 2: Green tea, Group 3: Lemon tea. *S. mutans: Streptococcus mutans*, SD: Standard deviation

Table 2: Mean and range of *L. acidophilus* zone of inhibition (mm) in each group for different concentrations

ANOVA test				
L. acidophilus	**Group**	**Mean±SD**	**F**	***P* value**
1.5 g concentration	1	0.0	564.250	<0.001
	2	15.7±0.6		
	3	16.0±1.0		
5 g concentration	1	6.7±0.6	481.333	<0.001
	2	17.3±0.6		
	3	20.7±0.6		
7.5 g concentration	1	11.7±0.6	522.333	<0.001
	2	24.3±0.6		
	3	25.3±0.6		

Group 1: Black tea, Group 2: Green tea, Group 3: Lemon tea. *L. acidophilus: Lactobacillus acidophilus*, SD: Standard deviation

Lemon tea showed highest antibacterial effectiveness followed by green tea and black tea against test organisms. At different concentrations, lemon tea showed maximum zone of inhibition and was statistically significant except for green tea (Tables 3 and 4).

Discussion

During the last few years, an increased attention has been focused on the natural plant extracts, especially those containing phenolic compounds with antimicrobial and antioxidant properties. Tea is one of the important dietary sources of these compounds.[16] Tea is reported to contain nearly 4000 bioactive compounds of which one-third is contributed by polyphenols.[8] It contains flavonoids[17,18] (alkaloids-caffeine, theophylline, and theobromine),[3] saponins, tannins, catechins and polyphenols,[18] amino acids, carbohydrates, proteins, chlorophyll, volatile compounds, minerals, trace elements, and other unidentified compounds.[3,8] Flavonoid components are the aflavins, bisflavanols, and theaflavic acids.[2] Four polyphenol compounds, epigallocatechin gallate (EGCG), epicatechin gallate (ECG), EGC and EC[3,18] are significant antioxidants

Table 3: Comparison between different groups of commercially available tea in relation to test organisms at different concentrations

Multiple comparisons: Bonferroni test				
S. mutans	**Group A**	**Group B**	**Mean difference (A-B)**	**P value**
1.5 g concentration	1	2	−17.7	<0.001
	1	3	−19.0	<0.001
	2	3	−1.3	0.149
5 g concentration	1	2	−15.3	<0.001
	1	3	−17.3	<0.001
	2	3	−2.0	0.946
7.5 g concentration	1	2	−13.7	<0.001
	1	3	−15.3	<0.001
	2	3	−1.7	0.343

Group 1: Black tea, Group 2: Green tea, Group 3: Lemon tea. *S. mutans: Streptococcus mutan*

S. mutans zone of inhibition	At 1.5 g, 5 g and 7.5 g concentration
	Group 2 > Group 1, (*P*<0.001)
	Group 3 > Group 1, (*P*<0.001)
	Group 2 = Group 3, (*P*>0.05)

S. mutans: Streptococcus mutan

Table 4: Comparison between different groups of commercially available tea in relation to test organisms at different concentrations

Multiple comparisons: Bonferroni test				
Lactobacillus acidophilus	**Group A**	**Group B**	**Mean difference (A-B)**	***P* value**
1.5 g concentration	1	2	−15.7	<0.001
	1	3	−16.0	<0.001
	2	3	−0.3	1.000
5 g concentration	1	2	−10.7	<0.001
	1	3	−14.0	<0.001
	2	3	−3.3	0.001
7.5 g concentration	1	2	−12.7	<0.001
	1	3	−13.7	<0.001
	2	3	−1.0	0.234

L. acidophilus zone of inhibition	At 1.5 g and 7.5 g concentration	At 5 g concentration
	Group 2 > Group 1, (*P*<0.001)	Group 2 > Group 1, (*P*<0.001)
	Group 3 > Group 1, (*P*<0.001)	Group 3 > Group 2, (*P*<0.001)
	Group 2 = Group 3, (*P*>0.05)	Group 3 > Group 2, (*P*<0.001)

constituents, and these compounds are responsible for the inhibition of pathogens.[18] These constituents are oxidized during fermentation to yield a complex mixture of secondary

polyphenols. The polyphenols, catechins, gallic acid, and the aflavins are introduced as antibacterial agents of tea.[10] Tea contains 300-2000 ppm fluoride of which more than 50% is extracted into the tea infusion.[11]

Literature search shows that most of the previous studies have been done on organic extracts of tea or extracts made using tea leaves. Very few have been done on commercially available tea. Since most of people use commercially available tea and it is consumed regularly in aqueous form, the present study was carried out to determine antibacterial efficacy of aqueous extracts of tea. Earlier it was suggested that fluoride is an important contributing factor in tea which is responsible for caries inhibition. The recent investigations show that flavonoids have antibacterial properties which over rules the low fluoride concentration in tea.[3]

Three different commercially available tea of single brand at three different concentrations were selected for the present study. These three tea varieties at three different concentrations were more effective on *S. mutans* than *L. acidophilus* but at 5 g concentration black tea shows more inhibitory effect on *L. acidophilus* than *S. mutans*, this observed difference might be a result of genetic differences among the organisms. Hence, further research should be done to know the reasons behind the same.

Although the varieties of tea used in this study were manufactured from leaves of the same plant species, *Camellia sinesis*, the catechin content depends on the leaf process before tea drying, the geographic location of the farm, soil, climate, and the type of tea such as blended or decaffeinated.[10] Although we had taken all tea of same brand for the present study, we cannot assure/guarantee of tea leaves taken from the same geographical area under same climatic conditions. Hence, further research is necessary to overcome this limitation. The absorption of elements such as aluminum may be increased under acidic conditions as in the presence of citric acid in tea with lemon.[12] Citrus flavonoids have a large spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer, and antiviral activities.[19] The peel of citrus fruits is a rich source of flavonoid glycosides, coumarins, and volatile oils.[20] The fiber of citrus fruit also contains bioactive compounds, such as polyphenols, the most important being Vitamin C (or ascorbic acid), and they certainly prevent and cure Vitamin C deficiency.[21] This may be the reason lemon tea is more effective besides geographical or climatic conditions. The primary difference between green tea and black tea is in the fermentation process required to produce tea. In case of black tea, the leaves and buds are fully fermented or oxidized after they have been dried. In green tea, leaves are steamed after they are dried.[16] Green tea also contains gallic acid, quercetin, kaempferol, myricetin, caffeic acid, and chlorogenic acid.[22] Black tea and green tea contains same amount of flavonoids, their chemical structure is different.[10] The green tea is non-fermented thus contains highest concentrations of polyphenols[22] whereas the black tea undergoes fermentation process which oxidizes many of polyphenols catalyzed by polyphenol oxidase, degrading EGCG, and reducing tea's antibacterial potency. EGCG and ECG are present in minute amounts in black tea compared to green tea; this may be due process of oxidation.[8,23] This may be the reason why black tea exhibits less inhibitory action.[3] This is in accordance with many previous studies conducted on black tea and green tea. Shehab showed aqueous extract of green tea was more effective than black tea on oral bacteria in pregnant women,[24] Subramaniam *et al.* showed aqueous extracts of oolong tea and green tea showed greater inhibitory effect than black tea and chlorhexidine against *S. mutans*.[3]

The alkaloids, flavonoids, tannins, EGCG, and polyphenols are reported to have inhibitory effect. The alkaloids are said to interfere with microbial cell division whereas flavonoids possess anti-glucosyltransferase activity and inhibit bacterial adherence. Tannins, on the other hand, inhibit bacterial growth with their strong iron binding capacity and also inhibit glucosyltransferase activity and bacterial adhesion.[3] Tea polyphenols exert different actions by acting as a slow release source of catechins and theaflavins, which inhibit growth and adherence of *S. mutans* to the surface. EGCG and EC have been reported to disrupt reconstituted bacterial membranes.[3] EGCG is primarily responsible for the inhibitory activity.

It is also seen that different extracts were different in their antimicrobial effectiveness depending on the extractive solvent used. Oloke and Kolawole showed that bioactive components of any medicinal plant may differ in their solubility depending on the extractive solvents used.[25] Our study also shows significant inhibitory effect of all tea on *L. acidophilus* which is contradictory to Taylor *et al.*[26] and Margaret[22] who showed drinking green tea appears to protect against dental caries. Mushtaq showed that recent research indicates that tea can counter some of the microorganisms such as *S. mutans, L. acidophilus* that can form plaque and biofilms on teeth, resulting in tooth decay.[11]

Use of hot water is the traditional way of brewing tea and previous studies have shown that hot water is an efficient way of extracting tea. Water temperature is an important factor when extracting tea. Higher temperature reduces the polarity of water, thus increasing its extraction efficiency and capability to dissolve less polar compounds. Raising the temperature of water also reduces its surface tension and viscosity, which increases the diffusion rate and the rate of mass transfer during extraction.[27]

Conclusion

Lemon tea possessed maximum antimicrobial effect against test organisms at all concentrations taken in the present study. Maximum zone of inhibition was seen at 7.5 g concentration for lemon tea followed by green tea and black tea.

Recommendation

The present study has its own limitations. This study was performed on standard microorganisms. Further studies on these commercially available teas on *S. mutans, Lacidophilus* present in oral cavity are necessary with minimal inhibitory concentration for therapeutic use.

Dental public health significance

Because of many health benefits, lemon tea could be advocated as an alternative to black and green tea as lemons have strong antibacterial, antiviral, and immune boosting powers which build immunity and fight infection. Its antibacterial property inhibits oral bacteria responsible for causing many dental diseases.

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